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Full Length Research Paper

Effects of two growth hormones and three manure formulas on a variety of papaya (*Carica papaya* L.) in the Sudano-Sahelian zone of Mali

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This study was conducted in the orchard of the Rural Polytechnic Institute for Training and Applied Research (IPR / IFRA) in Katibougou to assess the growth and production of solo papaya (*Carica papaya* L., 2n = 18) in Sudano-Sahelian zone of Mali as well as the effects of the hormone and manuring formulas. A split plot device, comprising 2 factors with 3 levels of variation each, was used. The main factor was the hormone represented by gibberellic acid (GA3) and the synthetic auxin Trichloro phenoxi acetic acid (2-4-5-T). The secondary factor was the manure formulas consisting of organic manure (OF); organic + mineral manure (FOM) and Liquid manure (FL). The test was characterized by 9 treatments (GA3 + FO, GA3 + FOM (Organic and mineral manure), GA + FL (Liquid manure) ; 2-4-5-T + FO, 2-4-5-T + FOM, 2-4-5-T + FL; SH (No hormone) + FO (Organic manure) ; SH + FOM and SH + FL). It was carried out on a ferruginous tropical hydromorphic soil with a strong flapping of water tables. Each treatment was applied to 3 rows of 4 plants. It appears from the study that the application of the combination of hormones (GA3 + 2-4-5-T) and the formula of organic manure + mineral manure stimulates growth and that the application of hormones (GA3 and 2-4-5-T) on the papaya tree makes it possible to reduce planting - flowering cycle and early fruit setting.

Key words: Biomass, flowers, height of plants, number of leaves, planting-flowering.

INTRODUCTION

Agriculture represents a huge challenge for the development of Mali. In fact, around 80% of the population earn their income there. The majority of it is made up of small vulnerable family farms practicing mainly food crops (Sidibé and Ballo, 2013).The practice of industrial, vegetable and fruit crops is also

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considerable. In Mali, fruits are a source of food necessary to fight malnutrition and poverty, even if their production is still relatively low. Indeed, according to a survey of food consumption standards in West Africa, the consumption of fruit by a Malian is 3 kg / year. According to CIRADGRET (2006), papaya production was 13.7 million tons worldwide and 54,664 tons in Mali (TECHNISEM, 2014). The main papaya production areas in Mali are the regions of Sikasso, Ségou, Koulikoro, and the District of Bamako. The papaya tree (*Carica papaya* L.) is said to be from Mexico. It is cultivated in many tropical countries, notably in the Antilles, Brazil and Central America (CRFG, 1998 ; Fabert, 2011). From the nutritional point of view, it should be remembered that the consumption of papaya is particularly recommended; generally not treated with pesticides. It is classified as one of the best digestible tropical fruits. It can be eaten fresh, as a salad or as a dessert; or made into juice, syrups, or jam. Papaya is not very energetic because its sugar content is low (33 kcal versus 55 kcal on average for other fruits). Thanks to its richness in vitamin C, beta carotene (70%) and its very remarkable mineral concentration (rate high of calcium, potassium and magnesium), it contributes very effectively to the defense of the organism, and to fight against infections. For a proportion of 100 g of the fruit, up to 85 % of the recommended nutritional intake is ensured (which is 80 mg for adults), which contributes to meeting the body's needs for provitamin (A) around 30% (caribfruits.cirad.fr/fruits_des_antilles/papaye). Despite these multiple advantages, the culture is confronted with several constraints in Mali which are among others the yield, the choice of an adequate fertilization, the unsuitability of the soils, the poor control of irrigation, the varieties and their resistance to diseases (ITV, 2003). These problems can only be resolved by agricultural research and technical progress, particularly with regard to plant breeding and conservation techniques (Dankoro, 2018; Sidibé et al., 2017). In addition, given the abundance of poultry droppings due to the expansion of poultry farms on the outskirts of Koulikoro, it seemed appropriate to us to seek a manure formula based on poultry droppings favorable for the production of papaya. This is the context of this study, which will attempt to answer the following research questions: a) does the spraying of hormones on the papaya tree improve its growth? b) does spraying the hormones on the papaya tree shorten its cycle ? and c) what is the best combination of hormone and manure formula which can be used on papaya?

MATERIALS AND METHODS

Experimentation site

The experiment was done in the orchard of the Rural Polytechnic Institute for Training and Applied Research (IPR/IFRA) in Katibougou, Mali. This area is characterized by a ferruginous tropical hydromorphic soil with strong beating of aquifers. This soil has a clayey-silty texture, poor in nitrogen and phosphorus, moderately rich in potassium and slightly acidic.

Plant materials

The plant material used consisted of seeds of *Carica papaya* L. (Solo 8 variety). The seeds of this variety were purchased from authorized resellers, in this case, Green Seed - Mali. At the beginning of the rainy season, the seeds were sown in the seed bed.

Hormones and fertilizers used

Hormones were:

(i) Gibberellin acid (GA3): it is powdery in nature, whitish in color and dissolved in alcohol, and;
(ii) 2-4-5-T Trichloro-phenoxy-acetic acid (2-4-5 -T) solid crystalline water soluble.

Fertilizers were:
(i) Urea (46% nitrogen);
(ii)Potassium sulphate (50% potash);
(iii) Poultry manure, and;
(iv) Liquid and organic fertilizers (Aton AZ, Turbo root, Codabor, Coda maxi and Boramin Ca).

Experimental setup and size of the plots

The experimental device used was the split-plot, with 3 repetitions; the hormones were the main factor with 3 levels of variation which constitute the main treatments (Gibberellic acid (GA3), Trichloro-phenoxy-acetic acid (2-4-5-T)), and a control (hormone free or No hormone); the manure formula was taken as a secondary factor at 3 levels of variations which constitute the secondary treatments: organic manure formula, organic manure formula + mineral manure, and the liquid fertilizer formulas. The repetitions were separated by aisles of 1 m, subdivided into main plots of 6 m × 6 m and secondary plots of 6 m × 2 m. The test plot covered an area of 360 m², 20 m long and 18 m wide.

Observations and measurements

Observations were carried out every two months on two plants of the central line of each sub-plot and related to the measurement of the height of the plant (cm), the counting of the number of leaves, the dry leaf biomass, the number of flowers, planting-flowering cycle (number of days between planting and flowering on 50% per elementary plot), average number and diameter of the fruits.

Statistical analysis

The results of the various observations were subjected to analysis of variance using the GENSTAT software with the application of the Newman - Keuls 5% test for the comparison of treatment averages.

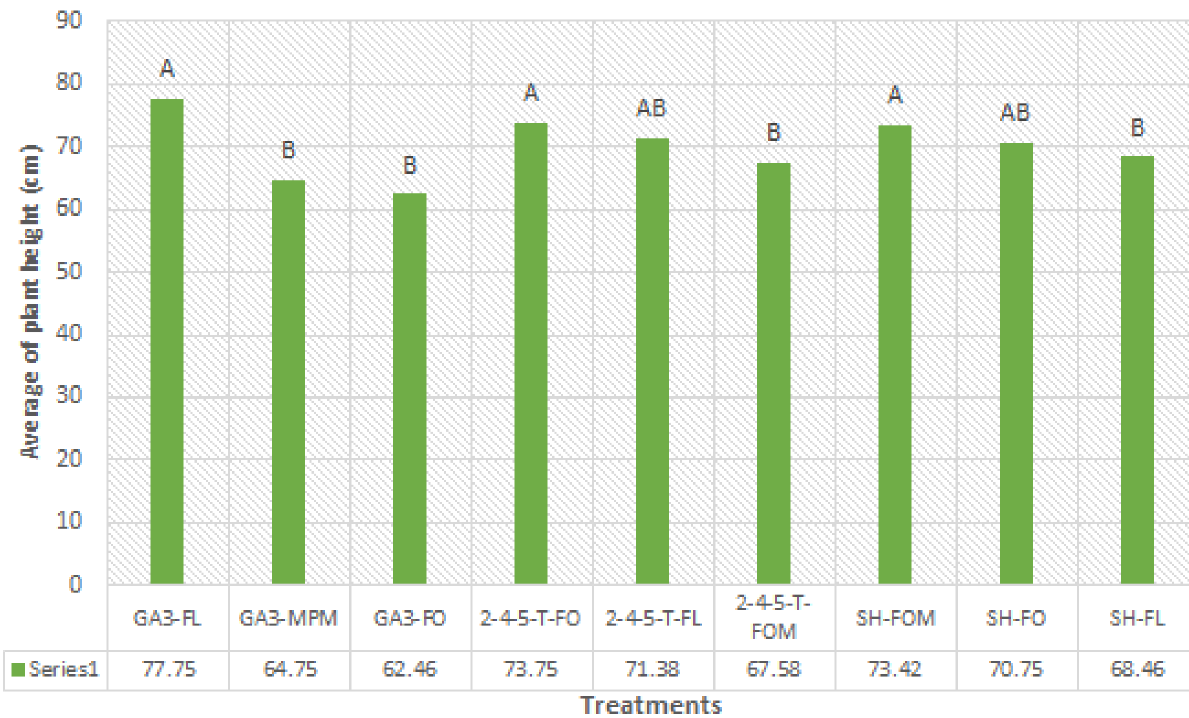

Figure 1. Effects of the manure formulas and the interaction between the hormones on the height of the plant 2 months after recovery. FOM, Organic manure + mineral manure + Mineral; FO, Organic manure; FL, Liquid manure; SH, No Hormone), GA3 (Giberrellic acid), 2-4-5-T (Trichloro-phénoxy-acetic acid).
Columns with the same letters are not statistically different.

RESULTS

All the measurements made on the plants allowed us to draw bar graphs of their growth or development after processing the variance data.

Analysis of plant height data 2 months after recovery

Analysis of the variances in the data revealed no significant difference between the effects of the hormones. It revealed a highly significant difference between the effects of the fertilizer formulas and their interaction between the effects of the hormones (Figure 3). According to the Newman-Keuls test, we have seen that the plants which received the GA3 + liquid manure obtained the largest size than those which received the combinations GA3 + formulas of organic manure and mineral manure + organic manure. The combination of 2-4-5-T + organic manure gave plants larger than those given 2-4-5-T and formula of organic manure + mineral manure. Without hormones, the plants having received the formula of organic manure + mineral manure obtained the greatest height than the plants treated with the formula of liquid manure (Figure 1).

Analysis of plant height data 4 months after recovery

The analysis of variance revealed a significant difference between the effects of hormones and the effects of mineral formulas. It was not detected a significant difference between the effects of the interaction of hormones and manure formulas. The classification according to the Newman-Keuls test made it possible to observe that the plants that received GA3 obtained the largest size while without hormones the largest plants were observed on the plots that received the formula of organic manure + mineral manure (Figures 2 and 3).

Analysis of plant height data 6 months after recovery

The analysis of variance revealed not only a significant difference between the effects of fertilizer formulas and those of the interaction between hormones and fertilizer formulas, but also a non-significant difference between the effects of hormones. The classification according to the Newman-Keuls test made it possible to notice the plants that received GA3 and formulas of organic fertilizer + mineral fertilizer obtained a larger size in height than those with combinations of GA3 and organic

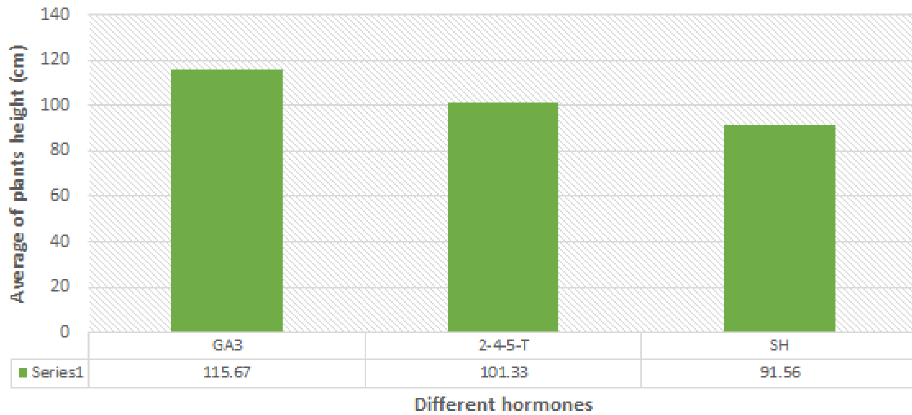

Figure 2. Effect of hormones formula on plant height 4 months after recovery.

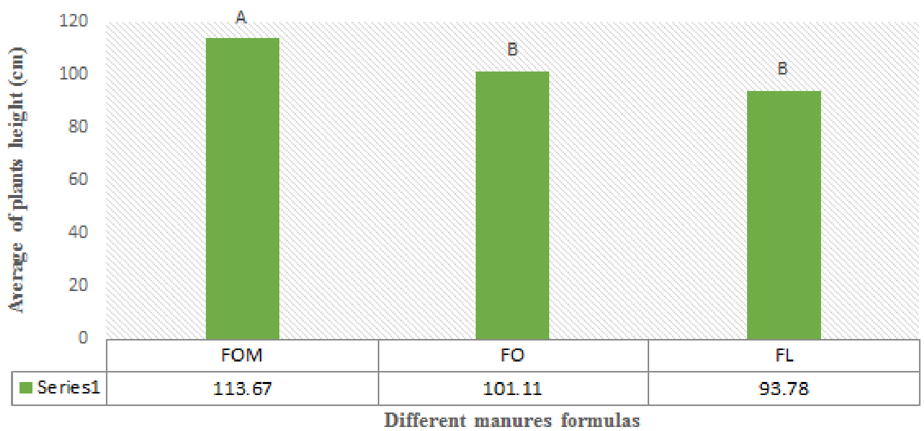

Figure 3. Effects of the manure formula on the height of the plant 4 months after recovery.

fertilizer; GA3 and liquid manure formula.

The plants that received 2-4-5-T and the organic manure + mineral manure formulas were larger than those that received the combinations of 2-4-5-T and liquid manure formula; 2-4-5-T and organic fertilizer formula. Plants without hormones that received organic manure + mineral manure obtained the greatest height as against those that received organic manure and / or liquid manure (Figure 4).

Analysis of the data on the number of leaves 2 months after recovery

The analysis of variance of the obtained data found no significant difference between the effects of hormones and fertilizer formulas or between the effects of their interaction. It should be noted that the plants have the same number of leaves whatever the treatment.

Analysis of the data on the number of leaves 4 months after recovery

The analysis of variance in the data found no significant difference between the effects of hormones and fertilizer formulas or between the effects of their interaction. It should be noted that the plants have the same number of leaves regardless of the treatment.

Analysis of the data on the number of leaves 6 months after recovery

Analysis of variance in the data revealed a highly significant difference between the effects of hormones, the effects of fertilizer formulas, and those of the interaction between hormones and fertilizer formulas. The classification, according to the Newman-Keuls test, made it possible to notice the plants that received the

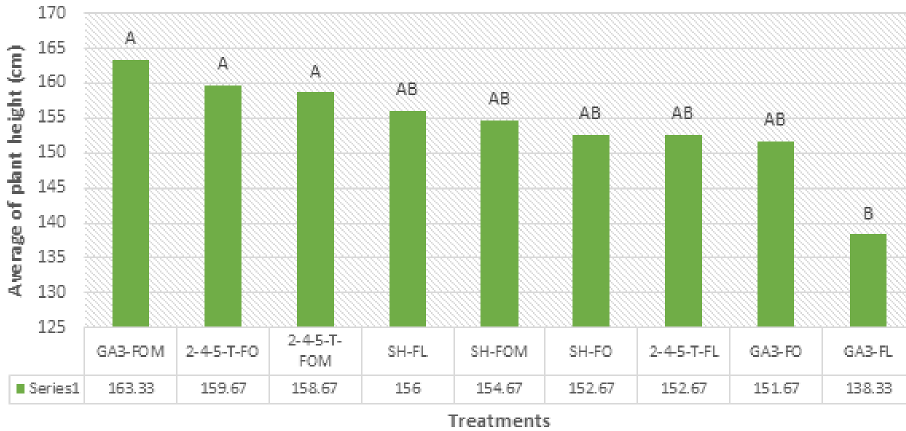

Figure 4. Effects of the interaction of hormones and fertilization formulas on the height of plants 6 months after recovery.

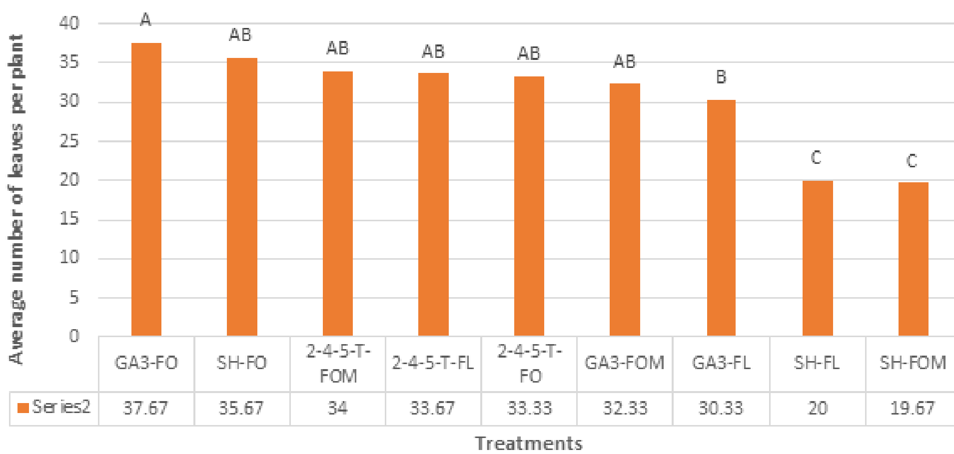

Figure 5. Effect of the interaction of hormones and manure formulas on the number of plant leaves 6 months after resumption.

combination of GA3 and formulas of organic manure emitted more leaves than the plants without hormones and those which received GA3 + liquid manure (Figure 5).

Dry leaves biomass data 2 months after recovery

Analysis of variance in the data revealed a significant difference between the effects of the hormones. It made it possible to detect a highly significant difference between the effects of the manuring formulas and those of their interaction with hormones. The classification according to the Newman Keuls test made it possible to note that the plants that received the combination of GA3 + Liquid manure formula and GA3 + Organic and Mineral manure formula gave the highest amount of dry leaf biomass. However, the lowest amount was obtained in plants without hormones and those that received GA3 + organic manure formula (Figure 6).

Analysis of data on dry leaves biomass 4 months after recovery

Analysis of variance in the data revealed a highly significant difference between the effects of the hormones and a significant difference between the effects of the interaction and the fertilizer formulas. The difference between the effects of the fertilizer formula was statistically insignificant. The classification, according

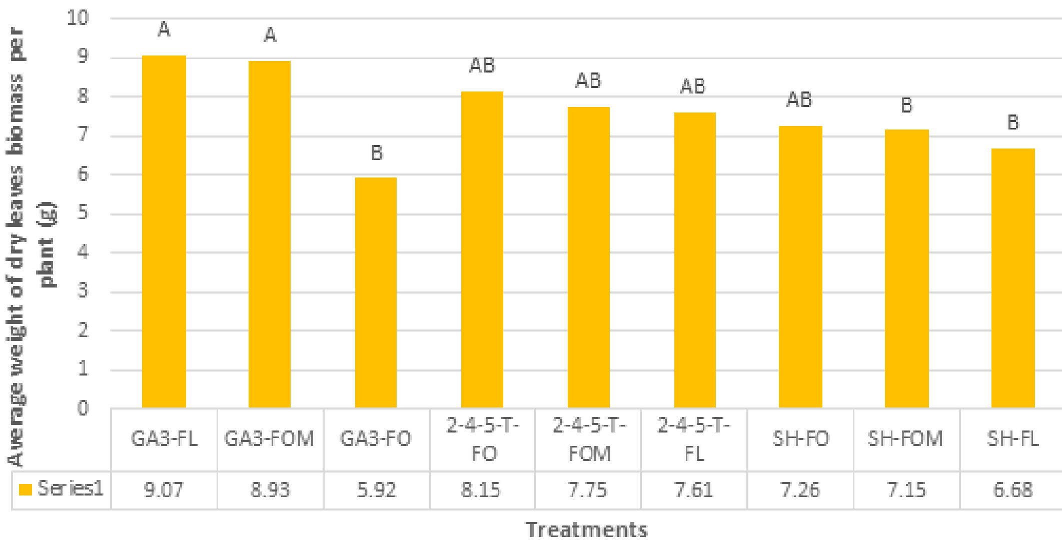

Figure 6. Effects of the interaction between hormones and fertilization formulas on dry biomass 2 months after recovery.

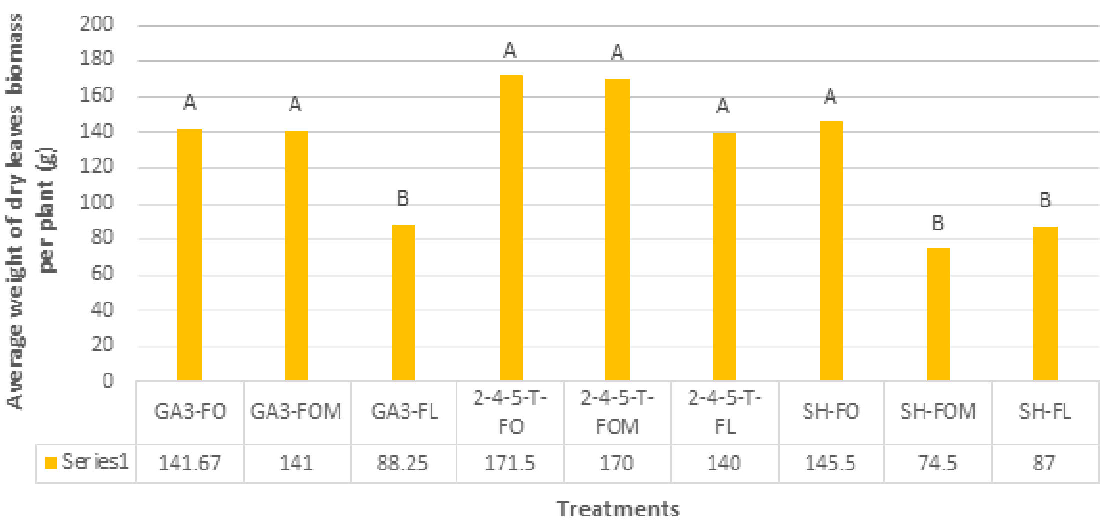

Figure 7. Effects of the hormone interaction and manure formula on dry leaves biomass 4 months after recovery.

to the Newman-Keuls test, made it possible to note that the greatest quantity of dry biomass was obtained by the plants having received the combinations GA3 + formulas of organic manure and GA3 + formulas of organic manure + mineral manure. Plants that received 2-4-5-T combinations had the same amount of dry biomass. Without hormone, the plants that received the organic manure + mineral manure formula produced more dry biomass than those having received the combinations GA3 + liquid manure formula and organic manure + mineral manure; Hormone-free liquid fertilizer formula (Figure 7).

Analysis of the dry leaf biomass data 6 months after recovery

Analysis of variance in the data revealed a significant difference between the effects of the fertilizer formulas and those of the interaction between hormones and

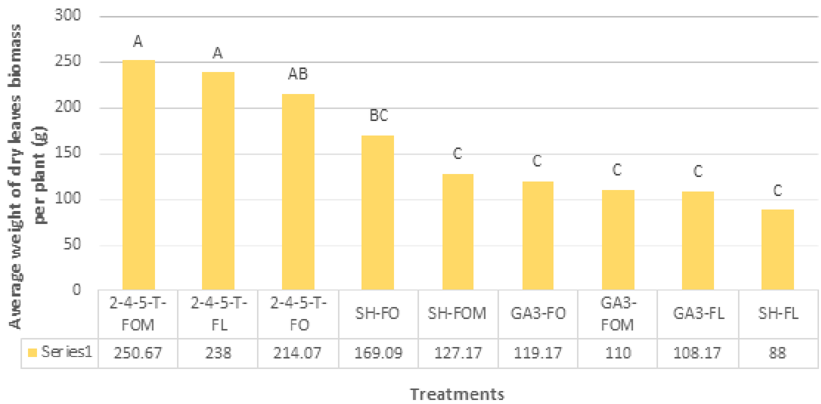

Figure 8. Effects of the interaction of hormones and fertilizer formulas on dry leaf biomass, 6 months after recovery.

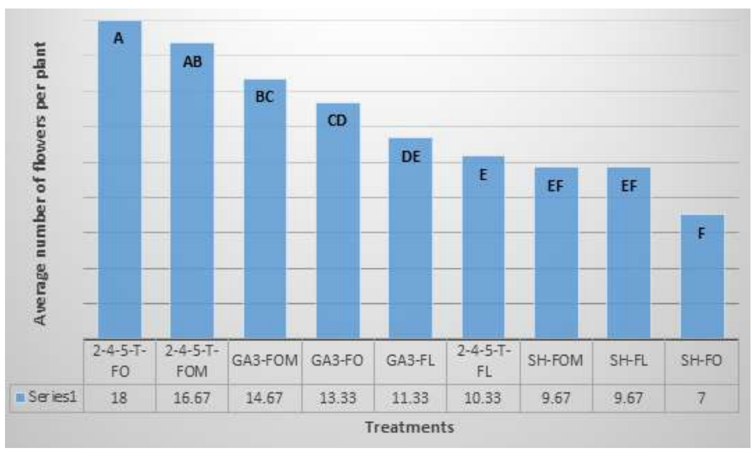

Figure 9. Effects of the interaction of hormones and manures formulas on the number of flowers 6 months after resumption.

fertilizer formulas. It also revealed a highly significant difference between the effects of hormones. The classification according to the Newman-Keuls test made it possible to note the plants having received the combination of 2-4-5-T + formulas of organic manure + mineral manure and that of 2-4-5-T + liquid manure provided the highest amount of dry leaf biomass ; they are followed by plants having received the combination of 2-4-5-T + organic manure. The plants that provided the lowest dry biomass were obtained from the plots that received the other combinations (Figure 8).

Number of flowers

The analysis of variance revealed a significant difference between the effects of the fertilizer formulas and those of the interaction between hormones and fertilizer formulas. It, also revealed a highly significant difference between the effects of hormones. The classification according to the Newman-Keuls test made it possible to note the plants having received the combination of 2-4-5-T + formulas of organic manure + mineral manure and that of 2-4-5-T + liquid manure provided the largest number of flowers ; they are followed in descending order by the plants having received the combinations of 2-4-5-T + organic manure, GA3 + organic and mineral manure, GA3 + organic manure, GA3 + manure and 245T + liquid manure. The plants with the lowest number of flowers were obtained from plots that did not receive hormones (Figure 9).

Planting - flowering cycle

Analysis of variances showed a highly significant

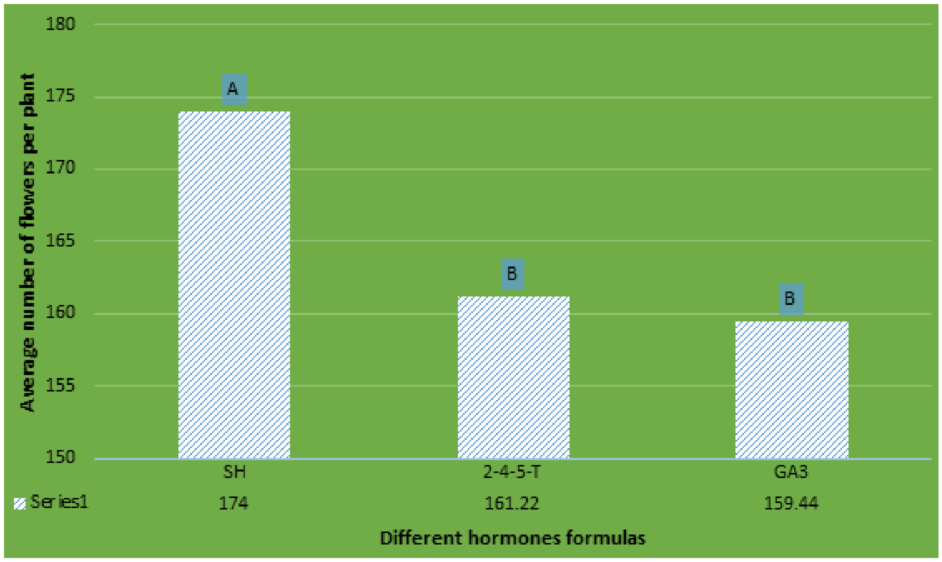

Figure 10. Effects of hormones formula on the flowers number.

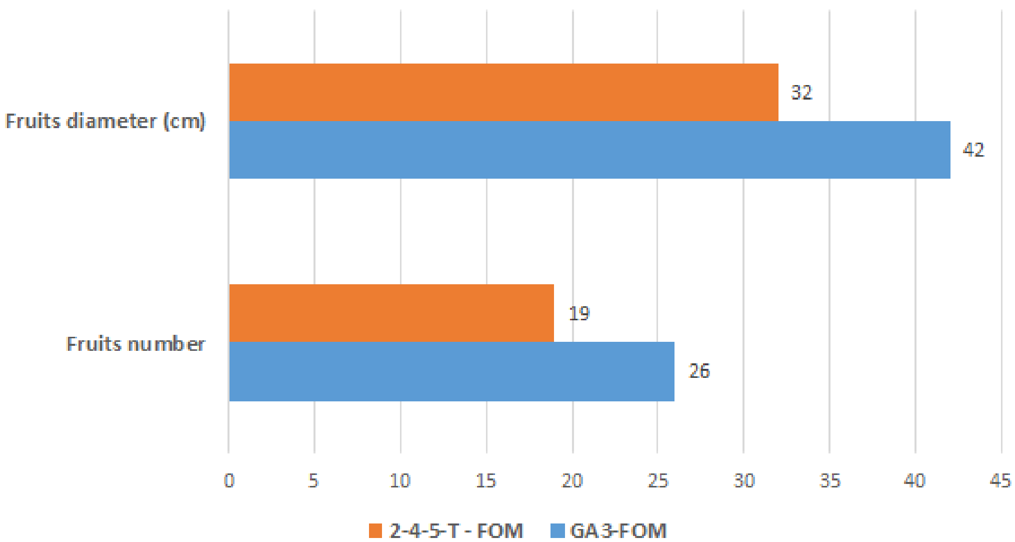

Figure 11. Hormones effect on the average of fruits number and the fruits diameter (in cm).

difference only between the effects of hormones. The Newman Keuls test allowed us to know that the contribution of the hormones reduced the vegetation cycle of the plants independently of the fertilization formula (Figure 10).

Number and diameter of fruits

We noted the formation of the fruits on the plants having received the hormones GA3 + organic manure + mineral manure and 2-4-5-T + organic manure + mineral manure. Plants that received AG3 appear to produce more fruit than those that received 2-4-5-T (26 versus 19). Plants that received AG3 appear to provide larger diameter fruits than those obtained on 2-4-5-T (4.2 cm versus 3.2 cm) (Figure 11).

DISCUSSION

The results obtained by this experiment show that the plants which received GA3 + the formula of organic manure + mineral manure and those which received 2-4-

5-T + the formula of organic manure + mineral manure had the greatest pruning, highest amount of dry biomass, highest number of flowers, shortest planting-flowering cycle and earliest fruit production. Our results corroborate with a study conducted on the behavior of bean plants, *Phaseolus vulgaris* (Fortin and Nadeau, 2002), under the influence of growth hormones: auxin and gibberellic acid. Growth hormones have the effect of growing plants faster, industrially producing seedless fruit in addition to several other applications (Sidibé et al., 2012). The plants under their influence are disproportionate and extremely large since the hormones act in particular on the roots, stem and the proliferation of the leaves. However, to observe this phenomenon, it is necessary to grow enough plants for the experiment to be statistically valid. In addition, the growth period must be long enough to see a major difference and all plants must absorb an adequate amount of the hormone (Sidibé et al., 2012; Fabert, 2011). According to the hypothesis, plants treated with auxin of concentration 1 x 10 - 4 mol / L, and those treated with gibberellic acid of concentration 500 ppm have faster growth.

Conclusion

FRom the results obtained, the plants of larger diameters were obtained at the level of the plots that received the formulas of organic manures + mineral manure and organic manure. Plants that received GA3 + organic fertilizer formulas + mineral fertilizer produced the highest number of leaves than those that received other combinations of GA3 2-4-5-T which did not stimulate leaf production in the plants. Without hormones, the plants that received the organic fertilizer formula had the highest number of leaves than those that received organic fertilizer + mineral fertilizer and liquid fertilizer. The greatest amount of dry biomass was obtained by the plants that received the combinations 2-4-5-T + formulations of organic manure + mineral manure and 2-4-5-T + formulations of manure. The lowest amount of dry biomass was provided by the plants that received the combinations GA3 + organic manure formula and GA3 + liquid manure formula. The addition of hormones reduced the plant's cycle of vegetation regardless of the fertilization formula. Fruit formation was obtained on plants that received the hormones GA3 + organic manure + mineral manure and 2-4-5-T + organic manure + mineral manure. Plants that received GA3 appear to produce more fruit than those that received 2-4-5-T (26 versus 19). Plants that received GA3 appear to provide larger diameter fruits than those that obtained 2-4-5-T (4.2 cm versus 3.2 cm).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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African Journal of Agricultural Research

Full Length Research Paper

Estimation of magnitudes of heterosis for grain yield and yield contributing traits of conventional maize (*Zea mays* L.) single cross hybrids

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This study was initiated with the objective of estimating magnitude of heterosis of selected conventional maize inbred lines. Ten elite inbred lines were selected based on over per se performances. The crosses were done in a 10 x 10 half-diallel mating design to produce 45 F_1 single crosses hybrids during 2016. The experiment was conducted at Bako National Maize Research Center in 2017 main cropping. The experimental material consisted of 45F1 single crosses and three standard checks with a total of 48 genotypes. The quantitative agronomic data were recorded following standard protocols of CIMMYT. Percent of mid-parent (MP), better parent (BP) and standard heterosis was estimated for agronomic traits that revealed significant under analysis of variance. Maximum percent of mid-parent (240.34%), better parent (220.85%) and standard heterosis of 18.79% were detected for grain yield. Crosses of L1 x L4, L1 x L5 and L2 x L4 showed significant heterosis over the best two standard checks for grain yield.

Key words: Heterosis, economic heterosis, grain yield, maize

INTRODUCTION

Maize (*Zea mays* L.) occupies a prestigious place in the world agriculture. It is a miracle crop in view of its widespread usage as food and nonfood items (Lone et al., 2016). It is also considered as the third important cereal crop in the world after rice and wheat (Devi et al., 2016). The world maize production area was around 196.08 million hectares, and that of wheat and rice was 220.83 million and 163.00 million hectares, respectively (USDA, 2020).

Despite a remarkable increase in maize yield starting from late 1990s, maize yield is still low relative to that of the developed countries and world average. According to USDA (2020) for example, the national average grain yield for USA, Canada, Turkey, Argentina, Egypt, and world average was 10.21, 10.21, 11.42, 8.06, 8.00 ton and 5.93 ton ha^{-1}, respectively.

Maize has a significant importance in the food security and diets of rural Ethiopia and gradually penetrated into

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urban centers. This is particularly evidenced by green maize being sold at roadsides throughout the country as a hunger-breaking food available during the months of February to May annually (Twumasi et al., 2012).

In Ethiopia, during 2017/18 main cropping season, maize was cultivated on 2.13 million hectares from which 8.4 million tons of maize grain was produced (CSA, 2017, 2018). Even though, more than forty hybrids were released by national maize research program and other agricultural research centers, the demand for maize is increasing from time to time due to the high food demand associated with increased human population. From 2015 (CSA) to 2017 (USDA) (USDA, 2017), the population of Ethiopia increased from 90.08 million to 103.9 million people out of which maize growers increased from 7.49 million to 10.86 million at household level. To ensure food security for the ever increasing human population in Ethiopia and the maize growing agro-ecologies of the country, information on heterosis of maize germplasm is essential in maximizing the effectiveness of hybrid development.

The phenomenon of heterosis has provided the most important genetic tool improving yield potential of crop plants. Heterosis breeding is primary based to the identification of parents and their cross combinations capable of producing the highest level of transgressive segregates. The magnitude of heterosis depends on the extent of genetic diversity between parents and helps in choosing the parents for superior Fl hybrids (Dhoot et al., 2017). Many scholars defined heterosis as the superiority in performance of hybrid individuals compared with their parents which implies that the hybrids obtained have more vigour than their parents. For example, Falconer et al. (1996) defined heterosis or hybrid vigour as the difference between the hybrid and the mean of the two parents. This difference is called mid-parent heterosis. The type of parents chosen and measurement of trait determines the level of heterosis in maize, hence it is important to select superior parents (Abuali et al., 2012). Better parent heterosis quantifies the performance of the F1 hybrid over the better performing parent (Springer and Stupar, 2007). Standard heterosis refers to the superiority or inferiority of F_1 crosses over the standard check hybrids and it indicates the usefulness or uselessness of the crosses. Both relative heterosis (MP) and heterobeltiosis (BP) are important parameters as they provide information about the presence of dominance and over dominance type of gene actions in the expression of various traits. Heterosis has been used in breeding and production of many crops, and in maize, an estimated 15% per annum on yield increase has been reported through the use of hybrids (Iqbal et al., 2010).

The study of heterosis can provide the basis for the exploitation of valuable hybrid combinations in the breeding program (Shrestha and Gurung, 2018). The present study aimed to estimate the magnitude of heterosis and yield advantage of the crosses for grain yield and related traits.

MATERIALS AND METHODS

Experimental locations

The experiment was conducted at Bako National Maize Research Center during 2017 main cropping season. Bako is located in East Wollega zone of the Oromia National Regional State, Western Ethiopia. The center lies between 9°6' North latitude and 37°09' east longitude in the sub-humid agro-ecology, at altitude of 1650 m above sea level (m.a.s.l). It is 250 km far from Addis Ababa, the capital city of the country. The mean annual rainfall in the last half century is 1238 mm. The rainy season covers April to October and maximum rain is received in the months of July and August. The mean minimum, mean maximum and average air temperature is 12.8, 29.0, and 20.9°C, respectively; and relative humidity is 51.04% (Appendix Table 1). The soil is reddish brown in color and clay and loam in texture (Wakene, 2001). According to USDA (2015) soil classification, the soil is Alfisols developed from basalt parent materials, and is deeply weathered and slightly acidic in reaction (Wakene, 2001).

Experimental materials

Ten inbred lines namely, L1, L2, L3, L4, L5, L6 and L7 from BNMRC (Bako National Maize Research Center), L8 and L9 from CIMMYT and L10 from IITA were used in this study. The inbred lines were cross pollinated in a half diallel fashion to develop 45 single cross hybrids. A total of 48 hybrids, 45 single cross hybrids and three commercial standard checks (BH546, BH547 and SPRH1) were evaluated during 2017 main cropping season for grain yield and related agronomic traits.

Experimental design

Each 48 hybrid was sown in 5.1 m-long rows with row to row and plant to plant spacing of 75 cm and 30 cm respectively. The experiment was laid out in alpha lattice (0, 1) with two replications.

Collected data

The agronomic data were recorded on ten random competitive plants following standard protocols of CIMMYT (Magorokosho et al., 2009). The recorded quantitative traits were;

Days to anthesis (AD): The number of days from planting date to 50% pollen shedding was recorded.

Days to silking (SD): The number of days from planting date to when 50% of the plants in a plot have grown 2-3 cm silk length.

Plant height (PH): The height from the soil surface to the first tassel branch of ten randomly taken plants from each experimental unit was measured in centimetres. Like ear height, this was also measured two weeks after pollen shedding had ceased from the same plants that EH measured.

Ear height (EH): The height from the ground level to the upper most ear-bearing node of ten randomly taken plants from each experimental unit was measured in centimetres. The measurement was made two weeks after pollen shedding ceased.

Table 1. Range of mid- parent and better parent heterosis of F_1 crosses for yield and yield contributing traits.

Traits	Mid-parent heterosis (%)						Better parent heterosis (%)					
	G. Mean	Minimum	Cross	Maximum	Cross	SE (±)	G. Mean	Minimum	Cross	Maximum	Cross	SE (±)
GY	70.01	-15.56	L9 x L10	240.34	L3 x L7	0.78	40.94	-36.45	L9 x L10	220.85	L3 x L7	0.9
AD	-8.6	-15.52	L3 x L4	-3.01	L2 x L10	1.48	-6.84	-14.53	L3 x L4	0.62	L5 x L7	1.71
SD	-9.5	-17.42	L3 x L4	-4.07	L8 x L10	1.21	-7.87	-16.95	L3 x L4	-0.6	L8x L10	1.4
PH	74.11	32.36	L9 x L10	109.18	L7 x L8	6.73	91.24	32.36	L9 x L10	134.12	L5 x L8	7.77
EH	79.11	44.52	L2 x L9	142.08	L3 x L8	6.33	111.84	45.53	L2 x L9	184.09	L3 x L6	7.31
ED	17.56	2.41	L2 x L6	38.12	L3 x L7	0.09	8.73	-6.32	L3 x L6	33.38	L3 x L7	0.1
EL	29.05	-1.44	L9 x L10	61.51	L3 x L7	0.82	20.83	-7.81	L9 x L10	55.47	L3 x L7	0.95
TKW	25.03	-8.33	L9 x L10	68.42	L1 x L5	23.74	15.4	-14.47	L8 x L9	50	L1 x L5	27.41
SHP	5.71	0.09	L4 x L8	12.09	L2 xL8	1.35	2.9	-3.87	L2 x L5	8.14	L3 x L6	1.56
HI	-23.65	-48.68	L3 x L4	-4.54	L6 x L6	5.82	-29.64	-57.92	L3 x L4	-7.86	L6 x L8	6.73

GY = Grain Yield (tonha^{-1}); AD = 50% Days to Anthesis; SD = 50% Days to Silking; PH = Plant Height (cm); EH = Ear Height (cm); ED = Ear Diameter (cm); EL = Ear Length (cm); TKW = Thousand Kernel Weight (g); SHP = Shelling Percentage; HI = Harvest Index (%); G. mean = Grand Mean; SE (±) = Standard Error.

Grain weight per plot (GY): Ears were removed from all plants in each plot leaving other crop residues (husk, leaf, stem and tassel) intact. The total field weight from all the ears of each experimental unit was recorded and converted to ton ha^{-1}.

Ear diameter (ED): This was measured at the mid-section along the ear length, as the average diameter of ten randomly taken ears from each experimental plot in centimetres using digital calliper.

Ear length (EL): Length of ears from the base to tip was measured in centimetres. Data recorded represents the average length of ten randomly taken ears from each experimental unit.

Thousand kernel weight (TKW): After shelling each ten randomly selected ear, random kernels from the bulk of shelled grain in each experimental unit was taken and thousand kernels were counted using a seed counter and weighed in grams and then adjusted to 12.5% grain moisture.

Harvest index (HI) (%): The ratio of grain yield to total above ground dry biomass yield multiplied by 100.

$$\mathrm{HI} = \frac{\text{Grain yield per plot}}{\text{Total above ground dry biomass per plot}} \text{ x } 100$$

Where, GY = Grain yield per plot in tons, AGDB = above ground dry biomass in tons.

Shelling percentage (SHP): The ratio of weight of ten sampled cob after shelling to the weight of ten sampled cob before shelling multiplied by 100.

$$\mathrm{SHP} = \frac{\text{Shelled sampled grain yield}}{\text{Not shelled sampled grain yield}} \text{ x } 100$$

Data analysis

Heterosis was estimated over the mid-parent, better parent (Heterobeltiosis), and over the checks (Economic heterosis) for traits revealed significant under analysis of variance and test of significance was carried out. The significance of heterosis was determined as the least significant differences (L.S.D) at 0.05 and 0.01 levels of probability according to formula suggested by Steel et al. (1997).

Estimation of heterosis

Mid parent heterosis (MPH), better parent heterosis (BPH) and standard variety heterosis (SH) were calculated for the character that showed significant differences between genotypes (crosses and parents) following the method suggested by Falconer et al. (1996).

Mid parent heterosis (%) = $\frac{F_1 - MP}{MP} \times 100$

Better parent heterosis (%) = $\frac{F_1 - BP}{BP} \times 1000$

Standard Variety heterosis (%) = $\frac{F_1 - SV}{SV} \times 100$

Where, F_1 = Mean value of the crosses
MP = Mean value of the two parents
BP = Mean value of the better parent
SV = Mean value of standard varies.

The standard error of the difference for heterosis is calculated as follows:

SE (m) for MP = $\pm\sqrt{\frac{3Me}{2r}}$

SE (m) for BP = $\pm\sqrt{\frac{2Me}{r}}$

SE (m) for SH = $\pm\sqrt{\frac{2Me}{r}}$

SE (d) for MP = SE (m) for MP x t value at error degree of freedom,
SE (d) for BP = SE (m) for BP x t value at error degree of freedom,
SE (d) for SH = SE (m) for SH x t value at error degree of freedom.

Test of significance for heterosis was done by comparing (F_1-MP) with SE (d) for mid parent, (F_1 -BP) with SE (d) for better parent heterosis and for standard heterosis (F_1 – SV) with SE (d). Where, SE (m) is standard error of the mean, SE (d) is standard error of the difference, Me is error mean square and r is the number of replications.

RESULTS AND DISCUSSION

The range of percent of mid-parent and better parent for all traits under the study as respects crosses was summarized in Table 1 whereas, for standard heterosis in Table 2. The extent of mid parent, better parent and standard heterosis for best five hybrids for yield and yield contributing traits at 10% selection intensity was summarized in Table 3.

Significance of heterosis was tested and presented in Appendix Table 2 (for mid parent and better parent heterosis) and Appendix Table 3 (for standard or economic heterosis).

Percent of mid and better parent heterosis

Heterosis may be defined as the superiority of an F_1 hybrid over both of its parents in terms of yield and other characteristics (Bhat and Singh, 2005). Krivanek et al. (2007) declared that heterosis is prerequisite for developing a good economically viable hybrid maize variety.

In the present study, most crosses showed positive significant mid and better parent heterosis for grain yield and related traits (Appendix Table 2). In this study, maximum mid and better parent heterosis obtained for grain yield was 240.34% and 220.85% respectively in Table 1. Similar results were reported by Tollenaar et al*.* (2004) who estimated heterosis in maize hybrids and their parental inbred lines for grain yield and its components and also reported 167% heterosis for grain yield.

Similarly, Gudeta (2007) found that all the crosses manifested positive and highly significant heterosis over the mid-parents while most of the crosses manifested positive and highly significant heterosis over the better parents; he also showed maximum mid parent heterosis (259.17%) and better parent heterosis (226.68%). Berhanu (2009) also reported, all crosses showed positive and significant MPH and HPH and can get 202.34% of MPH and HPH value for grain yield. Besides, Wende (2014) reported out of 81 crosses, thirty-three hybrids displayed positive mid-parent heterosis up to 250% and thirty-three crosses displayed positive high parent heterosis up to 235% for grain yield.

Majority of the crosses showed negatively significant heterosis over mid parent and better parent for days to anthesis and silking indicating the progenies are earlier than their respective inbred lines. Crosses that exhibited negatively significant heterosis over mid and better parent had gene combinations that reduce 50% days to anthesis and silking. In general, almost all crosses showed significant difference and had negative mid and better parent heterosis for days of anthesis and silking (Appendix Table 1). The present result is in agreement with Bayisa (2004), Dagne et al. (2007) and Berhanu (2009) who, reported that almost all crosses showed negative and significant MPH and BPH for days to anthesis and silking indicating F_1 crosses were earlier on days to anthesis and silking than their parents and Dagne et al. (2013) also reported all the hybrids showed negative MPH and BPH for days to anthesis and silking at Bako and Harare environments.

For mid and better parent heterosis for 50% days to physiological maturity, all crosses depicted negative heterosis whereas majority of crosses showed negative heterosis respectively. Similar findings were previously reported by Habtamu (2015) that both mid-parent and better parent heterosis for days to maturity are negative for all the crosses. This showed that hybrids tend to be earlier in maturity compared to their parents.

All crosses expressed positive highly significant heterosis over both mid and better parent heterosis for plant and ear height (Appendix Table 2). The result indicates vigorousity of the crosses over their parents. This result is in agreement with earlier findings of Bayisa et al*.* (2005), Gudeta (2007) and Berhanu (2009). They reported that the positive MPH and BPH for plant and ear height in all crosses except some crosses. Dagne et al*.* (2013) also reported positive MPH for plant and ear height was positive at Bako and Harare locations and an average of 50.2 and 62.6% for the two locations respectively. The current result is in disagreement with Amanullah et al*.* (2011). They reported less positive heterotic values for plant height and ear height in their F_1 population. The reason could be due to the materials used in making crosses which might be population or early generation inbred lines rather than fixed and homozygous lines.

Heterosis over mid and better parent for ear diameter varied from 2.41% (L2 x L6) to 38.12% (L3 x L7) and -6.32% (L3 x L6) to 33.38% (L3 x L7) respectively. All 45 crosses showed highly significant positive heterosis over the mid parent whereas, most crosses showed significant positive better parent heterosis. For ear length also, almost all crosses manifested passively significant mid and better parent heterosis into desired direction (Appendix Table 2). Similar results were reported by Bayisa (2004) who reported positive and significant heterosis over better-and-mid-parent for ear length in most of the crosses across Ambo and Kulumsa locations. Gudeta (2007) reported that most crosses showed positive and significant heterosis over the better parent while more than 98% of the crosses showed positive and significant heterosis over the mid parent in combined analysis of three locations. According to the result of

Table 2. Range of standard (economic) heterosis over three standard checks for yield and yield contributing traits.

Traits	Checks	G. mean	Minimum	Cross	Maximum	Cross	SE (±)
GY	BH546	-13.16	-41.16	L8 x L10	18.79	L1 x L4	0.90
	BH547	-12.98	-41.04	L8 x L10	19.03	L1 x L4	
	SPRH1	11.72	-24.30	L8 x L10	52.82	L1 x L4	
AD	BH546	-1.34	-6.37	L3 x L4	5.10	L8 x L10	1.71
	BH547	1.23	-3.92	L3 x L4	7.84	L8 x L10	
	SPRH1	-1.35	-6.37	L3 x L4	5.10	L8 x L10	
SD	BH546	-1.22	-6.96	L3 x L4	4.43	L8 x L10	1.40
	BH547	1.34	-4.55	L3 x L4	7.14	L8 x L10	
	SPRH1	-1.85	-7.55	L3 x L4	3.77	L8 x L10	
PH	BH546	-2.28	-18.78	L1 x L3	14.15	L2 x L5	7.77
	BH547	1.09	-15.98	L1 x L3	18.08	L2 x L5	
	SPRH1	-0.17	-17.03	L1 x L3	16.61	L2 x L5	
EH	BH546	3.44	-16.10	L1 x L3	26.88	L5 x L10	7.31
	BH547	-7.64	-25.08	L1 x L3	13.30	L5 x L10	
	SPRH1	1.01	-18.06	L1 x L3	23.91	L5 x L10	
ED	BH546	1.30	-14.60	L3 x L8	11.18	L2 x L4	0.10
	BH547	-10.90	-24.89	L3 x L8	-2.21	L2 x L4	
	SPRH1	12.27	-5.36	L3 x L8	23.21	L2 x L4	
EL	BH546	-4.59	-18.35	L9 x L10	7.99	L3 x L7	0.95
	BH547	6.28	-9.05	L9 x L10	20.29	L3 x L7	
	SPRH1	8.33	-7.29	L9 x L10	22.62	L3 x L7	
TKW	BH546	0.84	-24.05	L8 x L10	25.32	L7 x L9	27.41
	BH547	-2.85	-26.83	L8 x L10	20.73	L7 x L9	
	SPRH1	20.71	-9.09	L8 x L10	50.00	L7 x L9	
SHP	BH546	-2.48	-11.17	L2 x L5	3.19	L3 x L8	1.56
	BH547	0.26	-8.68	L2 x L5	6.09	L3 x L8	
	SPRH1	-3.54	-12.14	L2 x L5	2.07	L3 xL8	
HI	BH546	-7.73	-40.56	L4 x L9	21.77	L4 x L6	6.73
	BH547	1.01	-34.94	L4 x L9	33.30	L4 x L6	
	SPRH1	-7.64	-40.51	L4 x L9	21.89	L4 x L6	

GY = Grain Yield (tonha^{-1}); AD = 50% Days to Anthesis; SD = 50% Days to Silking; PH = Plant Height (cm); EH = Ear Height (cm); ED = Ear Diameter (cm); EL = Ear Length (cm); TKW = Thousand Kernel Weight (gm); SHP = Shelling Percentage; HI = Harvest Index (%); G. mean = Grand mean; SE (±) = Standard.

Dagne et al. (2007), all crosses showed positive mid parent heterosis for ear length. Besides, Berhanu (2009) also reported positive and significant mid and better parent heterosis values for ear length. Habtamu (2015) noticed that ear length showed relatively higher and positive mid and better parent heterosis.

For thousand kernels weight, most crosses showed above 20% mid parent hetrosis while crosses which showed above 40% of mid-parent heterosis was found in L1 x L2, L1 x L4, L1 x L5, L1 x L7, L2 x L7, L3 x L5, L3 x L7 and L5 x L7 with maximum of 68.42%. In this case, crosses involving L1 and L7 as one of their parents have expressed high positive mid parent heterosis. This might be the dominant effect of alleles which is found in line L1

Table 3. Extent of mid parent, better parent and standard heterosis for best five hybrids for yield and yield contributing traits at 10 % selection intensity mid-parent heterosis.

Crosses	Traits									
	GY	AD	SD	PH	EH	ED	EL	TKW	SHP	HI
					Mid parent heterosis					
L3 x L7	240.34	-10.51	-13.12	87.89	118.36	38.12	61.51	45.9	5.38	-32.19
L7 x L8	227.81	-10.39	-11.63	109.18	138.97	30.17	54.35	23.73	3.61	-33.8
L3 x L8	156.14	-8.05	-9.3	99.49	142.08	17.86	47.78	18.52	8.9	-17.46
L3 x L5	137.27	-10.6	-13.48	74.12	84.21	22.34	31.15	50	7.07	-19.82
L1 x L7	136.2	-7.32	-7.46	92.71	90.12	32.48	37.04	48.28	7.48	-29.65
SE (±)	0.98	1.34	1.6	6.38	6.31	0.09	0.82	23.74	1.35	5.82
					Better parent heterosis					
L3 x L7	220.85	-7.45	-10.24	98.42	159.55	33.38	55.47	34.85	2.38	-32.75
L7 x L8	180.85	-6.21	-8.43	109.18	169.71	27.14	49.88	10.61	2.4	-42.42
L3 x L8	131.27	-6.98	-9.04	103.68	153.64	16.48	38.3	14.29	4.61	-28.7
L1 x L4	99.16	-8.98	-8.88	109.27	103.87	16.06	36.02	27.78	-2.17	-32.49
L2 x L8	91.01	-7.19	-9.83	102.69	76.31	-0.42	21.06	14.52	3.93	-9.25
SE (±)	1.13	1.55	1.85	7.37	7.29	0.1	0.95	27.41	1.56	6.73
					Standard Heterosis over Best check (BH546)					
L1 x L4	18.79	-3.18	-2.53	-2.68	-0.86	9.87	-0.52	16.46	-8.5	-6.51
L2 x L4	7.46	-1.27	-1.9	-2.52	-3.6	11.18	1.05	8.86	-8.56	-2.8
L1 x L5	4.94	-3.82	-5.7	-2.2	-2.05	6.95	0.52	21.52	-3.24	-20.25
L7 x L8	2.72	-3.82	-3.8	1.19	11.3	-8.96	-3.67	-7.59	1.01	-12.61
L4 x L6	1.71	0	-0.63	3.9	12.33	7.15	0.79	10.13	-2.66	-29.87
SE (+)	1.13	1.55	1.85	7.37	7.29	0.1	0.95	27.41	1.56	6.73

GY = Grain Yield (tonha^{-1}); AD = 50% Days to Anthesis; SD = 50% Days to Silking; PH = Plant Height (cm); EH = Ear Height (cm); ED = Ear Diameter (cm); EL = Ear Length (cm); TKW = Thousand Kernel Weight (gm); SHP = Shelling Percentage; HI = Harvest Index (%); SE (±) = Standard.

and L7. Most crosses showed positive MPH and BPH for this trait. This result corresponds with the previous finding of Berhanu (2009),Gudeta (2007) and Habtamu (2015) who reported most crosses showed positive heterosis over the better and mid parent for the trait.

All crosses showed positive and significant heterosis over the mid parent heterosis for shelling percentage while 86.67% of crosses manifested positive significant heterosis over better parent with maximum of 8.28% (L3 x L6) in desired way. For harvest index, all crosses exhibited negative mid and better parent heterosis indicating that harvest index of inbred lines was higher than that of F_1 crosses.

Standard (economic) heterosis

Standard heterosis to grain yield, L1 x L4, L1 x L5, L2 x L4, L4 x L6, L7 x L8 and L7 x L9 crosses were relatively high positive significant standard heterosis over the best two checks (BH546 and BH547) (Appendix Table 3). In the case of breeding program, hybrids performing better than the best standard variety could be used as a commercial production (Table 2). The result is similar to the previous finding of Legesse et al. (2008) and Habtamu (2015).

For 50% days to anthesis and silking, most crosses showed negative significant heterosis over BH546 and SPRH1 standard checks indicating that, these crosses exhibited earliness for this trait than those standard checks (Appendix Table 3). The current finding was in line with Shushay (2014) who reported both positive and negative significant heterosis for days to anthesis and silking.

Most crosses showed negative significant standard heterosis for plant and ear height over BH546 and SPRH1 standard checks. Negative value indicated shortness of the checks while high positive heterosis also indicated the tallness from the checks. The current finding agreed with Shushay (2014) who reported both positive and negative significant levels of heterosis for plant and ear height was observed.

For ear diameter, most crosses exhibited positively significant standard heterosis over BH546 and SPRH1 standard checks. All crosses showed significant negative standard heterosis over BH547 indicating that the ear diameter of all crosses is lower than this check. In the case of ear length, most crosses showed negative standard heterosis over BH546 check in undesired direction. Most crosses manifested positive highly significant heterosis over BH547 and SPRH1 standard checks for ear length. For thousand kernel weight, most crosses manifested positive highly significant standard heterosis over SPRH1 than BH546 and BH547 standard checks indicate that 1000 kernels weight of those crosses were higher than that of checks. Similar to the current study, both undesirable and desirable heterosis for 1000 kernel weight in maize has been previously reported by Shushay (2014).

Most crosses exhibited positive highly significant standard heterosis over BH547 than BH546 and SPRH1 standard heterosis for shelling percentage (Appendix Table 3), indicating that the crosses showed higher shelling percentage than the three checks in desired direction. For harvest index, 33.3, 60 and 33.3% of crosses showed positive standard heterosis over BH546, BH547 and SPRH1 respectively in desired direction.

CONCLUSION AND RECOMMENDATION

Hybrid namely L1 x L4 was superior hybrids, exhibited >18.79% standard heterosis over best check hybrids (BH546) for grain yield. Both mid parent and better parent heterosis were higher in L3 x L7 hybrid for yield and yield attributing traits such as ear diameter and length. Therefore the findings of this study suggested that farmers cultivate L1 x L4 hybrid for commercial utilization in achieving higher maize grain yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX

Table 1. Mean monthly rain fall, temperature and relative humidity of Bako areas in 2017.

Month	Rainfall (mm)	Air temperature (°C)		Average	Relative Humidity (%)
		Minimum	Maximum		
January	0	8.8	32.2	20.5	46
February	57.8	9.5	31.5	20.5	47
March	33	9.7	33.2	21.45	48.6
April	155.8	10	33.4	21.7	47
May	146.5	14.2	28.6	21.4	49
June	270	14.3	27.8	21.05	52.3
July	240.7	14.4	26.9	20.65	57
August	291.3	14.2	24.7	19.45	55.3
September	230.2	14.8	25.1	19.95	55
October	86.4	14.7	26.5	20.6	54
November	86.3	14.3	27.4	20.85	52.3
December	0	14.5	30.8	22.65	49
Total	1598	153.4	348.1	250.75	612.5
Mean	133.2	12.8	29.0	20.9	51.04

Source: Bako Agricultural Research Centre, Unpublished

Table 2. Mid-parent and better parent heterosis for grain yield and yield contributing traits of 45 F_1 single crosses.

Crosses	Traits									
	GY		AD		SD		PH		EH	
	Percent Mid parent (MP) and Better parent (BP) heterosis									
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
L1xL2	70.87**	64.03**	-10.18	-10.18	-11.70	-10.65	65.48**	94.41**	54.87**	76.41**
L1xL3	52.74**	16.55**	-11.50	-10.18	-11.56	-9.47	74.80**	74.96**	94.44**	122.73**
L1xL4	105.40**	99.16**	-11.37	-8.98	-11.49	-8.88	94.32**	109.27**	90.77**	103.87**
L1xL5	89.71**	87.32**	-12.21	-9.58	-14.37	-11.83	69.08**	110.31**	61.13**	101.41**
L1xL6	41.13**	25.21**	-11.64	-11.38	-11.50	-11.24	60.16**	105.24**	58.60**	115.85**
L1xL7	136.20**	73.11**	-7.32	-5.59	-7.46	-6.63	92.71**	103.32**	90.12**	96.48**
L1xL7	70.18**	40.65**	-9.62	-7.19	-10.09	-7.69	88.35**	92.13**	107.62**	126.14**
L1xL9	22.64**	11.57**	-8.71	-8.43	-9.79	-9.52	73.34**	87.59**	68.22**	90.14**
L1xL10	1.29**	-18.72	-6.63	-6.06	-6.87	-6.02	63.05**	98.25**	76.24**	119.37**
L2xL3	117.66**	71.07**	-10.32	-8.98	-11.43	-10.40	85.85**	118.56**	103.77**	170.00**
L2xL4	93.29**	80.15**	-9.62	-7.19	-11.93	-10.40	67.46**	55.31**	64.14**	74.30**
L2xL5	76.08**	71.13**	-9.30	-6.59	-9.66	-8.09	73.01**	81.87**	67.30**	81.82**
L2xL6	30.78**	11.98**	-11.64	-11.38	-11.95	-11.18	44.90**	56.35**	46.01**	71.35**
L2xL7	104.80**	54.25**	-8.54	-6.83	-9.14	-7.23	64.61**	82.36**	66.37**	82.84**
L2xL8	123.44**	91.01**	-9.62	-7.19	-11.11	-9.83	76.44**	102.69**	111.92**	76.31**
L2xL9	20.08**	5.31**	-7.51	-7.78	-9.09	-7.74	54.10**	66.37**	44.52**	45.53**
L2xL10	21.64**	-5.33	-3.01	-2.42	-4.42	-2.41**	51.35**	55.96**	58.52**	71.63**
L3xL4	115.15**	60.73**	-15.52	-14.53	-17.42	-16.95	88.63**	103.33**	106.63**	155.00**
L3xL5	137.27**	82.66**	-10.60	-9.30	-13.48	-12.99	74.12**	116.81**	84.21**	170.45**
L3xL6	53.98**	8.36**	-9.41	-8.33	-10.09	-11.86	65.73**	112.61**	76.30**	184.09**
L3xL7	240.34**	220.85**	-10.51	-7.45	-13.12	-10.24	87.89**	98.42**	118.36**	159.55**
L3xL8	156.14**	131.27**	-8.05	-6.98	-9.30	-9.04	99.49**	103.68**	142.08**	153.64**
L3xL9	42.74**	2.14**	-8.28	-6.63	-8.99	-6.55	81.89**	97.02**	94.12**	155.00**
L3xL10	19.03**	-21.55	-5.04	-3.03*	-6.71	-3.61*	61.29**	96.32**	73.87**	154.09**

Table 2. Contd.

L4xL5	67.11**	60.05**	-12.18	-11.93	-12.85	-12.85	80.28**	106.36**	72.76**	100.31**
L4xL6	54.12**	40.60**	-8.72	-6.55	-10.03	-7.65	64.48**	93.64**	61.58**	103.10**
L4xL7	120.33**	58.36**	-8.61	-4.35	-10.14	-6.63	83.32**	86.93**	88.50**	94.72**
L4xL8	90.99**	54.05**	-9.09	-9.09	-10.36	-10.11	91.08**	101.51**	107.80**	143.15**
L4xL9	2.16**	-4.35	-8.19	-5.42	-8.93	-5.95	77.68**	76.88**	66.81**	75.85**
L4xL10	6.49**	-12.46	-8.50	-5.45	-8.99	-5.42	61.19**	80.61**	56.30**	80.50**
L5xL6	48.73**	30.50**	-7.83	-5.36	-8.31	-5.88	58.17**	62.16**	54.97**	66.43**
L5xL7	82.27**	34.69**	-4.14	0.62**	-4.93	-1.20**	69.13**	103.62**	73.11**	108.25**
L5xL8	118.78**	82.66**	-9.92	-9.66	-10.36	-10.11	87.11**	134.12**	101.80**	179.25**
L5xL9	48.55**	33.60**	-8.45	-5.42	-9.51	-6.55	56.79**	83.63**	57.91**	72.91**
L5xL10	13.21**	-10.01	-6.43	-3.03	-7.25	-3.61*	58.44**	65.69**	74.56**	75.18**
L6xL7	66.63**	13.37**	-6.38	-4.35	-7.14	-6.02	106.46**	106.46**	75.00**	128.71**
L6xL8	58.74**	19.50**	-8.14	-5.95	-8.62	-6.47	99.35**	106.05**	68.22**	154.77**
L6xL9	25.17**	21.73**	-10.18	-9.64	-11.24	-10.71	88.16**	92.76**	47.82**	74.86**
L6xL10	9.53**	-2.45*	-6.91	-6.06	-7.14	-6.02	69.88**	94.49**	49.12**	60.76**
L7xL8	227.81**	180.85**	-10.39	-6.21	-11.63	-8.43	109.18**	109.18**	138.97**	169.71**
L7xL9	114.72**	48.34**	-8.26	-6.83	-7.78	-7.23	85.41**	96.47**	93.65**	111.22**
L7xL10	52.38**	-2.34*	-3.07*	-1.86**	-4.22	-4.22*	80.34**	114.29**	102.75**	142.90**
L8xL9	24.53**	-4.42	-5.85	-3.01*	-5.20	-2.38**	67.87**	67.87**	86.64**	131.95**
L8xL10	-8.89	-36.45	-3.23	0.00**	-4.07	-0.60*	50.44**	67.72**	73.49***	139.00**
L9xL10	-15.56	-26.61	-6.34	-6.06	-5.99	-5.42	32.36**	32.36**	48.02**	61.45**
SE (±)	0.98	1.13	1.34	1.55	1.60	1.85	6.38	7.37	6.31	7.29

	ED		EL		TKW		SHP		HI	
	Percent Mid parent (MP) and Better parent (BP) heterosis									
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
L1xL2	17.21**	12.2**	19.47**	12.94**	41.07**	27.42**	6.44**	4.74**	-24.88	-31.09
L1xL3	20.00**	9.81**	26.90**	26.07**	32.08**	25.00**	5.63**	3.40**	-46.82	-49.54
L1xL4	20.09**	16.06**	38.63**	36.02**	50.82**	27.78**	1.34**	-2.17**	-21.17	-32.49
L1xL5	23.13**	21.09**	30.71**	20.48**	68.42**	50.00**	7.84**	4.72**	-38.20	-42.41
L1xL6	12.59**	4.28**	21.74**	15.66**	27.42**	6.76**	9.47**	7.02**	-17.97	-28.05
L1xL7	32.48**	17.45**	37.04**	31.10**	48.28**	30.30**	7.48**	2.29**	-29.65	-32.73
L1xL7	21.79**	10.26**	43.14**	33.15**	31.37**	28.85**	7.99**	1.64**	-47.82	-52.76
L1xL9	13.09**	8.36**	18.18**	13.02**	9.52**	-9.21**	6.47**	3.27**	-30.49	-37.96
L1xL10	14.89**	14.37**	9.17**	-2.04**	3.39**	-10.29**	8.78**	3.79**	-20.84	-28.62
L2xL3	21.04**	6.47**	34.51**	26.37**	35.59**	29.03**	4.14**	0.35**	-32.08	-40.59
L2xL4	16.27**	15.12**	32.82**	27.86**	28.36**	19.44**	2.86**	-2.23*	-9.29**	-15.90*
L2xL5	16.77**	10.01**	24.72**	21.43**	28.57**	26.56**	0.56**	-3.87	-13.89*	-15.34*
L2xL6	2.41**	-1.07	6.04**	5.47**	25.00**	14.86**	10.22**	6.08**	-16.26	-20.30
L2xL7	23.89**	5.74**	37.92**	25.04**	42.19**	37.88**	7.04**	0.33**	-19.73	-29.30
L2xL8	14.37**	-0.42	37.09**	21.06**	24.56**	14.52**	12.09**	3.93**	-7.93**	-9.25**
L2xL9	12.00**	11.89**	14.52**	13.13**	4.35**	-5.26**	4.54**	-0.17**	-8.00**	-10.74**
L2xL10	10.07**	4.9**	7.91**	2.10**	4.62**	0.00**	4.66**	-1.65**	-36.03	-37.22
L3xL4	16.79**	3.62*	43.75**	40.14**	31.25**	16.67**	5.75**	4.25**	-48.68	-57.92
L3xL5	22.34**	13.68**	31.15**	20.17**	50.00**	40.63**	7.07**	6.18**	-19.82	-28.82
L3xL6	9.73**	-6.32	35.98**	28.39**	21.54**	6.76**	8.28**	8.14**	-32.52	-43.42
L3xL7	38.12**	33.38**	61.51**	55.47**	45.90**	34.85**	5.38**	2.38**	-32.19	-32.75
L3xL8	17.86**	16.48**	47.78**	38.30**	18.52**	14.29**	8.90**	4.61**	-17.46	-28.70
L3xL9	12.76**	-0.73	19.10**	13.19**	15.15***	0.00**	6.04**	5.05**	-42.44	-50.94
L3xL10	16.34**	6.9**	16.93**	4.32**	12.90**	2.94**	6.66**	3.90**	-29.78	-39.56
L4xL5	19.35**	13.51**	32.26**	24.10**	33.82**	26.39**	5.33**	4.69**	-6.83**	-14.96
L4xL6	8.08**	3.4**	33.21**	28.90**	19.18**	17.57**	5.43**	4.07**	-30.96	-32.86

Table 2. Contd.

L4xL7	28.55**	10.64**	53.38**	44.09**	36.23**	30.56**	3.08**	1.57**	-5.12**	-21.70
L4xL8	22.59**	7.66**	38.43**	26.52**	22.58**	5.56**	0.09**	-2.50*	-13.95*	-19.15
L4xL9	10.17**	9.2**	29.62**	26.27**	18.92**	15.79**	4.27**	3.75**	-42.69	-45.32
L4xL10	15.87**	11.49**	15.42**	5.36**	11.43**	8.33**	2.08**	0.86**	-23.33	-27.66
L5xL6	11.56**	1.75**	26.34**	22.37**	37.68**	28.38**	7.45**	6.71**	-14.50	-19.92
L5xL7	28.44**	15.57**	34.69**	19.23**	43.08**	40.91**	5.54**	3.38**	-18.11	-26.78
L5xL8	29.06**	18.63**	49.28**	28.81**	39.66**	26.56**	6.61**	3.24**	-31.33	-33.44
L5xL9	12.24**	5.85**	18.20**	13.73**	12.86**	3.95**	7.81**	7.69**	-9.87*	-13.98**
L5xL10	14.04**	12.66**	11.70**	8.46**	27.27**	23.53**	2.60**	0.77**	-26.69	-29.24
L6xL7	16.81**	-3.11	37.81**	25.54**	31.43**	24.32**	5.46**	2.60**	-4.94**	-19.76
L6xL8	11.84**	-5.44	40.00**	24.20**	19.05**	1.35**	7.99**	3.86**	-4.54**	-7.86**
L6xL9	5.14**	1.46**	18.31**	17.50**	5.33**	3.95**	5.06**	4.21**	-6.49**	-8.32**
L6xL10	7.48**	-0.87	5.63**	-0.56**	0.00**	-4.05**	7.04**	4.40**	-12.80*	-15.48*
L7xL8	30.17**	27.14**	54.35**	49.88**	23.73**	10.61**	3.61**	2.40**	-33.80	-42.42
L7xL9	28.93**	10.14**	40.53**	28.82**	39.44**	30.26**	5.54**	3.50**	-23.74	-34.55
L7xL10	26.31**	12.43**	27.94**	10.39**	10.45**	8.82**	2.83**	2.55**	-16.68	-27.79
L8xL9	15.23**	0.42**	27.19**	13.53**	1.56**	-14.47**	5.80**	2.56**	-36.84	-37.84
L8xL10	14.43**	4.03**	17.60**	-1.01*	0.00**	-11.76**	3.71**	2.23**	-21.11	-21.46
L9xL10	7.67**	2.72**	-1.44*	-7.81	-8.33	-13.16**	3.96**	2.22**	-36.04	-36.78
SE (±)	0.09	0.10	0.82	0.95	23.74	27.41	1.35	1.56	5.82	6.73

Table 3. Economic heterosis over the best standard checks for grain yield and yield contributing traits of 45 F_1 single crosses.

Crosses	Traits									
	GY	AD	SD	PH	EH	ED	EL	TKW	SHP	HI
	Standard Checks (BH546)									
L1xL2	-8.11	-4.46	-4.43*	-9.59*	-14.21*	8.36**	-10.75	0.00**	-8.83	-4.57*
L1xL3	-34.71	-4.46	-3.16*	-18.78	-16.10*	-3.02	-11.27	-11.39**	-6.04	-22.16
L1xL4	18.79**	-3.18*	-2.53*	-2.68**	-0.86**	9.87**	-0.52**	16.46**	-8.50	-6.51**
L1xL5	4.94**	-3.82	-5.70	-2.20**	-2.05**	6.95**	0.52**	21.52**	-3.24*	-20.25
L1xL6	-9.42	-5.73	-5.06	-4.55**	4.97**	8.06**	-9.57	0.00**	-2.49**	-0.36**
L1xL7	-3.02	-3.18*	-1.90**	-5.45**	-4.45**	3.73**	-7.73	8.86**	-1.45**	2.09**
L1xL7	-21.21	-1.27**	-1.27**	-10.65**	-6.68**	-2.62	-6.29	-15.19**	0.26**	-34.58
L1xL9	-23.73	-3.18*	-3.80*	-12.76*	-7.53**	4.43**	-12.84	-12.66**	-4.36	-14.08*
L1xL10	-24.74	-1.27**	-1.27**	-7.80**	6.68**	1.01**	-13.24	-22.78**	-0.53**	-1.14**
L2xL3	-11.84	-3.18*	-1.90**	1.46**	1.71**	2.82**	-0.13**	1.27**	-8.81	-8.36**
L2xL4	7.46**	-1.27**	-1.90**	-2.52**	-3.60**	11.18**	1.05**	8.86**	-8.56	-2.80**
L2xL5	-6.55	-0.64**	0.63**	14.15**	13.01**	6.24**	1.31**	2.53**	-11.17	1.26**
L2xL6	-18.99	-5.73	-4.43*	-1.87**	6.51**	2.52**	-16.64	7.59**	-3.35*	-7.88**
L2xL7	-20.50	-4.46	-2.53*	-5.85**	-5.14**	2.11**	-1.18**	15.19**	-3.33*	7.29**
L2xL8	-1.56	-1.27**	-1.27**	-1.95**	9.59**	-3.83	-4.33	-10.13**	2.52**	4.89**
L2xL9	-28.01	-1.91**	-1.90**	-9.92**	-10.79**	8.06**	-10.59	-8.86**	-7.55	3.17**
L2xL10	-12.34	2.55**	2.53**	-2.11**	6.68**	1.31**	-9.57	-13.92**	-5.75	-27.44
L3xL4	-4.13	-6.37	-6.96	-5.61**	-3.94**	-1.91	2.49**	6.33**	-2.49**	-35.09
L3xL5	-0.25**	-0.64**	-2.53*	0.65**	1.88**	-2.92	0.26**	13.92**	-1.89**	9.80**
L3xL6	-21.61	-1.91**	-1.27**	-1.30**	7.02**	-2.92	0.39**	0.00**	-1.47**	-12.71*
L3xL7	-5.44	-5.10	-5.70	-7.89**	-2.23**	-2.22	7.99**	12.66**	-1.36**	3.75**
L3xL8	-15.42	1.91**	1.90**	-5.45**	-4.45**	-14.60	-3.93	-18.99**	3.19**	9.99**
L3xL9	-30.18	-1.27**	-0.63**	-8.54**	-3.94**	-4.33	-12.71	-3.80**	-2.72*	-24.32
L3xL10	-27.36	1.91**	1.27**	-8.86**	-4.28**	-6.45	-7.60	-11.39**	-0.43**	-6.77**

Table 3. Contd.

L4xL5	-4.53	-1.27**	-1.27**	10.73**	10.79**	7.45**	3.54**	15.19**	-2.08**	1.72**
L4xL6	1.71**	0.00**	-0.63**	3.90**	12.33**	7.15**	0.79**	10.13**	-2.66*	-29.87
L4xL7	-5.54	-1.91**	-1.90**	-3.50**	1.03**	4.73**	5.37**	18.99**	-2.14**	18.82**
L4xL8	-8.11	1.91**	1.27**	-2.52**	0.34**	1.91**	-7.47	-3.80**	-3.82	-9.22**
L4xL9	-34.61	0.00**	0.00**	-4.23**	-2.74**	5.24**	-2.62	11.39**	-2.96*	-40.56
L4xL10	-18.94	-0.64**	-0.63**	-3.09**	-0.17**	5.54**	-6.68	-1.27**	-3.35*	-19.49
L5xL6	-5.59	1.27**	1.27**	12.20**	21.40**	5.44**	2.10**	20.25**	-1.40**	-4.21**
L5xL7	-26.45	3.18**	3.80**	5.12**	8.05**	-1.31	-0.52	17.72**	-0.39**	11.11**
L5xL8	-0.25**	1.27**	1.27**	13.25**	15.24**	1.31**	7.47**	2.53**	1.84**	-20.38
L5xL9	-8.66	0.00**	-0.63**	-0.57**	5.99**	2.01**	-5.11	0.00**	-0.27**	2.90**
L5xL10	-16.68	1.91**	1.27**	10.33**	26.88**	-1.41	-3.93	6.33**	-3.44**	-15.36*
L6xL7	-17.98	-1.91**	-1.27**	6.59**	18.66**	0.40**	-1.83*	16.46**	-1.15**	21.77**
L6xL8	-13.55	0.64**	0.63**	-0.33**	5.14**	-2.01	-2.88	-5.06**	2.46**	3.45**
L6xL9	-11.94	-4.46	-5.06	-0.49**	7.19**	5.14**	-8.13	0.00**	-3.49*	-0.34**
L6xL10	-9.67	-1.27**	-1.27**	0.41**	16.44**	2.72**	-11.93	-10.13**	0.05**	-5.94**
L7xL8	2.72**	-3.82	-3.80*	1.19**	11.30**	-8.96	-3.67	-7.59**	1.01**	-12.61*
L7xL9	1.41**	-4.46	-2.53**	-4.96**	9.59**	6.14**	-0.66**	25.32**	-0.29**	-0.69**
L7xL10	-9.57	0.64**	0.63**	3.66**	26.03**	-1.61	-2.23	-6.33**	-1.20**	9.58**
L8xL9	-34.66	2.55**	3.80**	-9.11**	-4.28**	-3.22	-12.45	-17.72**	1.17**	-30.21
L8xL10	-41.16	5.10**	4.43**	-9.19**	-1.37**	-8.96	-12.32	-24.05**	0.85**	-11.82**
L9xL10	-32.04	-1.27**	-0.63**	-11.87**	-1.03**	-1.01	-18.35	-16.46**	-2.05**	-29.65
SE (±)	1.13	1.55	1.85	7.37	7.29	0.10	0.95	27.41	1.56	6.73

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Limestone application effects on common bean (*Phaseolus vulgaris* L.) yield and grain iron and zinc concentration on a Ferralsol soil in Uganda

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Soil acidification has major ramifications on crop production because low pH soils are less productive. The objective of this study was to determine the effect of limestone application on yield and grain iron (Fe) and zinc (Zn) concentration in newly released high iron and zinc, drought resilient varieties of common bean (NAROBEAN 1 and 3). Using a split-plot in time design, an experiment was set up using three common bean varieties (NABE 15, NAROBEAN 1 and NAROBEAN 3) as split plots and seven rates of limestone as main plots. The experiment was done for two rainy seasons on a Ferralsol soil in central Uganda. The results showed that soil pH, cation exchange capacity (CEC) and soil concentration of Ca and Na increased with greater amounts of limestone applied to the soil. On average, NAROBEAN 1 had 30 and 48% greater yield than NABE 15 and NAROBEAN 3 in both seasons, respectively. Grain Fe and Zn concentrations were not affected by limestone application. However, across seasons, NAROBEAN 3 seeds contained 12 and 15% more zinc and, 10 and 20% more iron than NAROBEAN 1 and NABE 15, respectively. Overall, limestone application did not impact yield or yield components of common bean.

Key words: Micronutrients, acidic soils, liming, biofortification, soil reaction.

INTRODUCTION

It is well documented that as soil pH declines, so does the supply of several essential plant nutrients, including calcium, magnesium and phosphorus (Goulding, 2016; Miller, 2016; USDA-NRCS, 2019). In many soils, this decline occurs alongside an undesirable increase in aluminum to levels toxic to plants (Harter, 2007; Miller, 2016). Free aluminum ions replace plant nutrient ions, such as potassium, calcium, and magnesium, on negatively charged soil colloids (Harter, 2007; Miller, 2016). While this process frees these minerals and makes them more available to plants in the short run, it also makes those nutrients more susceptible to loss from the soil due to leaching since they are unbound (Harter, 2007). In the tropics, most soil acidification is attributable to weathered soils associated with high rainfall (Kuylenstierna et al., 2001). These acidic soils are found

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where rainfall amounts exceed the level of evapotranspiration (Kamprath and Smyth, 2005). As excess water moves through the soil, Ca^{2+} and Mg^{2+} to which the balancing of negatively charged soil soluble anions (such as NO_3^- and Cl^-) is attributable, are leached in conjunction with soluble anions. Additionally, there is decrease in the percentage of basic cations on exchange sites. These sites, once occupied by Ca and Mg are then initially replaced with H^+ from soil organic matter decomposition and plant residues which then decompose and form Al-clays (Kamprath and Smyth, 2005). With highly weathered soils, phosphate fixation commonly occurs (Harter, 2007).

Highly weathered, poorly fertile soils are predominant in Masaka district of central Uganda, where years of continuous cropping, erosion and poor soil management have contributed to soil acidification. With average farm sizes of about 0.8 to 1.2 hectares per household in many farming communities in Masaka, continuous cropping through subsistence farming has become unavoidable (FAO, 2015). Low yields contribute to the common occurrence of food insecurity, hunger, and malnutrition. Masaka district is one of the major common bean (*Phaseolus vulgaris* L.) growing regions of Uganda (Kilimo Trust, 2012; Akpo et al., 2020; CASA, 2020). Common bean is embedded in the culture of the region and beans are a staple food and major source of protein. Although the majority of farms remain subsistence, beans also provide household income (Kilimo Trust, 2012; The Gatsby Report, 2014). However, common bean productivity is often constrained by highly acidic soils (MAAIF, 2019). While critical soil pH may vary with soil texture and crop cultivars (Goulding, 2016), common bean is reported to grow best in soils at a pH of 6 to 8 (Myers, 1999; Long et al., 2010). Studies done in Wisconsin by Fageria (2008), for instance reported achieving maximum grain yield when soil pH was 6.5 although chlorosis due to iron and zinc deficiencies could arise in some common bean varieties grown in soils at a pH above 7.2 (Hardman et al., 1990).

The negative impact of low soil pH on legume yield among other effects may be attributed to its interference with effective nodulation including reduction in the formation of nodules and their dry weight (Ferguson et al., 2013). Overall, yield and nodulation in common bean, faba bean (*Vicia faba* L.) and lupin (*Lupinis* spp.) was reported to be negatively affected by low soil pH (Frey and Blum, 1994; Denton et al., 2017). Frey and Blum (1994) explained that reduction in nodulation in low pH soils may be attributable to reduction in competitiveness of inoculant strains in acidic soils. Additionally, when soils are acidic, Ca^{2+} and Mg^{2+} loading are outcompeted by high concentrations of H^+ and Mn^{2+} (Horst, 1983). This affects nodulation by creating a steep concentration gradient between the rhizodermal cell and rhizosphere and thus favors anion uptake over Ca^{2+}, Mg^{2+} and K^+ (Horst, 1983). Vargas and Graham (1988) and Dejene et al. (2016) also explained that soil acidity associated with aluminum and manganese toxicity limited common bean nodulation because low soil pH affected abiotic and biotic factors for nodule formation except where acid-tolerant rhizobia strains existed. Hungria and Vargas (2000) added that soil acidity affected nodulation by limiting the survival and persistence of inoculant rhizobia. Slattery et al. (2001) also related root stunting to reduced nutrient and water uptake and overall decreased crop productivity to aluminum toxicities in acidic soils.

Liming is therefore recommended for common bean production where soil pH is below 5.5 (Long et al., 2010), as well as the use of wood ash to neutralize soil acidity (Demeyer et al., 2001; Park et al., 2005; Goulding, 2016; Dida and Etisa, 2019). Lime application is a long-established soil management practice to increase and maintain soil pH for optimal crop production with reports of positive impact on yield of most arable crops (Fageria et al., 2007; Connor et al., 2011; Goulding, 2016; Holland et al., 2019), including common bean (*P. vulgaris* L.) (Dida and Etisa, 2019). Lime is reported to improve soil structure and hydraulic conductivity by increasing Ca^{2+} concentration and ionic strength in the soil solution which in turn leads to flocculation of clays (Haynes and Naidu, 1998). Improved soil biodiversity was also reported following lime application due to increased biological (earthworm) activity leading to improved macro porosity and soil tilth over time (Bolan et al., 2003).

The global ramifications of mineral deficiencies on human health such as disease and health complications make the development of pulse crops with high seed mineral/nutrient concentration a necessity (Vandemark et al., 2018). Biofortification is one of the suggested strategies to reduce macro and micronutrient deficiencies. This may be achieved by application of agricultural management practices aimed at increasing mineral concentration in plant edible parts, development of new varieties with high concentrations of desired/target nutrients through conventional breeding, or the combination of both management practices and genetic approaches (White and Broadley, 2005).

The effects of iron and zinc deficiency are life long and contribute tremendously to the vicious cycle of poverty in many developing countries including Uganda. Iron Deficiency Anemia (IDA) is reported to affect mothers' mental health and mother-child interactions (Black et al., 2013). Maternal Iron Deficiency Anemia (IDA) during pregnancy also increases incidences of infant post and neo-natal deaths, affects child development and their general intelligence and cognitive functioning (Dibley et al., 2012; Black et al., 2013). Zinc deficiency also leads to preterm births and has long term effects on growth, and immunity of infants (King, 2011). In Uganda, iron deficiency affects one in two non-pregnant women and at least 50% of children below age five (HarvestPlus, 2016) and, about 20-69% and 21-29% of children and adults, respectively are zinc deficient in the country (Srinivasan, 2007). Good early nutrition is essential for children to attain their full development potential from which long-term human capital gains may be achieved alongside overall economic development of developing countries like Uganda (Black et al., 2013).

Supplementation programs are often used to overcome nutrient deficiencies and show promising results. Iron supplementation in children older than seven years resulted in improvement in their mental development (Sachdev et al., 2005). Other studies on iron also showed its benefits to motor development and some benefits to language in children below four years (Stoltzfus et al., 2001; Friel et al., 2003; Lind et al., 2003; Black et al., 2004). Additionally, zinc supplementation in pregnant mothers also reduced preterm births by up to 14% (Mori et al., 2015). Alongside supplementation programs, biofortified crops such as high iron and zinc common bean could be used to alleviate the burden of malnutrition. However, there are uncertainties about the impact of low soil pH on nutrient accumulation in edible plant parts especially on iron and zinc concentration in common bean grain of biofortified varieties. Therefore, combination of improved (biofortified) germplasm and improved agronomic management practices such as limestone and fertilizer application are important to ensure availability of these nutrients in the soil for plant root uptake. Grain iron for instance, is loaded in the seeds either via xylem vessels or phloem sieve tubes (Grillet et al., 2014). About 60-70% of iron loaded into seeds is as a result of root uptake from the soil and xylem transportation whereas 30-40% of total seed iron content is via the phloem stream from senescing leaves (Waters and Grusak, 2008; Grillet et al., 2014). This emphasizes the importance of soil conditions such as low pH and its effect on nutrient solubility and availability for plant uptake and, overall human nutrition and health.

An experiment was therefore set up using different rates of limestone for raising the pH of acid soil with the objective of determining the effect that these rates would have on grain yield and, grain iron and zinc concentrations in newly released high iron-high zinc, drought-resilient common beans.

MATERIALS AND METHODS

Site description

Experimental plots were established in Masaka district, at Kamenyamigo, Mukono Zonal Agricultural Research and Development Institute (MUZARDI) (0°18'12.1"S 31°39'56.0"E, 1242 m above sea level), Uganda. The site is located within the Buganda-Catena with predominantly shallow and skeletal soils which are believed to have developed from either summit or upper slope ironstone of quartzite and deep red/reddish brown clay loams occurring on pediments (ESG et al., 2001). Although not characterized by the US Soil Taxonomy, FAO characterizes the soils at the experimental site as Ferralsols (TAXOUSDA, 2014; Bulyaba et al., 2020). These high iron Ferralsols are vulnerable to erosion under poor management. The site receives annual averages of about 367 to 291 mm of rainfall in the MAM (March-April-May) and SON (September-October-November) growing seasons, respectively (Mugume et al., 2016). The experimental site had previously been under maize (*Zea mays* L.) production. Pretreatment (before limestone and NPK application) soil samples were collected from 0 to 30 cm from each plot and analyzed for pH, nitrate, available P and K (Mehlich-3) and organic matter at Crop Nutrition Laboratory Service Ltd (CropNuts) in Nairobi, Kenya (Table 1) before starting the experiment.

Experimental design

The experiment used a split plot in time design with three replications (blocks) done over two years (2017 and 2018). Treatments included seven rates of limestone and three common bean varieties. The limestone rates were the main plots whereas the bean varieties were the split plots. The first year of the experiment (2017) was late planted at the end of a season typically characterized by long rains (referred to as season A in this study) whereas the second year (2018) was planted early in a typically short rain season (referred to as season B in this study).

Limestone samples were sent to an independent chemical analysis lab (CropNuts, Nairobi, Kenya) for chemical and physical analyses (Table 2). The concentration of Ca and Mg in lime were determined using spectroscopy and particle size gradation using mesh screens (Goodwin, 1979) (Table 2). Fertilizer NPK (17:17:17) was applied alongside all the limestone treatments, except the control, at 124 kg ha^{-1} according to NARO recommendations for pulse production in Uganda and adjustments made for Ferralsols (Sunday and Ocen, 2015). The limestone and NPK (kg ha^{-1}) treatments were 0, 0 (control), (0, 124), (1236, 124), (2471, 124), (4942, 124), (9884, 124), (19768, 124). Limestone treatments were applied once to the whole plots and the three improved common bean varieties were randomly assigned to sub-plots.

In 2017 (Season A: September, to November) and 2018 (Season B: March, to May), the three common bean varieties used were NABE 15, NAROBEAN 1 and NAROBEAN 3. These were released by National Agricultural Research Organization (NARO) in 2010 (NABE 15) and 2016 (NAROBEAN 1 and 3). NAROBEAN 1 (large-sized seeds that are white/greyish with dark black stripes) and 3 (medium-sized seeds that are light yellow) were bred and released for drought tolerance and high iron and zinc concentrations whereas NABE 15 (medium-sized seed that are red with dark red stripes) was released for drought tolerance and yield.

Site management

Prior to planting, the experimental site was deep ploughed with a tractor followed by harrowing to produce fine tillage and cultivation by hand hoeing. Limestone was applied and ploughed into the soil about 5-10 cm deep by hand hoeing three weeks before planting to allow for reaction time (Ball, 2002). Fertilizer NPK (17:17:17) was applied at planting to the furrow rows by banding and covered with a thin layer of soil to prevent seed-fertilizer contact. Fertilizers were applied at 124 kg ha^{-1} at planting. A peat-based Mak-bio-N fixer inoculant (Makerere University, Kampala, Uganda) was used to inoculate seeds just prior to planting in both seasons. Plot size was 7.6 m long by 3 m wide, and each individual plot had four rows. Season A plots were planted on 21 November 2017 and season B plots were planted on 3 March 2018. Seeds were planted in furrowed rows 50 cm apart. The furrowed rows were 3.8 cm deep and made using the string and stake technique (Lunze et al., 2012). Seeds were planted at 10 cm from seed to seed, one seed per hole and covered with soil.

Weeding in season A (2017) and B (2018) was done two weeks

Table 1. Pre-treatment soil chemical properties obtained at 0-15 cm and 15-30 cm soil depths.

Parameter soil depth	pH	EC ($\mu S\ cm^{-1}$)	CEC (meq 100 g^{-1})	OC (%)	Ca (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
0-15 cm	5.3	41.7	10.2	2.2	825	170	8	60	26
15-30 cm	5.3	38.4	9.9	2.1	817	167	6	49	27

Table 2. Physical and chemical characteristics of limestone used in the experiment.

Parameter	Unit	Limestone sample 1	Limestone sample 2
Calcium and magnesium content			
Calcium	%	36.7	37.2
Magnesium	%	0.32	0.34
Purity			
Calcium carbonate equivalent	%	87.7	88.4
Effective calcium carbonate equivalent	%	59.7	63.1
Speed of reaction/fineness			
Particle size (0.3-2 mm)	%	19.7	19.2
Particle size (< 0.3 mm)	%	48.5	52.2

after planting, before flowering, and additionally later in the both seasons as needed. Weeding was done by hand hoeing and pulling. Black bean aphids (*Aphis fabae* Scopoli) early in Season A were controlled using insecticide Dudu-Cyper® 5% EC (cypermethrin ((±) α-cyano-(3-phenoxyphenyl) methyl(±)-*cis-trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate). Application was done at 2.5 L ha^{-1} in 625 L H_2O ha^{-1}. A foliar wettable powder fungicide TATA MASTER® 72 (mancozeb 64% + metalaxyl 8%) was also applied that season at 2.5 kg ha^{-1}. Pesticides were sprayed using a knapsack sprayer. Other pests, including bean fly (*Ophiomyia phaseoli* Tryon.), which is an important pest in these areas was not observed in either season.

In 2017, the Season A experiment relied on natural rainfall and supplemental irrigation later in the season due to late planting that season. Hand-watering using watering cans was done on 19 and 23 December 2017 and 2 and 15 January 2018 (on the two middle rows) at 52,794 L ha^{-1}; the 2018 Season B experiment relied solely on natural rainfall.

Data collection

Soil samples were collected before the first season (before liming, before planting season A in 2017) and again before the second season (after 2017 liming and season A harvest, before 2018 season B planting) to determine the effect of limestone application on the soil (Table 3). Stand counts were taken at V4 (fourth trifoliate leaf stage) and again at R8 (full maturity) (Schwartz and Langham, 2010) stages of development. At V4 (fourth trifoliate leaf stage), stand counts were done using a randomly placed 5.3 m measuring rod between the two center rows along the length of each row whereas at R8, stand counts were done using a 1 m^2 quadrat.

Aboveground biomass was determined at harvest (R8-R9) by hand-clipping plants from 1 m^2 of each plot. Aboveground biomass samples were placed in a forced air oven at 60°C (NARO, Kawanda), dried to 0% moisture and weighed. Yield components (grain yield (kg ha^{-1}), pods (no. m^{-2}), seed (no. m^{-2}), seeds (no. pod^{-1}), seed (mg $seed^{-1}$)) from 1 m^2 at R8/R9 were determined from all plants. Pods were counted and hand threshed to remove seeds. Seeds were counted, oven dried at 60°C until dry, and weighed. Seeds were then packaged, labelled, and sent to CropNuts Laboratory for Fe and Zn analysis.

Data analysis

Data were analyzed by PROC GLIMMIX using SAS®9.4 (SAS institute Inc., Cary, NC). During analysis, blocks were

Table 3. Effect of different rates of limestone application on soil properties in the 0-15 cm and 15-30 cm soil horizons for soil samples collected October 15th 2017 and February 16th 2018.

Parameter	pH	EC (µS cm^{-1})	CEC (meq 100g^{-1})	OC (%)	Ca (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
0-15 cm soil									
Control (untreated)	5.1	98.7	10.1	2.3	775	178	9	58	6
Limestone rate (kg ha^{-1})									
0	4.9^{d}	254.7^{NS}	11.6^{c}	2.1^{NS}	837^{c}	160^{NS}	24^{NS}	124^{NS}	7^{c}
1236	5.1^{d}	300.3^{NS}	13.8^{bc}	2.4^{NS}	928^{c}	171^{NS}	26^{NS}	139^{NS}	8^{c}
2471	5.2^{cd}	250.4^{NS}	13.4^{bc}	2.2^{NS}	1256^{c}	167^{NS}	23^{NS}	125^{NS}	10^{c}
4942	5.8^{bc}	275.3^{NS}	15.3^{bc}	2.2^{NS}	1805^{bc}	171^{NS}	30^{NS}	141^{NS}	13^{bc}
9884	6.1^{ab}	236.2^{NS}	18.0^{ab}	2.0^{NS}	2568^{ab}	179^{NS}	27^{NS}	117^{NS}	19^{ab}
19768	6.5^{a}	279.1^{NS}	21.2^{a}	2.2^{NS}	3309^{a}	171^{NS}	30^{NS}	132^{NS}	23^{a}
Significance									
Lime (L)	***	NS	***	NS	***	NS	NS	NS	***
Covariate	**	NS	0.06	NS	*	***	NS	**	NS
15-30 cm soil									
Control	5.2	74.6	10.2	2.2	794	168	6	29	5
Limestone rate (kg ha^{-1})									
0	5.0^{b}	111.6^{NS}	10.1^{b}	2.3^{NS}	769^{b}	155^{NS}	10^{NS}	55^{NS}	5^{NS}
1236	5.0^{b}	157.1^{NS}	12.1^{ab}	2.1^{NS}	882^{b}	164^{NS}	10^{NS}	53^{NS}	6^{NS}
2471	5.1^{b}	195.2^{NS}	11.5^{ab}	2.2^{NS}	935^{b}	147^{NS}	12^{NS}	65^{NS}	7^{NS}
4942	5.2^{ab}	157.5^{NS}	13.2^{ab}	2.2^{NS}	1086^{ab}	166^{NS}	9^{NS}	48^{NS}	8^{NS}
9884	5.3^{ab}	129.1^{NS}	13.2^{ab}	2.1^{NS}	1273^{ab}	160^{NS}	9^{NS}	37^{NS}	9^{NS}
19768	5.6^{a}	162.5^{NS}	15.3^{a}	2.2^{NS}	1839^{a}	166^{NS}	13^{NS}	59^{NS}	10^{NS}
Significance									
Limestone (L)	**	NS	**	NS	**	NS	NS	NS	NS
Covariate	***	NS	*	**	*	***	NS	NS	NS

Means followed by the same letter (s) within a column indicate no significant difference at $p \leq 0.05$ by the least square means test. *Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, ***Significant at $p \leq 0.001$ and NS = not significant.NPK was applied at a rate of 124 kg ha^{-1} in all plots at planting.

treated as random elements in the model whereas lime rate and common bean variety were treated as fixed effects. Since seasons differed greatly, they were analyzed separately. The PDIFF procedure was used to test for differences among means when F-tests were significant for main effects or their interactions. Differences between treatments were evaluated at a significance level of $p \leq 0.05$, unless otherwise stated and covariate analysis done. The covariate in the model statement in SAS was the 2017 soil property such as, pH or EC. Soil samples used for SAS analysis for the covariate therefore included samples that were collected before limestone or NPK was applied in 2017 (limestone and NPK were then applied after soil sampling and season A planted) and, soil samples in 2018 that were collected after the first season A was harvested but before season B was planted. Linear regression was done using PROC REG for parameters that were influenced by limestone rate.

Table 4. Regression functions for limestone application rates predicting upper (0-15 cm) and lower (15-30 cm) soil pH, CEC, and Ca and Na concentrations, and grain yield in common bean.

Parameter	Function	r^2 or R^2
Upper soil pH	5 + 0.0002x	0.886
Upper soil CEC	12.609 + 0.0011x	0.957
Upper soil Ca concentration (mg kg^{-1})	959.57 + 0.3191x	0.949
Upper soil Na concentration (mg kg^{-1})	8 + 0.0021x	0.938
Lower soil pH	5 + 8E-05x	0.983
Lower soil CEC	11.174 + 0.0005x	0.836
Lower soil Ca concentration (mg kg^{-1})	797.71 + 0.1289x	0.995
NABE 15 (2017)	468 + 0.013x	0.341
NAROBEAN 3 (2017)	169 + 0.010x	0.644
NABE 15 (2018)	$431 + 0.318x – 0.000037x^2$	0.612
NAROBEAN 1 (2018)	877 + 0.110x	0.835

RESULTS AND DISCUSSION

Effect of limestone on soil properties at 0-15 cm and 15-30 cm

There were significant differences in the effects of treatments among the two soil horizons. Changes in pH were more prominent in 0-15 cm of soil than 15-30 cm depth. Regression analysis showed that soil pH increased with increases in limestone application rates (upper soil, r^2= 0.885; lower soil, r^2=0.983) (Table 4). In upper soil, the greatest increment in soil pH was observed when 19,768 kg ha^{-1} of limestone was applied and the lowest pH increments when limestone was applied at rates of 0 and 1,236 kg ha^{-1}. Similarly, in the deeper soil horizon, the highest limestone application rate of 19,768 kg ha^{-1} also had the greatest pH increment whereas, 2471, 1236 and 0 rates of limestone had much lower effects.

Agricultural limestone application did not affect electrical conductivity (EC) (Table 3). This may be because the soil did not have high levels of soluble salts. Although acceptable thresholds for these exist, they are also dependent on a number of factors such as crop, soil texture, among others (Gruttadaurio et al., 2013; Sonon et al., 2015). Provin and Pitt (2001) reported that saline soils often had an EC of 40,000 µS cm^{-1}. The EC of soils at our experimental site was between 98.7 µS cm^{-1} in topsoil and 74.6 µS cm^{-1} in subsoil therefore, compared to Provin and Pitt (2001), our experimental site had low EC. Low EC often exists alongside no/low salts in soils (Hanlon, 2015). Change in EC varied with soil depth and limestone application rate. Upper and lower soil EC ranges from our study were less than 1000 µS cm^{-1} and thus the soil at the experimental site would be considered non- saline after limestone was applied (USDA-NRCS, 2019). Thus, the conditions would not negatively impact crop growth and other important soil microbial processes such as nitrogen cycling, respiration and decomposition among others (USDA-NRCS, 2019). Low EC levels may also indicate low availability of plant nutrients. Optimal soil EC levels range between 1.1 to 5.7 µS cm^{-1}.

Limestone influenced CEC in 0-15 cm depth and 15-30 cm depth (Table 3). Regression analysis showed that upper and lower soil CEC increased with limestone addition (0-15 cm depth, r^2= 0.957; 15-30 cm depth, r^2=0.836) (Table 4). Similarly, Edmeades (1982) reported that effective cation exchange capacity (ECEC) increased with increasing soil pH. Lemire et al. (2006) reported that CEC increased as a direct function of the amount of lime added, if the final pH of the solution remained below 7. They further reported that a 1 cmol (+) kg^{-1} increase in CEC for every 2.1 t ha^{-1} of limestone added to all soils regardless of texture, organic matter or other soil properties. Similarly, Aitken et al. (1990) reported a linear relationship between pH and CEC for pH ranges between 4 to 6.5 for all soils in their study.

Regression analysis for the relationship between CEC and pH in our study had similar values to those of Aitken et al. (1990). However, they added that the relationship between CEC and pH became curvilinear in upper ranges with CEC increasing distinctly with relatively small pH increments. Bartlett and McIntosh (1969) explained that the increase in pH due to increasing lime rates leads to neutralization of positively charged polynuclear Al-OH complexes which further unblocks negatively charged sites. This process contributes to ECEC increase and the increased pH may also induce deprotonation of pH-dependent sites leading to a proportional increment in CEC as well as charge density (Goedert et al., 1975).

Agricultural lime rate had no influence on soil organic carbon (OC) in either soil depth (Table 3). Haynes and Naidu (1998) mentioned that few studies, if any, have found a causal link between effects of lime application and soil organic matter. Additionally, increase in soil OC is largely due to residue accumulation especially where mineralization and decomposition are lower than organic matter addition. Such a causal link could not easily be attained especially given the short duration of the experiment. Mehlich-3 Ca was significantly affected by limestone application in both soil depths (Table 3). Overall, Ca concentration in upper and lower soils increased with incremental rates of limestone (0-15 cm depth, r^2= 0.949; 15-30 cm depth, r^2=0.995) (Table 4). The Ca concentration in plots where 0, 1236 and 2471 kg ha^{-1} of limestone were applied did not differ in either depth. Chimdi et al. (2012) attributed the increase in exchangeable Ca and CEC when lime rates increased, to the enhancement of Ca^{2+} ion concentration and their replacement of H^+ and Al^{3+} from the soil solution and exchange complex in the soil.

Mehlich-3 extractable Mg was not affected by application of different rates of limestone (Table 3). This is because the magnesium concentration of the limestone used was less than 0.5% which is quite low compared to the Ca in percentage in the limestone. Simard et al. (1994) and Riggs et al. (1995) reported that calcitic limestone had no significant effect on Mg. However, unlike our study, they reported that the values of exchangeable Mg decreased after the first growing season to values lower than those recommended for agricultural crop productivity, values that were lower than exchangeable Mg values in the soil before limestone addition. Edmeades (1982) also reported a decrease in soil Mg concentrations following lime application. The author attributed this Mg decrease to an increase in the exchangeable Ca: Mg ratio following addition of calcitic limestone. Contrary to our findings, other researchers found an increase in exchangeable Mg following agricultural/calcitic lime application (Grove et al., 1981; Grove and Summer, 1985; Mayfield et al., 2001).

No differences were observed in Mehlich-3 soil P in upper or lower soil with different rates of limestone (Table 3). Reeve and Sumner (1970) also reported that liming had no effect on P sorption in Oxisols. Haynes (1982) explained that liming highly weathered acid soils could result in either increased, decreased and even sometimes no change in available soil phosphorus. The author explained that increases in phosphorus availability occurred following liming if there was formation of various phosphate compounds. Haynes (1982) reported that if limed soils desiccated before reaction with phosphate, a decrease in phosphorus sorption and increase in its availability would occur due to crystallization of amorphous hydroxy-Al polymers. Several of these processes are extremely slow and therefore changes in available soil phosphorus may be difficult to observe in the absence of long-term study.

Application of different rates of limestone had no significant effect on Mehlich-3 K in both soil depths (Table 3). Simard et al. (1994) reported that lime had no significant effect on K extractability under different tillage intensities. This is contrary to other studies that reported either decreases or increases in K levels when limestone was applied. Phillips et al. (1988) explained that liming could increase K concentrations in the soil solution although this could eventually lead to K loss through leaching over time. Further, the effect (increase or decrease) or no effect of lime application on K in strongly acidic soils was dependent on the initial degree of soil base saturation (Schmehl et al., 1950). MacIntire et al. (1927) reported a decline in supply of available K when lime was applied on three soils as did Bartlett and McIntosh (1969). Such K declines were attributed to the opening up of K-selective exchange sites, previously blocked by Al when soil pH was low (Nemeth and Grimme, 1972) or, due to a reduction in percentage of K saturation triggered by an increase in CEC when lime was applied (Bartlett and McIntosh, 1969). In contrast to these previous studies, we observed an increase in CEC with increased limestone rates, although this did not affect K levels in our experiment.

Agricultural lime application rates had significant impact on Na concentration in upper soil ($p \leq 0.001$) and no impact on Na in subsoil (Table 3). Regression analysis showed that the greater the lime application rate, the greater the Na concentration in upper soil (r^2=0.938) (Table 4). It is possible that Na was a constituent of the limestone and therefore increased when lime was added.

Common bean productivity season A

The interaction of limestone and variety was significant for yield but not for stand density (V4 and R8), aboveground biomass, pods m^{-2}, seeds m^{-2}, seeds pod^{-1}, seed weight and seed iron and zinc concentration (Table 5). Stand density at V4 and V8 did not differ for limestone rate or amongst the varieties. This may be attributable to good crop management practices such as adoption of recommended inter-row spacing (50 cm), timely weeding to avoid competition for sunlight and nutrients, and management of potential insect and disease infestations (MAAIF, 2019). Seeding in rows facilitates cultivation and weeding by mechanical methods (Goulden, 1975). However, high density stands can lead to greater incidences of foliar diseases (Heard et al., 1990, Sandoval-Avila et al., 1994) and increased intra crop competition.

Aboveground biomass differed amongst the three varieties (Table 5). NABE 15 and NAROBEAN 1 had similar biomass, 57 and 65% greater at R8-R9 than NAROBEAN 3, respectively. The greater aboveground biomass obtained from NAROBEAN 1 may be because of the indeterminate growth habit of this variety (Table 5). Kelly et al. (1987) reported that determinate dry bean cultivars had lower stability across environments compared to indeterminate cultivars under rainfed conditions. Generally, there is a positive relationship between biomass and grain yield. This may also be reflected in their yield.

Variety and the interaction of limestone rate by variety

Table 5. Stand density at V8 and R8, above ground biomass, yield and yield components and seed iron and zinc, season A (longer rainy season).

Parameter	Stand density V4 (no. m^{-2})	Stand density R8 (no. m^{-2})	Biomass R8-R9 (kg ha^{-1})	Grain yield (kg ha^{-1})	Pods (no. m^{-2})	Seed (no. m^{-2})	Seed (no. pod^{-1})	Seed (mg seed^{-1})	Seed Fe (mg kg^{-1})	Seed Zn (mg kg^{-1})
Control (untreated)	32	19	411	334	32	94	3	361	69	31
Limestone Rate (kg ha^{-1})										
0	33^{NS}	20^{NS}	456^{NS}	398^{NS}	36^{NS}	113^{NS}	3^{NS}	358^{NS}	68^{NS}	31^{NS}
1236	34^{NS}	20^{NS}	511^{NS}	455^{NS}	40^{NS}	134^{NS}	3^{NS}	346^{NS}	60^{NS}	29^{NS}
2471	32^{NS}	19^{NS}	433^{NS}	408^{NS}	37^{NS}	131^{NS}	3^{NS}	337^{NS}	62^{NS}	29^{NS}
4942	34^{NS}	20^{NS}	483^{NS}	431^{NS}	42^{NS}	137^{NS}	3^{NS}	328^{NS}	63^{NS}	31^{NS}
9884	33^{NS}	20^{NS}	594^{NS}	465^{NS}	44^{NS}	142^{NS}	3^{NS}	332^{NS}	73^{NS}	30^{NS}
19768	32^{NS}	19^{NS}	561^{NS}	523^{NS}	45^{NS}	148^{NS}	3^{NS}	338^{NS}	64^{NS}	33^{NS}
Variety										
NABE 15	33^{NS}	20^{NS}	572^{a}	510^{a}	40^{b}	138^{b}	3^{a}	371^{a}	60^{NS}	28^{b}
NAROBEAN 1	32^{NS}	20^{NS}	703^{a}	634^{a}	57^{a}	205^{a}	4^{a}	309^{b}	69^{NS}	29^{b}
NAROBEAN 3	34^{NS}	19^{NS}	244^{b}	196^{b}	25^{c}	59^{c}	2^{b}	340^{ab}	66^{NS}	34^{a}
Significance										
Limestone (L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Variety (V)	NS	NS	***	***	***	***	***	**	0.08	***
L × V	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

Means followed by the same letter (s) within a column indicate no significant difference at $p \leq 0.05$ by the least square means test. *Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, ***Significant at $p \leq 0.001$ and NS = not significant. NPK was applied at a rate of 124 kg ha^{-1} in all plots before planting.

influenced grain yield (Table 5). Yields of NABE 15 and NAROBEAN 1 did not differ, but were 62 and 69% greater than for NAROBEAN 3, respectively. Kelly et al. (1987) reported that determinate dry bean cultivars had lower seed yield than their indeterminate counterparts. Stebbins (1974) explained that indeterminate plants have inherent flexibility which enables them to form either few or many flowers depending on how long the growing season may be. This in turn provides a buffer against changes that may occur in environmental conditions, especially in rainfed cropping systems (Stebbins, 1974). Acosta-Gallegos and Adams (1991) further explained that indeterminate bean cultivars with early vigorous establishment, greater dry matter as the seed-filling period commenced and the potential for assimilate transfer during seed development stages were better suited for growing under rainfed conditions compared to cultivars that are determinate. Beaver et al. (1985) and Nleya et al. (1999) also reported that indeterminate bean cultivars were higher yielding and more productive than determinate cultivars. For the interaction of limestone rate and variety with regard to grain yield, two comparisons were fit by functions using a simple linear model. Regression modeling using limestone rate to predict yield was successful at explaining yield variation for NABE 15 and NAROBEAN 3 but not NAROBEAN 1 (Table 4). Regression analysis showed that NABE 15 and NAROBEAN 3 grain yield increased with greater limestone addition rates (NABE 15, r^2= 0.341; NAROBEAN 3, r^2= 0.644). Varietal increase in yield with greater limestone rate may be attributable to crop favorable changes in soil properties due to liming such as an increase in soil pH, improvement in soil biodiversity, structure and hydraulic conductivity (Haynes and Naidu, 1998; Bolan et al., 2003).

Agricultural limestone rate did not impact pods m^{-2} or seeds pod^{-1} (Table 5). However, pods m^{-2}

and seeds m^{-2} differed among the three common bean varieties ($p \leq 0.001$). NAROBEAN 1 had the greatest number of pods m^{-2} and seeds m^{-2}, followed by NABE 15; NAROBEAN 3 had the least. NAROBEAN 1 had 30 and 56% more pods m^{-2} as well as, 33 and 72% more seeds m^{-2} than NABE 15 and NAROBEAN 3, respectively. NAROBEAN 1 had 30 and 56% more pods m^{-2} than NABE 15 and NAROBEAN 3, respectively. These differences also may be attributed to the indeterminate growth habit of NAROBEAN 1. Limestone application did not impact seeds pod^{-1}. However, the number of seeds pod^{-1} differed by variety. NAROBEAN 1 and NABE 15 had the greatest seeds pod^{-1} and were similar whereas NAROBEAN 3 had the least. NABE 15 and NAROBEAN 1 had 33% and 50% more seeds per pod than NAROBEAN 3, respectively. Seed weight was not impacted by limestone application rate. However, seed weight was significantly different among bean varieties ($p \leq 0.01$). NABE 15 had the highest individual seed weight whereas NAROBEAN 1 seeds had the least weight. NABE 15 seeds weighed 8 and 17% more than NAROBEAN 3 and NAROBEAN 1 seeds, respectively. Perin et al. (2002) reported that large seeds often increased plant shoot and root biomass as well as plant height and leaf area index during early plant development stages. The authors postulated that larger seeds had larger reserves that enabled for more vigorous initial development such as biomass production compared to smaller seeds. However, initial plant vigour was not measured in our study.

Seed zinc concentration differed among the three bean varieties. NAROBEAN 3 seeds had the greatest zinc concentration whereas NABE 15 and NAROBEAN 1 had the least. Zinc concentration in NAROBEAN 3 seeds was 15 and 18% greater than that in NAROBEAN 1 and NABE 15, respectively. Additionally, the zinc concentration in the latter two varieties did not differ. NAROBEAN 1 zinc concentration from our study corresponded to that expected/predicted by NARO-Uganda, 31.4-34.3 ppm (Agona, 2017) although our NAROBEAN 3 grain zinc concentration was slightly lower than the predicted 35-38 ppm by NARO (Agona, 2017). We do not know why the biofortified NAROBEAN 3 had low zinc concentration. We did not observe differences in grain zinc concentration due to limestone application rate. Additionally, seed concentration of Fe did not differ for limestone application rate or variety (Table 5).

Common bean productivity season B

Plant stand density at V4 and V8 differed by variety but was not affected by limestone application rate or the variety × limestone rate interaction (Table 6). NAROBEAN 1 had the greatest plant stand density whereas NAROBEAN 3 had the least at both V4 and R8 stages. At V4, the stand density of NAROBEAN 1 was 10 and 13% more than NABE 15 and NAROBEAN 3, respectively. At R8, the stand density of NAROBEAN 1 was 11 and 17% more than NABE 15 and NAROBEAN 3, respectively.

Agricultural limestone rate, variety, and their interaction had significant effects on aboveground biomass at R8-R9 (Table 6). Regression analysis showed that aboveground biomass increased with increments in limestone application rates ($y = 192.77x + 1876.5$; $r^2 = 0.744$). NAROBEAN 1 had 37% and 41% more aboveground biomass than NABE 15 and NAROBEAN 3, respectively. This may be attributable to the NAROBEAN 1 having an indeterminate growth habit as explained in Kelly et al. (1987).

Common bean varieties differed for yield and there was a significant interaction of variety and limestone rate (Table 6). Yield of NAROBEAN 1 was 37% more than NABE 15 and NAROBEAN 3. Pods and seeds m^{-2} differed by variety. NAROBEAN 1 had 41 and 30% more pods m^{-2} than NABE 15 and NAROBEAN 1, and 37 and 35% more seeds m^{-2}, respectively. These differences in yield may be attributable to NAROBEAN 1 having an indeterminate growth habit which enables the variety to have yield superiority (Beaver et al., 1985; Acosta-Gallegos and Adams, 1991; Nleya et al., 1999). To examine the interaction between limestone rate and variety for grain yield, simple linear regression was done and limestone rate was used to predict yield. A simple linear model was successful at explaining yield variation for NAROBEAN 1 and a better prediction with a quadratic model/equation was used for NABE 15 although neither of the models was successful for NAROBEAN 3 (Table 4). Regression analysis showed that grain yield increased with greater limestone addition rates (NAROBEAN 1, r^2= 0.835; NABE 15, R^2= 0.612). This may be due to increases in pH, consequent increment in soil nutrients such as calcium, improvement in other soil properties and even fertilizer use efficiency. Holland et al. (2019) associated an increase in spring bean (*Vicia faba* L.) yield to an increase in soil pH following lime application. Liming low pH soils was also reported to increase fertilizer use efficiency leading to increased yield in barley and wheat (Von Tucher et al., 2018).

Seeds pod^{-1} and weight $seed^{-1}$ were not affected by either lime treatment or variety (Table 6). Seed concentration of iron and zinc was significantly different among varieties (Table 6). NAROBEAN 3 seeds contained 10 and 20% more iron than NAROBEAN 1 and NABE 15, respectively. Additionally, NAROBEAN 3 seeds contained 9 and 13% more zinc than NAROBEAN 1 and NABE 15, respectively.

Conclusions

Our study demonstrated that soil pH increased with greater limestone application rates along with increases in soil calcium and CEC, thus limestone application can raise soil pH for improved bean productivity. Despite

Table 6. Stand density at V8 and R8, aboveground biomass, yield and yield components and seed iron and zinc, season B (shorter rainy season), 2018.

Parameter	Stand density V4 (no. m-2)	Stand density R8 (no. m-2)	Biomass R8-R9 (kg ha-1)	Grain yield (kg ha-1)	Pod (no. m-2)	Seed (no. m-2)	Seed (no. pod-1)	Seed (mg seed-1)	Seed Fe (mg kg-1)	Seed Zn (mg kg-1)
Control	27	17	1350	976	52	165	3	522	65	31
Limestone rate (kg ha-1)										
0	31NS	17NS	1811b	560NS	67NS	212NS	3NS	262NS	71NS	30NS
1236	31NS	16NS	2639ab	912NS	90NS	303NS	4NS	324NS	63NS	30NS
2471	24NS	15NS	2461ab	830NS	82NS	307NS	4NS	271NS	61NS	28NS
4942	27NS	18NS	2528ab	830NS	87NS	283NS	3NS	295NS	64NS	31NS
9884	29NS	17NS	2844ab	1036NS	100NS	385NS	4NS	269NS	64NS	30NS
19768	28NS	17NS	3024a	996NS	105NS	350NS	3NS	275NS	63NS	29NS
Variety										
NABE 15	28ab	16ab	2170b	721b	68b	255b	4NS	281NS	57b	28b
NAROBEAN 1	31a	18a	3450a	1139a	116a	403a	3NS	281NS	64ab	29b
NAROBEAN 3	27b	15b	2033b	721b	81b	262b	3NS	286NS	71a	32a
Significance										
Limestone (L)	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
Variety (V)	**	**	***	***	***	***	NS	NS	**	***
L × V	NS	NS	*	*	NS	NS	NS	NS	NS	NS

Means followed by the same letter (s) within a column indicate no significant difference at $p \leq 0.05$ by the least square means test. *Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, ***Significant at $p \leq 0.001$ and NS = not significant. NPK was applied at a rate of 124 kg ha^{-1} in all plots at planting.

increasing soil pH and CEC, limestone addition did not improve bean yield in our two-year experiment. However, the newly released common bean varieties, NAROBEAN 1 and NAROBEAN 3 had greater yields and seed Fe and Zn concentrations than the older variety, NABE 15. These newer varieties should be used to improve livelihoods through better yields and human nutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Use of unmanned aerial vehicles (UAV) as an innovation in agriculture

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Facing up with the effects of climate change, increasing human population, reduction of natural resources, and degradation of soils the agricultural sector has the challenge of meeting the world's demand for food. In this scenario, the interest of scientific communities for the use of Unmanned Aerial Vehicles (UAV) in the agricultural sector has increased in recent years. The aim of this article is to provide a global view on how scientific communities interact and identify gaps and opportunities in research of UAV in the agricultural sector. The study reviewed the scientific publications of the Web of Science from 2000 to 2018 using a data-driven approach. According to specific search terms, the most relevant publications have been downloaded from specialized database and they have been analyzed from the point of view of bibliographic data through the VOSviewer software. Examining the network graphic of citations to describe the interaction and processing the terms of the titles and abstracts with the data mining techniques, in order to identify current trends research and gaps in the literature were identified. Scientific community is working to design technological platforms to support agriculture actors for making data-based decision schemes, where computational algorithms and visualization techniques are applied.

Key words: Agricultural innovation, technology applications, unmanned aerial vehicles (UAV), drones.

INTRODUCTION

Since 2011, Unmanned Aerial Vehicles (UAV), also called drones, represent a breakthrough for the agricultural sector, because they are more affordable and easier to use (Frankelius et al., 2017). Nowadays, practical applications for UAV are expanding faster than ever in the agricultural sector.

In contrast to the difficult and cost to obtain satellite imagery or conventional airborne data, recollect aerial images with UAV equipped with GPS and digital cameras mode is cheaper and faster to do it. UAV represents one of the most important emergent technological tools, recognizes to their flexibility of use as well increasing applicability.

Facing the challenges posed by the effects of climate

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change, increase in world population, decrease in natural resources and degradation of agricultural land (Bayala et al., 2017; Singh and Singh, 2017; Zilberman et al., 2018), the UAVs allow the agricultural innovation about planning and monitoring processes to strengthen food production. UAVs provide the opportunity to improve crop monitoring, soil, and field analysis. Using these aerial platforms, the monitoring task is developed in an easier, closer and precise way, reducing cost and time (Handique et al., 2017; Krienke et al., 2017, Hunt et al., 2018; Khan et al., 2018). With this innovation, it is possible to construct surface maps and digital models of crops (Hovhannisyan et al., 2018; Huuskonen and Oksanen, 2018).

Currently, some of the most common applications of UAV are: a) acquisition of crop data (using different types of cameras); b) monitoring of crop growth; c) sprayer (fertilizers, herbicides and pesticides); and d) capture of images to create 3D models. Based on a global report published by PricewaterhouseCoopers (PwC), the size of the UAV market in agriculture is estimated at $32.4 billion US dollars (Mazur et al., 2016). An important issue is considering that the UAV technology will allow for individual farms to be highly data driven, which will lead to increased productivity and yields.

UAV platforms are using within the agricultural ecosystems for data assimilation and monitoring agricultural production systems (Domingues et al., 2017). Low-cost aerial platforms collect images of crops to support discrimination of plants species (Hadique et al., 2017; Oldeland et al., 2017), monitoring based on phenotypes (Burkart et al., 2018) and diagnostic of water status (Martínez et al., 2017), determine the health status (Khan et al., 2018), calculate nitrogen and other nutriments level (Krienke et al., 2017), and identify pathogens (West et al., 2017).

In this context, it is relevant to know the works related to the use of UAV in the agricultural sector reported in the literature to identify the research fields that are being followed by the research communities. Additionally, to identify opportunities to develop new research and innovation projects in agricultural production systems is important know the characteristics, conditions, and benefits of the UAV applications in agriculture. The aim of this review of scientific literature was to provide a global view by (1) analyzing historical development and recent trends, (2) investigating through citation networks how scientific communities interact with each other and, ultimately, (3) identify gaps and opportunities in the scientific literature for research and application of UAV in the agricultural sector.

MATERIALS AND METHODS

Data selection

As source of data, the study considered scientific publications from Web of Science (WoS) of Thomson Reuters (Thomson, 2018). In an exploration test, the study found that publications prior to 2000 related to this topic of interest are minimal and not directly linked to current UAV platforms. A particular set of publications that correspond to the period between the years 2000 and 2018 were selected, analyzed, and classified. Logical combinations of search terms were used to collect relevant works from UAV as an innovation in the agricultural sector. For UAV applications in agricultura, the terms applied were: (agriculture*AND (unmanned aerial vehicle OR unmanned aerial system OR uav OR uas).

Search terms applied to collect papers related with the application of UAV in the monitoring and control of crops were: (crop* AND (unmanned aerial vehicle OR unmanned aerial system OR uav OR uas)). Finally, to include drone applications in the agricultural sector and in crop monitoring, the search terms considered were: (drone* AND (agriculture* OR crop*). Using these search terms, result was a set of 704 publications. The information of each publication was downloaded considering: title, abstract, author, citations, keywords, and year as a text file delimited by tabs for further processing with VOSviewer.

Data driven approach

Given that the agricultural sector and UAV have experienced a growing interaction of large number of communities, it is a challenge to carry out the holistic review. Instead, the VOSviewer software (available at www.vosviewer.com) was used, which is a free text mining software used to generate bibliometric maps of scientific fields (Van Eck and Waltman, 2013). Taking advantage of the bibliographic data and using the software functionality, the workflow of analysis was divided into data recovery, pre-processing, network extraction, normalization, mapping analysis and visualization. In the pre-processing test the temporal trends in the 704 publications retrieved from 2000 to 2018, geographical source and most used journals were analyzed.

A second analysis was performed for three techniques based in natural language processing algorithm used to generate the results applying the predefined thesaurus file to delete unrelated words and combined words. The first technique was based on keyword analysis producing a scientific map which was used to identify the scientific communities with a least of 15 occurrences. As a second technique, the citation information was employed to look into the interaction between authors and research communities. Finally, this work took advantage of data mining tools and Kernel algorithm (Perianes-Rodriguez et al., 2016), which was applied in the analysis of titles and abstracts of the considered set of research documents from 2014 to 2018 to investigate trends and identify gaps in the literature.

RESULTS AND DISCUSSION

Descriptive analysis

A set of 704 papers has been identified, which include UAV research and applications in the agricultural sector. The number of publications retrieved from 2000 to 2013 were 116, representing the historical development period. From 2014 to 2018, the number of publications has been increasing, 588 research works were published.

Increase in the amount of works published since 2014 relates to the reduction of costs of sensors, communication systems, and aerial platforms.

Additionally, the UAV camera systems have a larger spatial resolution increasing the capabilities to get better information about the agricultural environment. This situation allowed to scientific community access to different UAV platforms: fixed wing like Viewer Elimco and mX-Sight; multi-rotor as OktoKopter and Vario Benzin, and some cameras solutions as Micro-Hyperspec VNIR and MCA-6 Tetracam (Turner et al., 2012; Zarco-Tejada et al., 2013a, b).

Geographical analysis described that 94.3% of scientific works were published by researchers from 10 countries: 26.7% from USA (188), 16.9% from Spain (119), 14.6% from China (103), 7.2% from Germany (51), 5.9% from Australia (42), 5.8% from Italy (41), 4.8% from Brazil (34), 4.5% from India (32), 4.1% from France (29), and 3.5% from Netherlands (25).

In total, 271 sources of publications (journals and conferences) were identified. From obtained data, the advanced publications were reported in journals and a 15 group sources were responsible for 47% of all publications, while the other 256 sources were responsible for the remaining 53%. In the knowledge field under study, a significant amount of paper has been published in journals as Remote Sensing (12%), Sensors (5%), Precision Agriculture (5%), Computers and Electronics in Agriculture (4%), and International Journal of Remote Sensing (4%).

Historical development and interaction

To analyze the historical interaction of keywords, 3,563 keywords from the publications compiled by using VOSviewer were extracted. Of these, 38 occurred at least 15 times and were filtered through the thesaurus files to obtain the 25 most relevant keywords. Figure 1 presents the resulting scientific landscape considering the set of keywords, grouped by co-occurrences of the keywords of the documents. Five groups were identified and marked with different colors. Table 1 summarizes these groups; each group was named arbitrary (research topic) based on the observed keywords. For example, the red cluster labeled as Crop System include the keywords related to Crop. Green, blue, yellow, and purple groups were nominated Vegetation Index, Remote Sensing, Biomass Model, and Precision Agriculture, respectively.

As shown in Figure 1, it is clear that five constituted groups have a uniform distribution and the distances separating them were not large. The UAV keyword was the largest sphere, and it is at the center, indicating research collaborations between UAV with the communities working at Crop System (red), Vegetation Index (green), Precision Agriculture (purple), Remote Sensing (blue), and Biomass Model (yellow).

The keywords in the red cluster summarize the crop management systems developed in this group. First, the keywords UAV and crop occurred 375 and 107 times respectively, indicating the interest of the community to investigate the application of UAV in crops. Researchers have been interested in the issues of imagery (98), system (98), agriculture (53), management (51), classification (41), vegetation (37), identification (31), and photogrammetry (31). The community has been working on systems based on photogrammetry of agricultural fields, to identify and classify the vegetation growing in the fields with the purpose of strengthening the handling and management of production systems.

In the green cluster, the researchers interest was focused on vegetation indexes calculate from the aerial images collected by UAV in low altitude flying. The occurrence of the keywords chlorophyll (35), lai (69), ndvi (17), nitrogen (20), and reflectance (71) show that the scientific community has used these properties for the study of vegetable health and crop sanity. The keywords lai (Leaf Area Index) and ndvi (Normalized Difference Vegetation Index) occurred 69 and 17 times respectively, which were the vegetation index most used. Also, the work of the community has been addressed to study cereals: corn (43), grain (17), rice (15), and wheat (77) crops. Interest of researchers in the purple cluster, Precision Agriculture, was related to the keywords precision agriculture (168), hyperspectral (32), and multispectral (32). These terms include UAVs application to collect hyperspectral and multispectral images to describe different features of the crops that can determine the state of health, water levels and nutrients.

In the cluster of Remote Sensing (blue) the occurrence of the keywords temperature (56), conductance (17) and water-stress (19) indicate that the community's interest to measure, transmit, and even control a vast amount of variables and properties involved in crops. Besides, the keyword variability (39) describes the tendency of remote sensing research to study variations in the properties of crops during different stages of development. Keywords occurred in the yellow cluster include the interest of the research community to build models (40) of biomass (44) to describe the growth (23) and development (height, 23) of the vegetation (forest, 16) in agricultural production systems.

Bibliometric analysis

Bibliometric indicators of the analyzed scientific works included in the WoS citation report show that the total number of articles was 704, the total number of times cited was 9,336 and that the average number of citations per investigation was 13.26. Furthermore, the h-index of Hirsch (2005) allows to measure the quality of scientific works (based on the number of citations received) and the quantity of scientific production. The h-index of the whole set of publications was 51 and this represents that

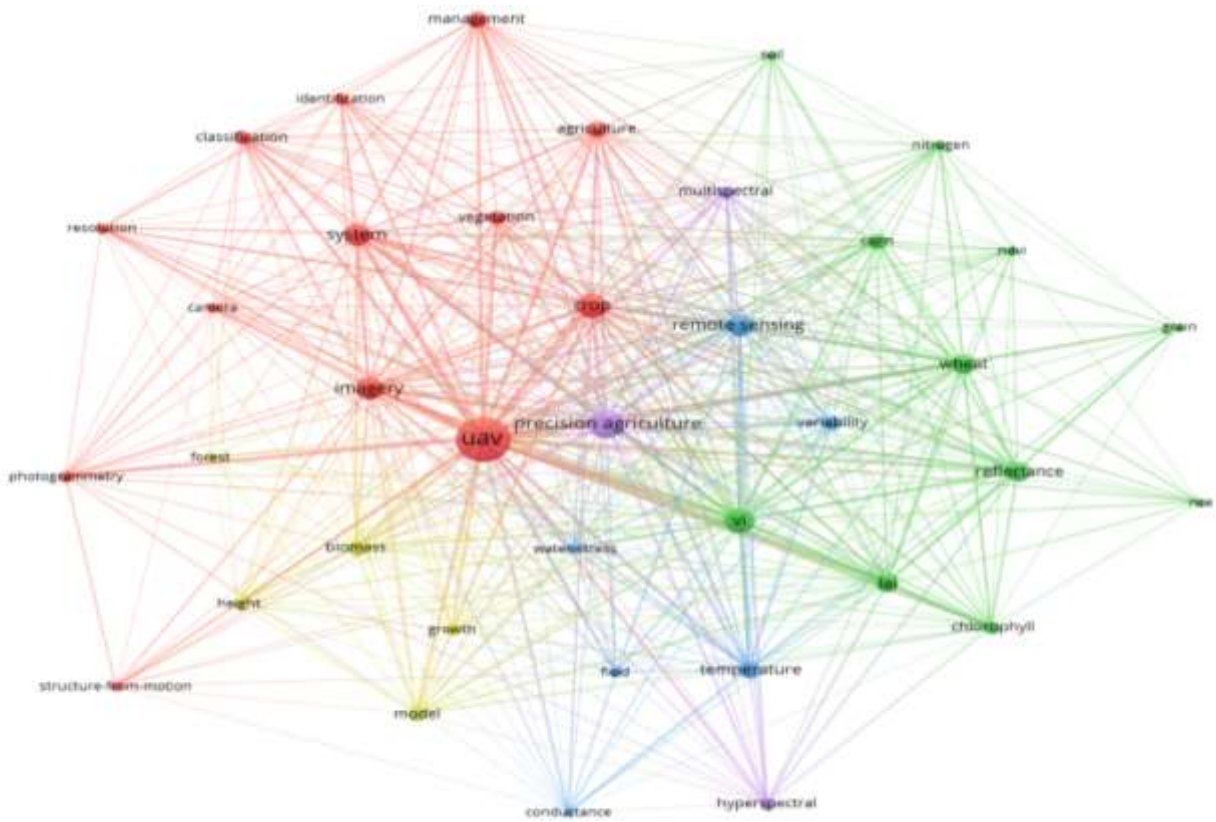

Figure 1. Scientific landscape of UAVs in agriculture (Keywords interaction).
Source: Authors (2020).

in the studied group there were 51 research works that have received at least 51 citations each. Table 2 presents the 10 scientific articles with the highest number of citations from the considered set of documents. As noted, the research topics that address the work were related to the techniques of image processing for crop monitoring and detection of water stress.

The most cited works deal with theoretical developments and conceptual references, conforming the historical development of the 2007 - 2014 periods. Also, other authors have researched the evolution of aerial platforms equipped with TIR (Thermal Infrared) sensors, multispectral cameras, and Global Positioning System (GPS). These technological platforms were used to analyze issues related to use of water and the estimation of vegetation index. In the same period, the first UAV sprayer systems for the application of chemical treatments on crops were reported (Huang et al., 2009; Zhu et al., 2010). From density scientific citation map of the set of publications (Figure 2), only works having at least 51 citations in correspondence with the h-index of the set were included. Some communities of authors were identified in the analyzed set.

A group of articles show the interest of the scientific community in the thermal and multispectral images application to get the vegetation indexes to determine the water stress in crops, yellow cluster.

Works published by the concerned community in application of image processing on agricultural sector, aquamarine cluster; and they have been expanded by other authors, where the construction of surface crop models were considerate.

Moreover, there were works reported by the scientific community developing strategies involving the UAV platforms application as a tool for precision agriculture techniques, particularly in monitoring and tracking tasks on crops, green cluster. Finally, the group exploring methods and technology based on the UAV applied to weeds detection and site mapping in crop fields has released, purple cluster.

Research trends

To analyze the research topics included in titles and abstracts of the works considered in the set of publications from 2014 to 2018. Thus, 17,805 terms have been extracted from the publications by using VOS

Table 1. Scientific communities in the publications of 2000 – 2018.

Cluster	Research topic	Keyword group
Red	Crop system	agriculture, camera, classification, crop, identification, imagery, management, photogrammetry, resolution, system, uav, vegetation, structure from motion
Green	Vegetation Index	chlorophyll, corn, grain, lai, ndvi, nitrogen, reflectance, rice, soil, vegetation index, wheat
Blue	Remote sensing	conductance, field, remote sensing, temperature, variability, water-stress
Yellow	Biomass model	biomass, forest, growth, height, model
Purple	Precision agriculture	hyperspectral, multispectral, precision agriculture

Source: Authors (2020).

Table 2. Most cited scientific publications.

References	No. citations	Research topic
Berni et al. (2009a)	377	Thermal and Multispectral Remoting Sensing
Zhang and Kovacs (2012)	318	Application of small UAV in agriculture. Review
Zarco-Tejada et al. (2012)	305	Vegetation index for water stress
Mulla (2013)	274	Remoting sensing in precision agriculture. Review
Turner et al.(2012)	200	Generating georectified mosaics based on SfM
Hunt et al. (2010)	176	NIR digital photographs for crop monitoring
Lelong et al. (2008)	145	Imagery for quantitative monitoring of wheat crop
Berni et al. (2009b)	137	Mapping canopy conductance and Crop Water Stress Index (CWSI)
Honkavaara et al. (2013)	113	Spectrometric, stereoscopic imagery for precision agriculture
Laliberte et al. (2011)	110	Image processing workflows

Source: Authors (2020).

viewer. Of these terms, 95 occurred at least 30 times. The resulting scientific landscape is presented in Figure 3 where the 50 most relevant terms were included. Three clusters of topics were identified, and then those groups of research works belonging to each one of the groups were considered.

Red cluster include the terms measurement (135), value (124), stage (95), estimation (93), season (85), and vegetation index (82). Existing proximity to terms effect (104), assessment (90), yield (103), and plot (86) describe the interest of scientific groups in the estimation of vegetation index in plots into stage in seasons of crops to measurement yields, estimations, effects, differences, relationship, and potential of biological control treatments, health vegetal monitoring, water stress vigilance, and nutrients provided.

Occurrences of the terms algorithm (105), detection (76), height (94), map (71), pixel (63), classification (55), and object (46) constitute the blue cluster. It describes that the research works have been focused to develop computational and statistical algorithms to crop maps construction useful to plants classification and object detection at field scale. Aerial images processing aims identification of crop interest features related to spatial location and natural resource in agroecosystem.

Green cluster include the terms application (201), unmanned aerial vehicles (197), development (137), technology (125), and precision agriculture (84) that describing the interest to implement different precision agriculture strategies to applying UAV into agricultural sector. Technological strategies focused on the impact (53), efficiency (67), quality (65), and advantage (46) describe research groups interest into low-cost (39) UAV application for development competitive advantage to farmers in the regional scale.

Interaction between Red, Blue, and Green clusters represent the scientific interest to application of UAV into farm production systems.

Considering the performed exploratory analysis, two periods can be defined as: historical development (2000-2013) and the current trends (2014 - 2018), with significant differences in the quantity of published works.

Scientific landscape of keywords identifies five communities (Table 1 has a uniform distribution centered on the term UAV and with small distances separating them, Figure 1). A recent trend is the design of agricultural management systems guided by data for smallholders (Maru et al., 2018), and it can be

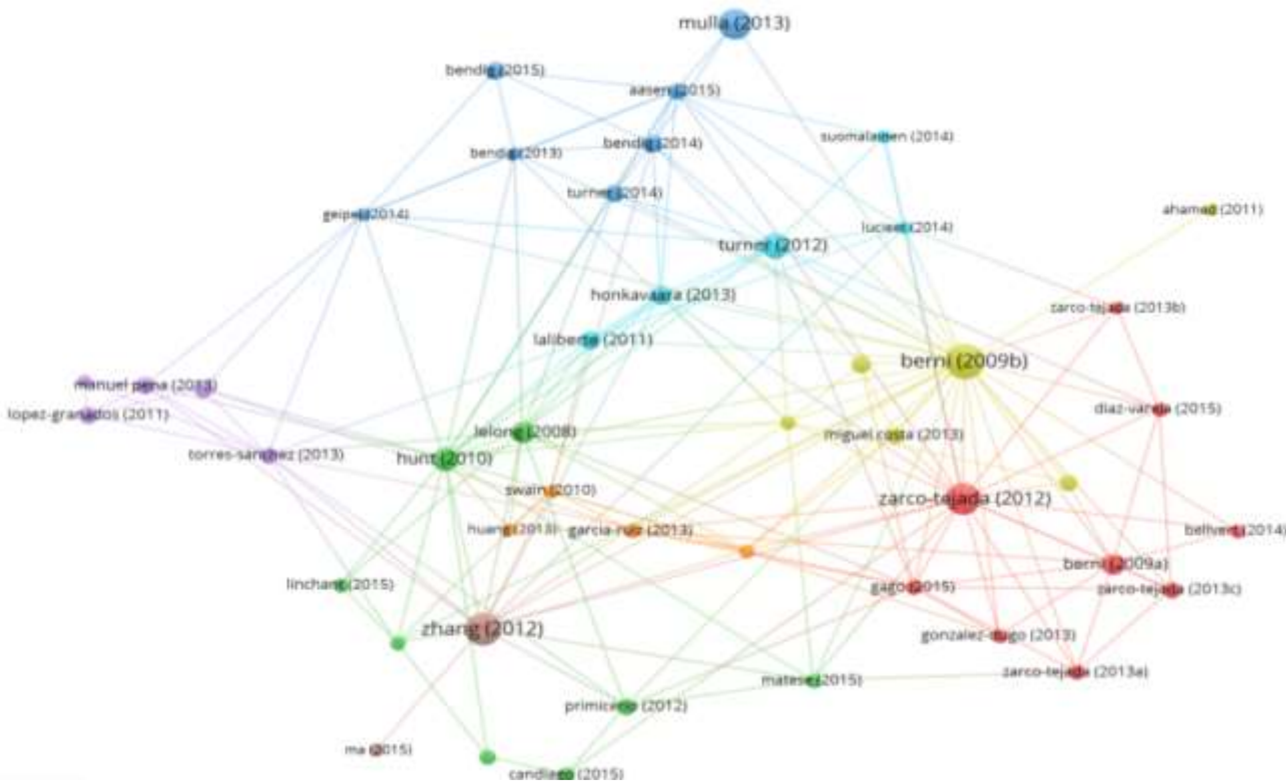

Figure 2. Scientific map landscape for publication citations.
Source: Authors (2020).

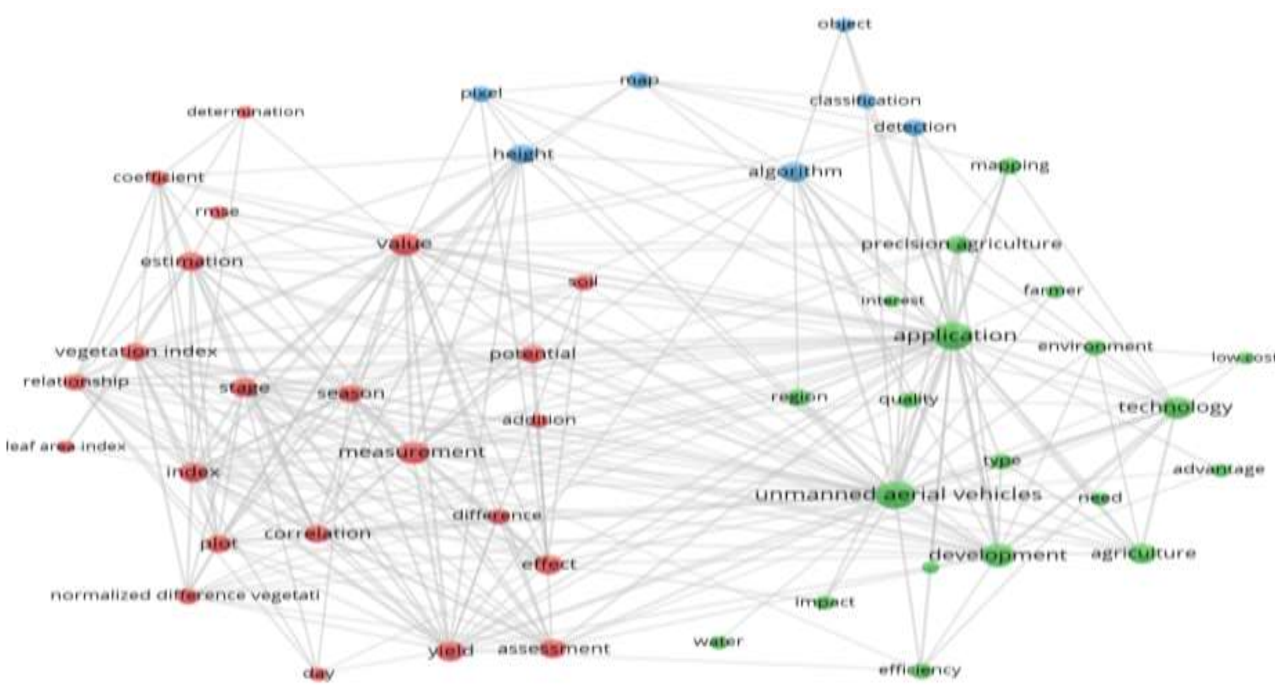

Figure 3. Scientific map of UAV trends in the agricultural sector.
Source: Authors (2020).

strengthened with the integration of strategies, techniques, and tools coming from the five described scientific communities.

Citation scientific map presents some communities of authors (Figure 2). The most cited research works consider the processing of digital images collected by UAVs as a fundamental tool to get vegetation index. An opportunity in the monitoring and evaluation of crop maps with plant disease detection (Lammoglia et al., 2018; Thomas et al., 2018) to integrate with risk management strategies for help to producers in the generation of guided action plans to solve these undesired effects based on collected data.

In the scientific landscape of research topics, three cluster were identified, then the set of documents belonging to each group was analyzed (Figure 3). It shows that the scientific community is currently interested in the existing correlation of vegetation index with the stages of crop development (Burkart at al., 2018), studying the spatial variability at the plot scale, and the relation of both with the treatments applied to the crops to evaluate their potential and measure their effects.

Recent trends promote active participation of agricultural producers in the design of technological solutions, design and implementation of monitoring, and analysis strategies at the plot level (Pallottino et al., 2018). Integration of low-cost technologies (Barrero and Perdomo, 2018; Schut et al., 2018), expansion of solutions with comprehensive data analytics capabilities (Lary et al., 2018), allows the configuration of software services with visualization technologies to strengthen decision-making based on data (Kamilaris and Prenafeta-Boldú, 2018; Rupnik et al., 2018).

On the other hand, a data-driven approach used in this work depends on the quality of data collection. Although, the set of considered publication has been built in a careful way through logical combinations of search terms, it was a challenge to ensure that all relevant paper was considered, because there could be exist alternative research terms. This study was limited to the search in the WoS database, whose search engine only explore and find matches in the title, abstract, and keywords of paper, without considering the main text. Therefore, a higher level systematic and inclusive approach is required in order to build a richer collection of publications, generating more precise data. However, a significant change in results and conclusions is not anticipated.

Conclusions

In this review, data-based method to search and analyze scientific publications was used to identify gaps in the literature and research opportunities in the application of UAVs in the agricultural sector. The relationship between the two fields of research was visualized through scientific maps of keywords, citations, and research topics.

Keywords analysis indicated that the scientific community has focused it is interest in the use of UAV to build technological tools that arise from collaborations between the areas of Crop System, Vegetation Index, Precision Agriculture, Remote Sensing, and Biomass Model. From the analysis of citations of the documents, communities of authors have been identified and they were interested in: (a) the application of thermal and multispectral images for the quantification of plant index and detection of water stress; (b) image processing techniques for the construction of surface models; (c) use of digital images for crop monitoring; and, (d) image analysis for the detection of weeds.

In analysis of data mining of scientific documents for the co-occurrence of research topics, the study found that the application of UAV in agricultural crops is an issue of interest to the scientific community, who was addressing the producer problems related to the costs and production processes at parcel scale. Analysis of the vegetation index, stages of crop development, and the variability in the crop field about the agricultural cycles and the effects of the treatments, were also performed.

Finally, this study allowed to identify trends in research topics in agricultural sector to use UAV. In contrast, terms about cost effectiveness, policy and management, UAV adoption, pollination process, pesticide application, and coordination of precision agriculture resources are not frequently present, underlining potential research gaps or under explored topics that are yet required to be filled.Application of the UAV in agricultural sector are expanding rapidly. To determine the appropriate strategies and UAV technologies for agricultural system and environmental conditions it is important to systematically and continuously conduct comparative studies to guide sustainable agricultural development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Managed bees as pollinators and vectors of bio control agent against grey mold disease in strawberry plantations

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Pollination by bees and other animals significantly increase both crop yields and quality. Bees also support the transfer of bio-control agents for suppression of crop pests and diseases through bio-vectoring technology that has not been applied in Africa. Two farms were set up to test the ability of managed bees to disseminate *Trichoderma harzianum* to control *Botrytis cinerea,* on strawberries. At on-station farm, three treatments (bee-vectoring inoculum, spraying and control) with 4 replicates each were set up; while on-farm, normal farmer practices were employed. A nuclear beehive fitted with a two-way dispenser was loaded with two grams of *T. harzianum* inoculum. Fifteen bees and flowers from each treatment were picked and cultured in the laboratory. Fruits and flowers infected with *B. cinerea* were recorded, while healthy fruits were counted, weighed and equatorial and polar diameter determined. Each bee carried $22.4 \pm 4.9 \times 10^2$ colony -forming units of *T. harzianum.* Flowers from the sprayed treatment had significantly higher Colony-Forming Unit's F (3,140), ($P<0.05$) than the bee-vectored treatment. Grey mold disease levels on fruits were significantly lower ($P>0.05$) in sprayed, bee-vectored and control treatment than in farmer's practice treatment. Fruits from spray treatment weighed significantly higher than those from control treatment F (3,2122), ($P< 0.05$). The number of seeds, equatorial and polar diameter per berry were significantly higher (F=3, 2122, $P<0.05$) in farmer's practice treatment. Managed bees proved effective in vectoring *T. harzianum* but, sufficient Colony-Forming units had to be delivered for effective control of the disease.

Key words: *Trichoderma harzianum*, grey mold, strawberry, biocontrol agent, managed bees, bio-vectoring technology.

INTRODUCTION

Garden strawberry, *Fragaria x ananassa* Duch*,* is a perennial, herb that belongs to the phylum Spermatophyte and family Rosaceae. The strawberry does well in tropical and subtropical regions of the world (Mir et al., 2019). Strawberry fruits are rich in well-balanced sugars and organic acids Guimarães et al. (2016), hence very

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nutritious for human consumption (USDA, 2015). Strawberry farming has picked over time and the annual world production greatly increased in quantity in the last twenty years to over 2.5 million tones (FAO, 2014). The demand and consumption have increased due to their antioxidants effect in human (Törrönen and Määttä, 2002).

In Kenya strawberry crop does well in acid soils ranging between 6-6.2 pH, and with good drainage capacity, but, irrigation is necessary during the growing period. However, due to poor crop management practices, strawberry pests and grey mold diseases caused by the fungi *Botrytis cinerea* are the major drawbacks in strawberry farming. According to IPM (2011), the convectional growers lose between 25-35% of their yield due to grey mold diseases as most of the fungicides used have become resistant to the pathogen. The fungicides further lead to the reduction of the market value of the strawberry due to their toxic residues in the fruit (Shen et al., 2008). According to Kovach et al. (2000) the viability of the strawberry reduces following the application of fungicides during the flowering stage as well as the harvestable yields. Further studies have also revealed that fungicides application is a great challenge to biodiversity conservation and a major threat to plant-pollinator interactions whose contribution to crop productivity is enormous (Ricketts, 2004).

Increasing production challenges have resulted in advancement of technology and innovation that ensured improved crop production in many countries of the world (Plan, 2016). However, it is necessary to validate such technologies in different geographic regions for the ease of adoption. Some of the notable new technologies that are transforming agriculture from a labor-intensive industry to capital intensive include bio-vectoring-technology (Mommaerts and Smagghe, 2011). This technology involves the use of insects as vectors of bio-control agents. The technology aims at minimizing the use of synthetic insecticides and development of pest resistance while maximizing on quality and yields of crops.

A successful outcome of entomovectoring technology has been realized mostly in some developed countries. The technology is especially useful in large variety of pollination-dependent crops. Managed bees, honey bees and bumble bees have been used to vector inoculum of fungi, bacteria and viruses from the hive to flowers (Kevan et al., 2003). The technology have been evaluated for the dissemination of *Trichoderma harzianum* T39 and *T. harzianum* 1295-22 against *B. cinerea* under field conditions using honey bees and bumble bees (Shafir et al., 2006; Kovach et al., 2000), *Metarhizium anisopliae* against pollen beetle, *Meligethes aeneus* and cabbage seed weevils, *Ceutorhynchus assimilis* the major pests of oilseed rape using honey bees (Carreck et al., 2007), *Trichoderma* spp against sunflower head rot, *Sclerotinia sclerotiorum,* the bacterium *Bacillus thuringiensis* against moth*, Cochylis hospes on* sunflowers (Escande et al., 2002; Jyoti and Brewer, 1999), and viruses to control *Heliothis* in clovers (Gross et al., 1994). However, despite this innovative approach, many African countries including Kenya have not embraced it as part of integrated pollinator pest management (IPPM).

The success of the technology, therefore, depends on the ability of the target vector to efficiently disseminate the biological control agent to the target crop. In the present study, we evaluated the effectiveness of African honey bees (managed bees) as vector of *T. harzianum* to strawberry crop under field conditions in central Kenya, evaluated the effectiveness of the *T. harzianum* against grey mold disease and determined the effect of simultaneous pollination and disease control using managed bees on strawberry quality and yields.

MATERIALS AND METHODS

Field sites and experimental design

The study was conducted on-station at the University of Nairobi (UoN) College of Agriculture and Veterinary Sciences (CAVS), and on-farm at Loresho in Kiambu county. The on-station farm is located off Kapenguria road, 15 km to the northwest of Nairobi at a geographical location E-1.25, S 36.742554 and elevation of 1840 m above sea level (ASL). The on station-farm is situated along Kaimoni road approximately five kilometres away from CAVS at a geographical location E-1.253, S 36. 751 and at an elevation of 1822 m ASL.

The on-station farm was divided into random experimental plots to form three treatments which included: Treatment 1- Open plot with one bee colony to enhance visits by managed bees and dispense the biological control agent, Treatment 2- Caged plot to enhance controlled manual spraying of crops with biological control agent (spray). The plot was caged using a stiff net that prevented entry of managed bees and other visitors into the plot, Treatment 3- Caged plot without managed bees and chemical spraying (control). The plot did not receive any biological control agent and there was no visitation by managed bees or other flower visitors. Each of the three treatments had 4 replicates. Loresho farm was classified as farmer normal practice. The plot was open to feral bees' visitation among other flower visitors.

Land preparation and strawberry planting

A piece of land measuring 50 m by 20 m at the on-station farm was cleared off the weeds. The plot was then divided into four blocks of 10 m by 20 m at two meters apart. Seven strawberry beds of one meter-wide and 10 m long at an interval of one meter- apart were made in each block. In each bed, twenty certified strawberry seedlings obtained from Kenya Agricultural and Livestock Research Organization (KALRO) were planted in a row. Spacing between plant to plant was 50 centimeters apart.

Weeding was done regularly to remove all the weeds that absorbed considerable nutrients and competed for space. Aerial irrigation was done for two hours daily during the first month of planting. Additionally, induced flower abortion was done on daily basis for the first two months after transplanting. This was done to enhance a healthy vegetative growth of the strawberry plants. To promote flowering and fruit set, Calcium Nitrate (CN) fertilizer was used (Republic of South Africa, 2008).

At on-farm site, two experimental plots each measuring 7 m by 10 m were established at an interval of 10 meters apart. The plots were then subdivided into two subplots each measuring 7 m by 2.5 m resulting to four replicates at 2 m apart. Three strawberry beds that measured one meter in width and 2.5 m in length were prepared on each subplot. The spacing between the beds and plant to plant was similar to that of on-station farm. In each strawberry bed, five certified strawberry seedlings obtained from KALRO were planted in each row resulting to fifteen strawberry seedling per subplot. Spacing was similar to that of on-station farm. Weeding was done weekly. Watering was done to supplement the little amount of rainfall received during the project duration. No chemical pesticides were applied to the strawberry plants on the on-farm site.

Installation of inoculated modified managed bee hive

A healthy colony of honey bees in a modified five- framed nuclear honeybee hive was installed at the on- station farm two weeks before flowering started. A modified two-way dispenser that consisted of an outlet (2 cm by 5 cm) and inlet (1 cm by 7 cm) pipes cylindrical in shape was fitted in the modified openings of the hive (Mommaerts et al., 2010). The outlet was made of transparent plastic material that transmitted light into the hive hence attracting bees to the exit, whiles the inlet had a landing platform from the outside that enabled bees to land on when coming back to the hive. The dispenser was designed to separate the outgoing bees from incoming bees for optimal dispensing of the biological control agent. At the onset of strawberry peak flowering, the dispenser was loaded with 2 g of *T. harzianum* inoculum prepared at 10^9 Colony-Forming Unit (CFU) g^{-1} with sterilized corn flour as a carrier- inert substance. The inoculum in the dispenser lasted for 6 days ensuring outgoing bees were well dusted with the inoculum on their hairy bodies (Freeman et al., 2004).

Bio-monitoring of *T. harzianum* transportation by managed bees

A sample of fifteen honey bees was picked when exiting the hive. The dispenser was refilled after every six days. The bees were captured using a sterile sweep net and picked using sterile forceps into sterile vials. Samples were taken to University of Nairobi (UoN) microbiology laboratory where they were cultured in potato dextrose agar (PDA) and incubated for four to seven days after which the total number of *T. harzianum* CFU was calculated.

Bio-vectoring of *T. harzianum* to strawberry flowers

A sample of fifteen strawberry flowers per treatments was excised using a sterilized pair of scissors and placed in manila paper bags. The samples were then taken for further processing at UoN, microbiology laboratory where the samples were cultured individually in PDA and after 4-7 days the number of *T. harzianum* CFU were calculated.

Determining grey mold disease incidence on flowers and fruits of strawberry

Healthy and grey mold diseased open flowers and ripe fruits from each plant per treatment were calculated and data recorded in the field data sheets. Strawberry fruits ripened on a weekly basis and hence data collection could only take place after every seven days for three months. The percent disease incidence was then calculated using the formula below as described by Waller et al. (2002):

$$\text{Disease incidence\%} = \frac{Number\ of\ Structures(\ flowers\ or fruits) with\ B.cinerea}{Total\ number\ of\ Structures(flowers\ or\ fruits)} \times 100$$

Determining the effect of simultaneous pollination and disease control using managed bees on strawberry quality and yield

The quality and quantity of strawberry fruits was determined using the following fruit parameters: total number of seeds per berry, the equatorial diameter, polar diameter and the weight of the berry (Colak et al., 2017). Harvesting of the fruits was done after every seven days for a duration of three months. Ripe berries characterized by red color were picked and packed for analysis at the National Museums of Kenya (NMK) in the Centre for Bee Biology and Pollination Ecology (CBBP). The diameter of the berries was measured using a vernier caliper in centimeters (cm) units, the weight was determined using analytical sensitive weighing balance in grams (g) units and the number of seeds per berry was counted from each fruit.

Statistical analysis

Data were analyzed using STATISTICA program (Stat Soft. Inc., 2007) version 8.0. The data were first tested for normality using Shapiro Wik; s W- test and histogram. Where data did not show a normal distribution it was subjected to a normal distribution curve to check for outliers and also transformed at $Log_{10}(x+1)$. Statistical tests were set at a significant level of 0.05. The data on effectiveness of managed bees as a vector of the bio control agent, effectiveness of *T. harzianum* against grey mold diseases and the simultaneous effect of inoculated managed bees as pollinators were analyzed using a one-way analysis of variance (ANOVA) to show whether there was any significant difference among means. Where data showed a significant difference in means, the results were further analyzed using Turkeys test to show the level of significance difference.

RESULTS

Effectiveness of managed bees as vectors of *T. harzianum*

From the study, it emerged that an individual honey bee carried $22.4 \pm 4.85 \times 10^2$ Colony-Forming units of *T. harziunum* (Mean ±SE) when exiting from the hive. Field observations showed that honey bees got dusted with the inoculum as they exited from the hives from the first day of refilling the dispenser with the inoculum. The highest mean of Colony-Forming Units ($25.8 \times 10^2 \pm 3.8 \times 10^2$) $P<0.05$ were observed during the first day compared to honey bees sampled on the second day and third day respectively ($23.2 \times 10^2 \pm 2.4 \times 10^2$),$18.2 \times 10^2 \pm 4.4 \times 10^2$) after refilling the dispenser with inoculum ($F=2,12$, ($P<0.05$) (Figure 1).

Colony-forming units of *T. harzianum* on strawberry flowers

There was a significant difference in number of CFU of

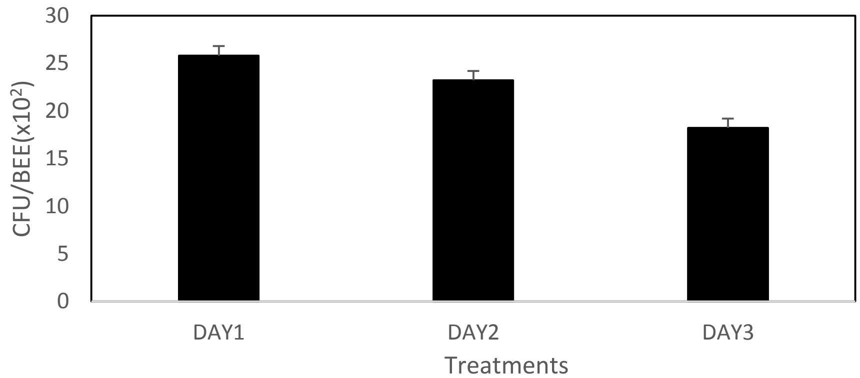

Figure 1. Number of *T. harzianum* spores per sampling day.

T. harzianum per strawberry flower among treatments (F=3,140, (P<0.05). Spray treatment recorded highest mean of CFU of *T. harzianum* per flower (20.56±0.48 (10^2) while bio-vectoring technology (BVT) treatment recorded 11.86±0.44 (10^2) CFU of *T. harzianum* per flower (P<0.05). Control treatment and farmers practice treatment recorded the lowest mean of CFU of *T. harzianum* per flower (0.36±0.11 and 0.33±0.08) 10^2) respectively (P>0.05).

Effectiveness of *T. harzianum* on Grey mold diseases control

It was evident that *T. harzianum* had significant effects on disease control on ripe fruits of strawberry. Fruits harvested from the four treatments showed a significant difference in their diseases incidence (F=3.12, P<0.05). Spray treatment showed significantly low fruit diseases incidence (16%) as compared to farmers practice plot (38%) P<0.05. There was no significant difference in fruit disease incidence among BVT (26%), control (26%) and spay plot. P>0.05. However, there was a significant difference in percentage disease incidence on strawberry flowers among treatments (F=3.12, P<0.05). The farmers practice plot recorded significantly lower mean of flower disease incidence (18%) as compared to BVT (30%) and control treatment (29%). There was no significant difference among spray plot (23%), BVT and control plot (P>0.05).

The effect of pollination by inoculated managed bees on strawberry quality and yield

Data on total harvest, weight, polar diameter, equatorial diameter and the number of seeds in a strawberry fruit were used in this study to assess the simultaneous effect of inoculated managed bees on strawberry quality and yield. There was no significant difference among the means of the total berry harvested in all the plots (P>0.05). However, the spray plot showed significantly the highest weight of 4.89±0.20 kg as compared to caged plot (4.13±0.22 kg) (F =3,2122, P< 0.05). There was no significant difference among weights of berries in BVT (4.84±0.18 kg), farmers practice (4.48±0. 42 kg) and spray plot (P>0.05). Polar diameter of fruits obtained from spray, caged and farmers practice showed a significant difference (F =3,2122, P<0.05). Fruits obtained from farmers practice plot recorded significantly the higher polar diameter (2.48±0.18 cm) as compared to caged plot (1.64±0.09 cm) P<0.05. Similarly, there was a significant difference in equatorial diameter of fruits obtained from all the treatments (F =3,2122, P<0.05). Fruits with the highest equatorial diameter were recorded from farmers practice (1. 93±0.20 cm), while the lowest mean of equatorial diameter was recorded from caged plot (1.31±0.05 cm). Lastly, the mean number of seed per berry had a significant difference among all the treatments P<0.05. Farmers practice recorded the highest mean (242±11) of the number of seeds while caged recorded the lowest mean number of seed per berry (125±6). (F=3, 2122, P<0.05) as shown in (Table 1). Wild bees and other flower vising insects recorded in the study sites before and after installation of managed bee's hives included managed bees, solitary bees, dipteran, lepidopteran, coleopteran, orthopteran, Aranea and wasps. Dipterans were proportionally the most numerous of the flower visitors in both the on- station and on-farm sites while solitary bees were many in the on-farm site, However, close observations confirmed that only bee species were in contact with both anthers and stigma while visiting the strawberry flowers and would thus be able to transfer pollen and hence enhancing cross-pollination.

DISCUSSION

From the results, it was evident that honey bees were

Table 1. Effect of pollination by managed bees and *T. harzianum* application on strawberry quality and yields.

Treatment	Mean ± SE					
	No. of flowers	**No. of fruits**	**Weight (g)**	**PD (cm)**	**ED (cm)**	**No. of seeds**
BVT	186.25 ± 21.07[a]	69.75± 2.78[a]	4.85±0.18[ab]	1.96±0.08[a]	1.55±0.04[a]	160±4.8[a]
Spray	204.5±28.6[a]	85.5±2.50[a]	4.89±0.20[a]	2.02±0.08[ac]	1.44±0.05[ab]	135±5.1[b]
Caged	157.25± 11.50[ab]	57.5 ± 3.38[a]	4.13 ± 0.20[b]	1.64±0.09[b]	1.31±.0.05[b]	125±5.8[b]
FP	88.75± 21.99[b]	54.25±20.14[a]	4.48± 0.42[ab]	2.48±0.18[c]	1.93±0.10[c]	241±11.1[c]

Means with same letters within a column are not significantly different (P>0.05).

loaded with *T. harzianum* spores as they exited the hive. The inoculum was heavily loaded on the upper part of their entire bodies and was dispensed consistently from the dispenser throughout the day. The findings agreed with the work done by Bilu et al. (2004) on use of Triwaks; a dispenser type that dispensed a high level of *Trichoderma* spp consistently. Flowers collected from all the treatment showed the presence of *T. harzianum* although at different concentrations. The results were an indication that honey bees were able to carry and disseminate the biological control agent to the target crop. The study results agreed with those of Kovach et al. (2000) who observed that honey bees could successfully deliver *T. harzianum* to strawberry plant for biological control of *Botrytis* fruit rot.

The study also revealed that honey bees carried more of the BCA on day one than on day two and three after loading the dispenser with the inoculum. It was evident that the inoculum was reducing every time the honey bees were exiting the hive hence the loading of the dispenser had to be done after every six days. The results concurred with findings of Freeman et al. (2004) where application of biological control agent after every two days resulted in better control of *B. cinerea* than less frequent application of every seven to ten days.

The findings of the study further revealed that spores of *T. harzianum* were recorded more on flower samples derived from sprayed and BVT plots as compared to those derived from control and farmers practice plots. BVT and spray treatments received biological control agent through managed bees and spraying respectively while control and farmers practice plot could have received the biological control agent as drift or as naturally occurring spores in the soil. That observation agreed with findings by Shafir et al. (2006) where on average 22000 CFU of *T. harzianum* per flower were delivered by honey bees to strawberry plants through a Triwaks dispenser. Even though the spray treatment showed the highest number of spores landing on the flowers, the practice is not economically viable for large scale or commercial farming. This is because the practice is labor-intensive and much amount of water is consumed leading to higher expenses. Shafir et al. (2006) found out a different challenge of application of biological control agent whereby frequent use of a sprayer may cause mechanical damage to the fruit and foliage. The use of managed bees to deliver the biological control agent requires little manpower and managed bees double up as pollinators of the target crop.

The flower samples obtained from BVT plot were assumed to have lost some *T*. *harzianum* spores. This was attributed to the movement of honeybees from one flower to another (Shafir et al., 2006). Additionally, biological control agent could have been lost during the process of capturing bees using a sweep net, drifting by the air or being swept away by sprinkled water during the irrigation process. However, traces of *T*. *harzianum* spores were found on flowers in the control and farmers practice plots. This observation provided evidence that the fungi (*T. harzianum)* could have naturally occurred in the soil at a very small concentration, or likely that the *T. harzianum* spores were drifted through the stiff net from the spray and BVT plots to the non *T. harzianum* loaded plots. Those findings were supported by work done by Shafir et al. (2006). Research done by Peng et al. (1992) and Kovach et al. (2000) indicated that drift effect of biological control agent occurred whereby non-target areas were found to have the inoculum. Similar observations were made by Elad and Yunis (1993) who also found a significant population of *T. harzianum* in untreated control plots when the biological control agent was sprayed in the treated plots of cucumber plants.

The presence of *B. cinerea* spores obtained from the laboratory after inoculating a gram of soil samples collected from the study sites indicated that the grey mold disease was present in the soil before the introduction of the biological control agent. Results from this study revealed that *T. harzianum* used in the strawberry plots either through usage of managed bees or spraying had *B. cinerea* disease suppression effect on strawberry fruits. When the biological control agent (*T. harzianum)* was applied on strawberry plants, it was evident that the results of the disease suppression were expressed more on fruits than on flowers. Those results could be justified by the fact that the transition from a flower to a fruit took place within one to three days while fruit ripening took between five to seven days. Additionally, high disease incidence on BVT flowers could be as a result of lower number of CFU of *T. harzianum* delivered by managed bees as compared to those delivered via spraying.

Finally, the effect of high humidity as a result of irrigation and increased managed bees may have contributed to the growth and multiplication of *B. cinerea* on BVT flowers. Dedej et al. (2004) found that the incidence of mummy berry disease increased as a result of increased honey bee density, but the mummy berry disease incidence was reduced when the honey bees disseminated *Bacillus subtilis* to blueberry plant.

The study also proved the effectiveness of *T. harzianum* against grey mold as also recorded by Hokkanen et al. (2015) who recorded a 66% grey mold reduction under light disease pressure when honey bees were used to disseminate *Gliocladium catenulatum* to strawberry plants. Similar findings by Peng et al. (1992) and Yu and Sutton (1997) also reported sixteen percent reduction of *B. cinerea* in strawberry using *Clonostachys rosea* vectored by honey bees. The use of biological control agent therefore, proved to be effective in suppressing *B. cinerea* that causes grey mold disease to strawberry plants. It was evident that the spray treatment recorded the lowest disease incidence as compared to the other treatments. Although the control treatment did not receive any biological control agent, disease incidence showed a decrease from what was recorded from flowers as compared to the fruits. Those findings revealed the protective effect of the stiff net in preventing entry of strong winds that would have drifted the pathogen, direct sunlight and water droplet or contaminated insects coming from open environment.

It was therefore apparent that pollination by insects and wind on strawberry plant played a significant role in influencing the quality and yield of strawberry crop. The findings of this study showed a significant difference in the number of seeds in a fruit, weight, total harvest and the diameter of strawberry fruit across the treatments. The number of managed bees, solitary bees and wind pollination could have influenced the quality and quantity of the strawberry fruits. Studies by Albano et al. (2009) showed a relationship between the number of fertilized ovules and berry size of strawberry when pollinated by both honey bees and solitary bees. Therefore, it may be assumed that the high number of pollinating solitary bees could have contributed to quality and quantity of strawberry yield from on-farm site. Comparison of the strawberry yield between BVT plot and farmers practice plot revealed a strong contribution of solitary pollinators. Managed bees alone were found to be effective in strawberry pollination. Colak et al. (2017) found a significant difference in weight for honey bee pollinated strawberry. However, reliance on several pollinators would lead to better quality and quantity of strawberry fruits. The results of this study agreed with the results of Albano et al. (2009) on diversification of pollination sources, avoiding the dependence on a single specific group of pollinators.

The consistent higher fruit weight, polar diameter and number of seeds in spray plot compared to control plot was likely to have resulted from the effects of *T. harzianum* as both plots were enclosed. Those findings agreed with those of Kovach et al. (2000) who showed that stiff net frequently used by fruits and vegetable growers, protected plants from cold and hence accelerated plant growth. However, despite the influence of pollinators on strawberry yield, it was confirmed from this study that the commercial strawberry cultivar had a degree of self-pollination and wind pollination. According to Albano et al. (2009) flowers of all the commercial strawberry are hermaphrodites and self-fertile. It was noted that flowers of both open (BVT and FP) and stiff-netted plots (control and spray) were similarly shaken under windy conditions, indicating that the stiff net was not a major barrier to wind pollination. That observation agreed with the findings of Kuvanci et al. (2010) that showed a significant increase in strawberry yields following honey bees, wind and native bee's pollination. It was further noted that on installation of honey bee's colony within or close to the BVT, solitary bees were displaced. Those findings agreed with Roubik (2009), who showed that increased managed bees in an area silently competed with solitary bees interfering with their foraging activity. However, higher quality of strawberry yield was recorded from on-farm site due to frequent pollination by solitary bees. That is in contrary to the findings revealed by Hansted et al. (2015) that showed a rise in fruit set and yield when bees were kept close or within an orchard.

CONCLUSION AND RECOMMENDATION

Conventionally, control of grey mold disease in strawberry plantation is achieved through application of chemical pesticides. The pesticides used are harmful to the environment, human and to beneficial insects such bees. In order to eliminate the negative effects of chemical pesticides in the environment, BVT where managed bees are used to disseminate biological control agent could be an alternative solution to pesticides application. The present study recommends: further research on the effect of increased managed bee to the environment, the long term effect of biological control agent (*T. harzianum)* to the bees and to the environment

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Farmers' preferences towards breeding objective for indigenous chickens in different agro-ecologies of Ethiopia

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A study was conducted in different agro-ecologies of Ethiopia with the objective of understanding the farmers' preferences towards breeding objectives in indigenous chickens. For the interview, 245 households (60 from lowland, 100 from midland and 85 from highland) were randomly selected. Farmers in lowland had significantly (p<0.05) lower chicken populations while comparing with the remaining agro-ecologies. The average age of village pullets at first egg was 6.54±0.063 months. There was significantly (p<0.05) higher egg production in midland. There was significant difference (p<0.05) in clutch number among the three agro-ecologies. Among the three agro-ecologies; midland showed significantly (p<0.05) higher number of eggs set/hen. Effective population size of village chickens per household was calculated as 4.43, 7.8 and 7.18 in lowland, midland and highland respectively. Most of the farmers (91%) were practicing culling their chickens for getting old, sickness, brooding frequency and low production for hens and getting old, sickness and fighting each other for cocks. Comparing the preferences of traits, female farmers preferred egg production, unlike the male farmers who gave equal emphasis both for egg and meat. Egg production for sale was prioritized by the farmers, especially for women, followed by live chicken sale. Body weight is the most considered trait to select male chickens for breeding, followed by plumage color, across thee agro-ecologies. For female chickens, brooding frequency is most considered in lowland (48.3%) and midland (37%) unlike in highland where age at first egg (47.1%) is prioritized. This study can be the base to design the breeding strategy of the chicken population in the study sites and beyond.

Key words: Agro-ecology, breeding objectives, effective population, farmer preference, inbreeding coefficient, local chickens.

INTRODUCTION

Local chickens have played a pivotal role in capital build up, poverty, malnutrition and hunger reduction among the resource poor rural households in developing countries because of their low input requirements for production,

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Table 1. Description of agro-ecological zones.

Agro-ecology	Features
Lowland	Hot semi-arid, 800-1100 m.a.s.l, low vegetation, rain fall (400-500 mm), agro-pastoral, poor infrastructure
Midland	Hot sub humid, 1501-2500 m.a.s.l, high vegetable, rain fall (1001-1200 mm/year), temperature (16-20°C), mixed farming system, moderate infrastructure
Highland	Humid and sub humid, 1600-3348 m.a.s.l, high vegetable, rainfall (>1300 mm/year), temperature (7-12°C), mixed crop farming, poor to moderate infrastructure

m.a.s.l = meter above sea level, °C **=** degree Celsius.
Source: Solomon et al., 2007; Sefa et al 2016.

short generation intervals, scavenging ability and adaptability to harsh environment conditions (Aberra and Tegene, 2011; Aberra, 2014). Village poultry are readily available assets to local populations throughout Africa and they contribute to food security, poverty alleviation and promote gender equality, especially in the disadvantaged groups, (HIV and AIDS infected and affected people, women and poor farmers) and less favored areas of rural Africa where the majority of the poor people reside. On top of these merits, village poultry can provide the start of the owner climbing the "livestock ladders'' leading to other livestock species such as goats and cattle or serve as "transport (transitional) bridge" from small livestock to large livestock species production (Dolberg, 2003)**.**

Small number of breeding cocks per household for different reasons like marketing breeding males might contribute in surging further inbreeding. The extremely small flock size of the breeding local chickens confirms the extreme drop in the total population of chicken in Ethiopia since the past decade (Nigussie *et al*., 2010).

According to the study conducted by Aboe *et al*., (2006), although indigenous village chicken is the most prominent class of livestock in the country and constitutes about 60-80% of the total poultry population, their productivity is low because of poor nutrition and low genetic potential. The local chicken production systems in Africa are mainly based on scavenging indigenous chickens found in almost all households in the rural areas. They are an integral part of the farming systems requiring low inputs with outputs accessible at both inter-household and intra-household levels. Village chickens also fulfill a number of other functions for which it is difficult to assign any monetary value. Ethiopia has an estimated 60 million chickens of which 96.83% are non-descriptive local ecotypes, 2.37% are hybrid chickens, and 0.8% are exotic breeds (CSA, 2015).

Objectives

i) To identify the preferences of village poultry keepers at different agro-ecologies.
ii) To identify breeding objectives of the local chickens at different agro-ecologies.
iii) To measure inbreeding coefficient of local breeding chickens.

MATERIALS AND METHODS

Basis of the study

The basis of this study was comparing the issues related with chicken production and breeding across three agro-ecologies of the country.

Agro-ecological zones

Identification of households

Three agro-ecologies were selected using a stepwise purposive sampling technique that was based on the local chicken populations, information on dissemination of exotic/crossbred chickens in the past, and the topographical accessibility.

Within the agro-ecologies, 245 households (60 from lowland, 100 from midland and 85 from highland) were randomly selected for filling the questionnaire on the productive and reproductive performance of village chickens, breeding objectives, culling practices, keeping purpose, selection criteria, and preferences of farmers, and management of indigenous chicken populations. Preferences were disaggregated by gender (Table 1). For identification of the locations and the respondents, assistance was sought from the local authorities of the selected study sites. Focus group discussions were conducted at each selected site with the selected members. Members of the focus groups included farmers experienced with rearing chickens and those have knowledge about past and present social and economic status of the area, including community elders, women, veterinarians, and extension agents. Furthermore, at the beginning of the study, informal discussions were conducted with village elders and those who have experience in poultry rearing to know about the types of local chickens that might be available in the area and also their physical descriptions and local names.

Data collection

A semi-structured questionnaire was developed, pretested and interviewed. Interviews were conducted with farmers who were associated with village chicken rearing. The survey included information on livestock demography.

Household members responsible for a part of the husbandry practices were identified and the enumerator tried to capture their

Table 2. Characteristic of the farmers in different agro-ecologies.

Descriptor	Agro-ecology						Average	
	Low (N=60)		Mid (N=100)		High (N=85)			
	N	%	N	%	N	%	N	%
Sex								
Female	44	73.3	81	81	77	90.6	202	82.4
Male	16	26.7	19	19	8	9.4	43	17.6
Education level								
Cannot read and write	26	43.3	28	28	13	15.3	67	27.3
Read and write	18	30	43	43	36	42.4	97	39.6
Primary	10	16.7	14	14	17	20	41	16.7
Above primary	6	10	15	15	19	22.3	40	16.4

experiences in that regard. Recall method was used to collect information on current flock demography and recent changes in flock structure after five years. The study also documented any special attributes of a genotype of chickens which have socio-cultural significance but may not relate to productivity or reproduction.

Data analysis

The data were analyzed using the SPSS and Microsoft Excel. The (Ne) and ΔF were calculated for male and female breeding chickens as follows

$$Ne = 4 \times Nm \times Nf/Nm + Nf$$

$$\Delta F = 1/Ne \times 2$$

Where; Ne = effective population size
ΔF = inbreeding coefficient
Nm = number of male chickens
Nf = number of female chickens.

RESULTS AND DISCUSSION

Details of general household characteristics are presented in Table 2. In this study, respondents from three ago-ecologies keeping local chickens participated. Of the total interviewed farmers, majority of them were females (82.4%). During the interview, there was communication with farmers to decide who would be the participant (male or female). After convincing, more women were interviewed, and for those did not have interest, male farmers were interviewed. The number of females was high in this survey because female farmers are better with the poultry production and breeding.

The large proportions (43.3%) of farmers in lowland area could not read and write; whereas, 43% in midland and 42.4% in highland responded as they can read and write. This survey result showed that more than half (51%) of the respondents were able to read and write, which indicates that farmers are in good condition to accept trainings on poultry production and local chickens conservation affairs, and also to easily implement opportunities in a relevant way.

Livestock under each agro-ecology

The livestock possession of the sampled households is summarized in Table 3. The reported mean chicken per household in this study was 4.82±0.28 in the lowland, 8.10±0.34 in the midland, and 7.67±0.348 in the highland. From this result, it is obvious that local chickens in lowland agro-ecology were enormously lower than the two remaining agro-ecologies (midland and highland). Farmers in lowland had owned significantly lower number of chickens but higher numbers of goats and mules in lowland agro-ecology. This result is higher than the report of Meseret (2010) in which the average flock size per household in Gomma district is 6.23. Whereas it is lower than the report of Fisseha *et al.,* (2010) who reported that the mean flock size of chicken was 9.2 chickens per household in Dale district. This difference might be due to the study sites coverage. Both studies were conducted in a single district for each unlike the current study which covered wider areas of different agro-ecologies.

Productive performance of indigenous chickens

There were no differences in age at first egg of local pullets in the lowland and highland agro-ecologies (Table 4). The average age pullet at first egg is 6.54±0.063 months. Age at first egg of pullets is significantly ($p<0.05$) shorter at midland agro-ecology. This is a longer while comparing to the study conducted by Melaku (2016) who reported that 5.68 months in Southern Wollo for pullets to lay the first egg. Also congruent with the report of Gebreegziabher and Tsegaye, (2016) who stated that the age at first egg was 6.3, 6.2 and 7 months in lowland,

Table 3. Livestock possession of farmers across different agro-ecologies of Ethiopia.

Descriptor	Agro-ecology (Mean ± SE)			Average (Mean ± SE)	Significance
	Lowland	Midland	Highland		
Chickens	4.82±0.28[a]	8.10±0.34[b]	7.67±0.348[b]	7.15±0.214	0.000
Cattle	8.85±0.376[a]	7.93±0.349[a]	7.74±0.454[a]	8.1±0.232	0.166
Sheep	1.28±0.16[a]	1.95±1.57[b]	3.35±0.2[c]	2.27±0.115	0.000
Goats	3.67±0.356[b]	1.49±0.118[a]	1.15±0.133[a]	1.91±0.127	0.000
Donkeys	0.98±0.056[a]	0.97±0.39[a]	0.96±0.035[a]	0.07±0.024	0.957
Horses	0.57±0.065[a]	0.43±0.05[a]	0.45±0.054[a]	0.47±0.032	0.217
Mules	0.43±0.069[b]	0.20±0.04[a]	0.31±0.05[ab]	0.29±0.03	0.008

[a,b] Different superscripts with in row indicate the presence of significant difference (p<0.05); [a,a or b,b] same superscripts with in row indicate the absence of significant difference (p>0.05) and [a, b, ab] indicate that absence of significant defiance (p>0.05) of [ab] with [a] and [b].

Table 4. Productive performance of the local chickens across three agro-ecologies of Ethiopia.

Variable	Agro-ecologies (Mean ± SE)			Average
	Lowland	Midland	Highland	
Average age of pullet at first egg (months)	6.67±0.123[b]	6.21±0.095[a]	6.85±0.1[b]	6.54±0.063
Average number of eggs per hen per clutch	13.75±0.19[b]	14.94±0.18[c]	13.02±0.177[a]	13.98±0.119
Average number of clutches per year	3.52±0.077[a]	3.94±0.076[b]	4.59±0.119[c]	4.06±0.061
Total number of eggs per hen per year	57.32±0.68[a]	65.36±0.71[c]	61.05±0.81[b]	61.89±0.48
Clutch length (days)	32.6±0.53[b]	30.14±0.26[a]	31.98±0.29[b]	31.38±0.21

[a,b] Different superscripts within row indicate the presence of significant difference (p<0.05); but [a,a or b,b] same superscripts with in row indicate the absence of significant difference (p>0.05).

midland and highland, respectively in Wolaita Zone; whereas, Addisu *et al,* (2013) in North Wollo reported that the shorter age at laying 5.43 for pullets.

Average egg production per hen per clutch was 13.75, 14.94 and 13.02 eggs in lowland, midland and highland, respectively. This was higher than the report of (Berhanu and Temesgen, 2019) who stated that mean egg production/clutch is 10.73eggs/hen in Hadiya Zone. This might be due to the scope of the study; the current study covered all types of agro-ecologies and the wider sites to gather information while comparing with the mentioned report.

The number of eggs across the three agro-ecologies was different. Number of eggs in the midland was significantly (p<0.05) higher than the remaining two agro-ecologies. The total annual egg production/hen of local hens, under existing farmer management condition, is estimated to be 61.89±0.48. This was higher than the report of Addis et al, (2013) and Mekonen, (2007) in North Gonder and in Southern Ethiopia, reported that the annual egg production per hen is 51.08 and 55.2, respectively. This might be due to the site difference and also due to better clutch numbers.

The average numbers of clutches per hen in this study were 3.52±0.077, 3.94±0.076 and 4.59±0.119 per year in lowland, midland and highland, respectively. There was significant difference (p<0.05) in clutch number among the three agro-ecologies. Chicken in highland agro-ecology showed significantly higher clutch number than the remaining agro-ecologies. The current study agreed the report of Gebreegziabher and Tsegaye, (2016) in which the mean clutch number of local chickens in Wolaita zone, of SNNPRs was 3.6, 4.1 and 4.59 per year in lowland, midland and highland, respectively.

Reproductive performance of local chickens

The average number of eggs set/hen was 11.67 ± 0.162, 12.68 ± 0.138 and 11.88 ±0.137 in lowland, midland and highland areas, respectively. Among the three agro-ecologies; midland showed significantly (p<0.05) higher number of eggs set per hen.

On average 8.17±0.177, 10.2±0.187 and 8.64±0.197 eggs were hatched in lowland, midland and highland, respectively. The current study is lower for the eggs set per hen per clutch than the report which was conducted by Fisseha et al. (2010) who stated that, 13 eggs set per hen per clutch in lowland, 13 eggs set per hen per clutch in midland and 14 eggs set per hen per clutch in highland, in Bure district of North West Ethiopia. Overall mean of hatched chicks in this study was 9.16. This

Table 5. Reproductive performance of the local chickens across three agro-ecologies of Ethiopia.

Variable	Agro-ecologies (Mean ± SE)			Overall
	Lowland	Midland	Highland	
Number of eggs laid per hen	11.67±0.162[a]	12.68±0.138[b]	11.88±0.137[a]	12.16±0.088
Number of eggs hatched per hen	8.17±0.177[a]	10.2±0.187[b]	8.64±0.197[a]	9.16±0.124
Number of chicks raised per hen	5.15±0.178[a]	6.68±0.187[b]	4.87±0.159[a]	5.68±0.117
Hatchability (%)	70.1±1.2[a]	80.2±0.98[b]	72.5±1.27[a]	75±0.72
Survivality (%)	62.97±1.7[b]	65.3±1.3[b]	56.6±1.4[a]	61.7±0.87

[a,b] Different superscripts within row indicate the presence of significant difference ($p<0.05$); but [a,a or b,b] same superscripts with in row indicate the absence of significant difference ($p>0.05$).

Table 6. Effective population size and inbreeding coefficient of local chickens in different agro-ecologies.

Agro-ecology	Flocks	Total chickens	N_m	N_f	N_e	ΔF
Lowland (N=60)	Combined total	289	104	185	266.3	**0.002**
	Mean per HH	4.82	1.73	3.08	4.43	**0.113**
Midland (N=100)	Combined total	810	327	483	780	**0.0006**
	Mean per HH	8.1	3.27	4.83	7.8	**0.128**
Highland (N=85)	Combined total	652	244	408	610.75	**0.00082**
	Mean per HH	7.67	2.87	4.79	7.18	**0.07**

N_{e} = effective population size, N_m = number of breeding male, N_f = number of breeding female and ΔF = change in inbreeding coefficient.

report is congruent with the study conducted by Sefa et al., (2016) who stated that 9.33 chicks hatched per set in Lemo district, Hadiya Zone of Southern region, Ethiopia.

Among the hatched chicks, 5.15±0.178, 6.68±0.187 and 4.87±0.159 were raised in lowland, midland and highland areas, respectively. This result showed that, 70.1±1.2, 80.2±0.98 and 72.5±1.27 were hatched and from these, 62.97±1.7, 65.3±1.3 and 56.6±1.4 were raised in lowland, midland and highland, respectively. This result is higher than Aberra et al., (2013) who reported that the survivable rate of chickens in highland and lowland agro-ecological zones of Ethiopia were 55.0% and 55.1%, respectively.

There was significant ($p<0.05$) difference in hatchability of chicks in lowland area in this study, this was better than the remaining two agro-ecologies. High hatchability can improve poultry production when there is good chick survival (Table 5).

Generally, the survival rate of chicks in this study was high unlike to the report of Wondmeneh et al. (2014) who reported that the survival rates of chicks kept under natural brooding conditions is very low in Ethiopia.

Based on the information reported in Tables 3 to 5, midland agro-ecology is more favorable for chicken production considering most of the parameters. This is for relative betterment of feed resources, disease outbreaks, and awareness of farmers towards improving management, market access and environment than the remaining agro-ecologies.

Effective population size and inbreeding coefficient

Effective population size and inbreeding rates of local chickens in this study are presented in Table 6.

While comparing inbreeding rate of household mean chickens and the whole chicken in the study, there was higher for mean per HH which leads to more genetic drift on household flock mean across the three agro-ecologies.

Effective population size and increase in inbreeding over next generation were calculated based on chicken flocks of the farmers. Effective population size of the indigenous chicken flocks per household ranged from 4.43, 7.8 and 7.18 in lowland, midland and highland agro-ecological zones respectively.

Inbreeding coefficients of the chickens were recorded as 11.3, 12.8 and 7% for lowland, midland ad highland areas respectively. This is comparable with the study conducted by Nigussie, 2011 for lowland and midland who reported 12% of inbreeding coefficient; however, there was lower inbreeding coefficient in low altitude. In order to modify inbreeding coefficient of the chickens,

Table 7. Breeding objective and culling experiences of farmers in different agro-ecologies.

Variable	Study site			Average [N (%)]
	Lowland (N=60)	Midland (N= 100)	Highland (N= 85)	
	N (%)	N (%)	N (%)	
Breeding objectives				
Egg	37 (61.7)	47 (47)	47 (55.3)	131 (53.5)
Meat	8 (13.3)	13 (13)	19 (22.4)	40 (16.3)
Both egg and meat	15 (25)	40 (40)	19 (22.4)	74 (30.2)
Culling practices				
Yes	52 (86.7)	94 (94)	77 (90.6)	223 (91)
No	8 (13.3)	6 (6)	8 (9.4)	22 (9)
Culling reason for females				
Age	15 (25)	13 (13)	12 (14.1)	40 (16.3)
Sickness	10 (16.7)	23 (23)	23 (27.1)	56 (22.9)
Frequent broodiness	23 (38.3)	42 (42)	26 (30.6)	91 (37.1)
Low production	12 (20)	22 (22)	24 (28.2)	58 (23.7)
Culling reason for males				
Age	36 (60)	66 (66)	42 (49.4)	144 (58.8)
Sickness	16 (26.7)	22 (22)	36 (42.4)	74 (30.2)
Fighting	8 (13.3)	12 (12)	7 (8.2)	27 (11)

increasing chickens number is needed. However, increasing the local chickens may not be profitable strategy for local chickens are of poor genetic makeup. Therefore, it is better to develop breed improvement program so far.

Breeding objectives and culling practices

Breeding objective refers to the final goal of farmers to produce the chickens. As shown in Table 7, the main objective of the farmers to rear the indigenous chickens was egg production (53.5%) followed by rearing indigenous chickens for both egg and meat (for 30.2% of farmers).

About 91% of the farmers were practicing culling their chickens for getting old, sickness, brooding frequency and low production for female and getting old, sickness and fighting to each other for male indigenous chickens.

Preferences by sex of the farmers

As shown in Table 8 below, across all agro-ecologies, majority of the female farmers prefer egg production (79.6%, 74.1% and 48.1% in lowland, midland and highland) respectively. However, male farmers prefer both egg production and adaptation traits from local chickens strongly across three agro-ecologies.

While looking for the preferences up on the purposes of the chicken production, egg production for sale was prioritized by the farmers, especially for women farmers, across three agro-ecologies followed by live chicken sale. The traits egg for home consumption and meat were not preferred by most female farmers.

Farmers prefer multiple traits among the mentioned in Table 8, however, the rank of prioritization is different for most of the farmers.

Purpose of keeping chickens

The rank for purpose of chicken keeping is presented in Table 9. Chickens might be kept either for home consumption or income generation through different means, like eggs for home consumption, eggs for sale, eggs to be hatched, meat for home consumption, chickens for selling, and cultural issues. Among the mentioned ones, egg sale was the main purpose of chicken rearing across all agro-ecologies to generate income which accounted for 42%, 37%, and 40% farmers for lowland, midland and highland respectively. Unlike to lowland and highland, in the midland the farmers allow their chickens to incubate eggs (17%).

Next to egg sale, live chicken sale was the most common purpose given by farmers across the three agro-ecologies towards keeping indigenous chickens. This indicates that most of the farmers those engaged in

Table 8. Preferences of male and female farmers towards different traits of local chickens.

Parameter	Agro-ecology					
	Lowland		Midland		Highland	
	Male (n=16)	Female (n=44)	Male (n=19)	Female (n=81)	Male (n=8)	Female (n=77)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Trait preference						
Egg	8 (50)	35 (79.6)	8 (42.1)	60 (74.1)	3 (37.5)	37 (48.1)
Meat	3 (18.75)	6 (13.6)	3 (15.8)	3 (3.7)	1 (12.5)	7 (9.1)
Adaptation	5 (31.25)	3 (6.8)	8 (42.1)	18 (22.2)	4 (50)	34 (44.2)
Purpose of traits						
Egg for home consumption	4 (25)	5 (11.4)	3 (15.8)	8 (9.9)	1(12.5)	5 (6.5)
Egg for sale	5 (31.25)	27 (61.4)	4 (21.1)	39 (48.1)	3 (37.5)	40 (51.9)
Egg for incubating	2 (12.5)	5 (11.4)	3 (15.8)	3 (3.7)	1 (12.5)	1 (1.3)
Meat	3 (18.75)	1(2.3)	3 (15.8)	6 (7.4)	0 (0.00)	1 (1.3)
Chicken sell	2 (12.5)	6 (13.6)	6 (31.6)	25 (30.9)	3 (37.5)	30 (39)
Genotype preference						
Local	4 (25)	9 (20.5)	7 (36.8)	22 (27.2)	2 (25)	27(35.1)
Exotic	4 (25)	23 (52.3)	6 (31.6)	38 (46.9)	4 (50)	35 (45.5)
Crossbred	8 (50)	12 (27.3)	6 (31.6)	21 (25.9)	2 (25)	15 (19.5)

N = number of the respondents.

Table 9. Purposes of rearing local chicken across different agro-ecologies of Ethiopia.

Variable	Agro-ecology											
	Lowland (N=60)				Midland (N=100)				Highland (N=85)			
	1st	2nd	3rd	Index	1st	2nd	3rd	Index	1st	2nd	3rd	Index
Egg (home consumption)	0	0	1	**0.02**	7	3	1	**0.11**	3	3	7	**0.15**
Egg (sell)	10	9	6	**0.42**	10	17	10	**0.37**	12	12	10	**0.4**
Egg (incubating)	2	3	2	**0.12**	7	2	8	**0.17**	5	6	0	**0.13**
Meat	1	2	0	**0.05**	2	6	2	**0.1**	4	3	2	**0.11**
Sell chickens	6	5	11	**0.37**	9	7	8	**0.24**	6	6	6	**0.21**
Cultural issues	1	1	0	**0.03**	0	0	1	**0.01**	0	0	0	**0.00**

Index = Sum of samples under 1st, 2nd and 3rd rank for each parameter divided by the sum of interviewed households under 1st, 2nd and 3rd per strata.

chicken rearing prioritized income generating through poultry production. The least common purpose of the farmers to rear the chickens was cultural issues across all agro-ecologies. Unlike to mid and highland areas, only 2% of the farmers in lowland preferred egg for home consumption. Generally, the most preferred trait across all agro-ecologies was assessed as eggs for sale.

Rural farmers keep indigenous chickens to fill the requirement of food and income either directly or indirectly like protein. This is in line with the study conducted by Tadelle et al. (2003 and Halima, 2007) who reported that chickens are the most widespread and almost every rural family owns chickens, which provide a valuable source of family protein and income in Ethiopia.

Selection criteria

All the interviewed farmers across the three agro-ecologies had a trend of selecting the breeding male and female chickens based on the traits body weight, color, brooding frequency and comb type accordingly. According to this study the traits used to select breeding chickens of both sexes are shown in Table 10. Body weight, plumage color, comb type and parental history were the most important traits to select breeding male chickens.

Male chickens that have convincing body weight and color for chicken rearing farmers are the most preferred by most of them across the three agro-ecologies. Of

Table 10. Criteria for selecting village chickens at different agro-ecologies of Ethiopia.

Criteria	Agro-ecology			
	Lowland (N=60)	Midland (N=100)	Highland (N=85)	Overall (N=245)
	N (%)	N (%)	N (%)	N (%)
For selecting male				
Body weight	31 (51.7)	41 (41)	30 (35.3)	102 (41.6)
Plumage color	13 (21.7)	33 (33)	27 (31.8)	73 (29.8)
Comb type	6 (10)	18 (18)	14 (16.5)	38 (15.5)
Parental history	10 (16.7)	8 (8)	14 (16.5)	32 (13.1)
For selecting females				
Age at first egg	13 (21.7)	36 (36)	40 (47.1)	89 (36.3)
Plumage color	18 (30)	27 (27)	18 (21.2)	63 (25.7)
Brooding frequency	29 (48.3)	37 (37)	27 (31.8)	93 (38)

course, color preference depends on a wish of the farmers. This indicates that some colors preferred by some farmers might not be preferred by some others and vice-versa. About 41.6% of the farmers based on the body weight to select breeding males.

Unlike for males, brooding frequency and age at first egg were the most highly considered traits in selecting breeding female chickens (38%) for brooding frequency and (36.3%) for age at first egg. The farmers prefer the hens with low brooding frequency in order to increase number of eggs per a year via increasing the number of clutches. They also prefer the chickens that start egg laying at an earlier age. However, using the color as selection criteria for the farmers was not even across the study agro-ecologies.

Management system of local chickens

As any other livestock production, chicken breeds require better housing, feeding and veterinary services to increase production, prevent chicken from predators, harsh climatic variables and disease.

More than 94% the farmers reporting supplementary feed such as maize, wheat, sorghum, residues of human food, barley and sometimes industrial by products for their indigenous chickens. However, according to most of the respondents, the veterinary service was poor.

Even though, all the farmers accessed the house for their chickens, only 10, 19 and 14.1% of the interviewed farmers had a separate house for their chickens in lowland, midland and highland agro-ecologies, respectively.

Improving management system towards the indigenous chickens is more important than introducing the exotic ones because adopting exotic chickens might be challenging in tropics. This is in line with the report of (Wondmeneh et al., 2014; Sefa et al., 2016; Teklemariam, 2017) who stated that the main constraints that limit the adoption of exotic poultry were susceptibility to diseases, susceptibility to predators, lack of vaccination access, higher feed requirement, needs more care, expensiveness of the breeding stock and market problem in Ethiopia (Table 11).

Special attributes of indigenous chickens

The special attributes through keeping indigenous chickens were adaptation, resistance to disease, low feed requirement, test of egg and meat test. Most of the participants (81.2%) during group discussion preferred adaptation and disease resistance from keeping the local chickens. This agreed report of (Aberra 2014; Wondmeneh et al., 2014; Wondmeneh, 2015) they stated that the Ethiopian indigenous chicken flocks are said to be disease resistant and adapted to their environment.

Challenges in managing chicks

Among the interviewed farmers, 91% of them did not practice egg selection for incubating. The chicks from all eggs might not be equal for their size, survival, and growth. Selecting egg is important to have the chicks with such features.

The farmers who were selecting eggs before incubating preferred the medium and large sized eggs to get productive and more surviving chicks to sustain the existing production, and to improve next generation productivity. The current study showed lower proportion of farmers practicing egg selection compared to the study conducted by Melaku (2016) who reported that 72.33% of the respondents had practiced selection of eggs for incubation in Southern Wollo. The farmers, not selecting eggs for incubation have reasons like protecting the eggs from the spoilage due to the contact.

Chick death was another considerable challenge in

Table 11. Management of local chickens under different agro-ecologies.

Parameter	Agro-ecology			Overall (N=245) [N (%)]
	Lowland (N=60)	Midland (N=100)	Highland (N=85)	
	N (%)	N (%)	N (%)	
Provision of supplementary feed				
Yes	53(88.3)	93(93)	81(95.3)	231(94.3)
No	7(11.7)	3(3)	4(4.7)	14(5.7)
Veterinary service				
Yes	11 (18.3)	40 (40)	33 (38.8)	84 (34.3)
No	49 (81.7)	60 (60)	52 (61.2)	161 (65.7)
Separate house only for chickens				
Yes	6 (10)	19 (19)	12 (14.1)	37 (15.1)
No	54 (90)	81 (81)	73 (85.9)	208 (84.9)

Table 12. Challenges in chick management at different agro-ecologies of Ethiopia.

Parameter	Agro-ecology			Average [N (%)]
	Lowland (N=60)	Midland (N=100)	Highland (N=85)	
	N (%)	N (%)	N (%)	
Selecting egg for incubation				
Yes	4(6.7)	11(11)	7(8.2)	22(9)
No	56(93.3)	89(89)	78(91.8)	223(91)
Age of chicks' death occurrence				
1^{st} - 3^{rd} week	54(90)	93(93)	81(95.3)	228(93.1)
4^{th} - 6^{th} weeks	6(10)	7(7)	4(4.7)	17(6.9)
Main causes of death of chicks				
Disease	32(53.3)	41(41)	47(55.3)	120(49)
Predators	21(35)	36(36)	25(29.4)	82(33.5)
Birds	7(11.7)	23(23)	13(15.3)	43(17.6)

chicken production. In this survey for more than 93% of the interviewed farmers, death of chicks might be occurred between the first and third week of their age (Table 12).

Conclusion

Indigenous chickens are well adapted to the tropics, resistant to poor management, feed shortages and tolerate to locally prevalent diseases. Mid land agro-ecology is the most suitable as compared with that of the low and highland areas for most parameters. Documenting the productive and reproductive performance of local chicken at different agro-ecologies could be considered as playing the pivotal role as a base for further research. Although the village chickens produce small number of eggs, most of the farmers, especially women preferred eggs production trait through poultry production for sale rather than using for home consumption. Inbreeding coefficient of the local chickens was higher and it needs increasing the number of chickens to decrease the chance mate to each other. However, escalating number of local chickens may not be the relevant strategy to optimize profitability. So, developing breed improvement program is advisable to improve profitability through increasing egg production and other relevant traits; because this study can be the base to design breeding strategy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of ploughing and weeding frequencies on growth, yield and yield components of Teff [*Eragrostis tef* (Zucc.) Trotter] in Mirab Abaya Area, Southern Ethiopia

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Inappropriate ploughing and weed control practices are among the major limiting factors for teff productivity in Ethiopia. Hence, this study was conducted to evaluate the effect of ploughing and weeding frequencies on growth and yield of teff varieties in Mirab Abaya district of southern Ethiopia during short cropping season of 2019. The treatments as combinations of three levels of ploughing (ploughing six times, four times and once), three levels of weeding frequency (no weeding, weeding once at tillering, weeding twice at tillering and stem elongation stages of the crop) and two varieties of teff (local and improved variety Quncho) which were laid out in split-split-plot design using ploughing as a main plot factor, weeding as subplot and variety as sub-subplot treatment with three replications. Data on phenological, growth and yield, and yield related parameters were collected and analyzed using General Linear Model (GLM) procedures of SAS 9.0. Weeding twice at tillering and stem elongation stage increased yield by 20% over the un-weeding treatment. The highest grain yield (1193 kg/ha) was obtained when ploughing six times combined with weeding twice for improved teff variety (Quncho) followed by (1135 kg/ha) the combination of ploughing four times and weeding twice for the local variety. However, ploughing four times combined with weeding twice at tillering and stem elongation stage for improved teff (Quncho) variety was found to be economical practice with the highest net benefit (27,503.4 Ethiopian birr/ha) and marginal rate of return (5800%). This practice, therefore, may be recommended for higher yield and profitability of Quncho variety in the study area and other similar environments.

Key words: Teff, grain yield, biomass yield, economic analysis, harvest index, Quncho.

INTRODUCTION

Teff (*Eragrostis tef*) plays a major role in the livelihood of most smallholder farmers in Ethiopia. It is adapted to

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Table 1. Some selected physicochemical properties of the soil at the experimental site (0-20 cm).

Sand	Silt	Clay	Textural class
3.73	24.8	71.43	Clay loam
Parameters	Soil test value	Rating	Reference
pH (1:2.5 H_2O)	7.42	Moderately alkaline	Tekalign (1991)
TN %	0.2	Medium	Tekalign (1991)
OC %	1.99	Medium	Tekalign (1991)
AP (mg/kg)	11.01	High	Olsen et al. (1954)
OM (%)	3.48	Medium	Murphy (1968)
CEC (cmol (+)/kg soil)	16.5	Moderate	Landon (1991)
K (meq /100 g)	15.69	Very high	FAO (2006)

TN: Total nitrogen, OC: organic carbon, AP: available phosphorus, OM: organic matter, K: potassium, CEC: Cation exchange capacity.

diverse agro-ecological regions of Ethiopia and grows better under stress environments than do other cereals known world-wide (Hailu and Peat, 1996). The area covered by teff during the main season is about 1.91 million ha, which stands first in area coverage and accounts for 29% of the total cereal area in Ethiopia (CSA, 2010). However, its productivity is far lower than that of other major cereal crops growing in the country. Average national yield of teff in 2014/2015 production season ranged from 1281 to 1575 kg/ha (Sharma and Adera, 2016). Lower teff grain yield is mainly attributed due to inappropriate ploughing and weed control practices (Habtegebrial et al., 2007; Oicha et al., 2010). Other studies also indicated that weed is one of the key limiting factors for attaining higher teff yield (Mersie and Parker, 1983).

Tillage has been an important aspect of technological development in the evolution of agriculture, particularly in food production. Tillage systems are site specific and depend on crop, soil type and the climate (Rasmussen, 1999). Ploughing is one of the methods for soil tillage and weed control. Repeated ploughing makes the soil light and increase the available pore space for aeration in soil and root development. Due to this increase in available pore space, soil compaction is reduced and water holding capacity of soil increased (Bargali et al., 1993a, 2018, 2009) which enhanced the decomposition processes (Bargali et al., 1993b; Bargali, 1996), nutrient cycling (Bargali, 1995; Bargali et al., 1992; Bargali and Singh, 1997; Manral et al., 2020) and influences the crop productivity (Padalia et al., 2018). Some crops need very high tillage processes. Teff also needs high tillage frequencies as compared to other cereal crops in Ethiopia. It also requires firm, and level seedbed, free from clods and stumps. With respect to teff cultivation on vertisols, several ploughings are necessary, occasionally as much as 12 times, relative to nitosols (Deckers et al., 1998). According to Kenea et al. (2001) the tillage frequency for teff in Ethiopia ranges from three to twelve times. Though some research results indicated teff grain yield increased with increasing number of ploughings (IAR, 1998; Melese, 2007).

Generally, farmers practice conventional tillage to control weeds, conserve moisture, and increase soil warming (Oicha et al., 2010). Other researchers (Mulumba and Lal, 2008; Rachman et al., 2003) noted that it is essential to select a tillage practice that sustains and favors successful growth of agricultural crops in a given environmental condition. An experiment conducted at DebreZeit Research Center in Ethiopia indicated that hand weeding once at early tillering or twice at early tillering and stem elongation stage depending on the degree of weed infestation of the crop was profitable (Seyfu, 1993). There is inadequate information on the effect of ploughing frequency and weed control practices on teff growth and yield in the low land areas of Gamo Zone. Therefore, the objective of this study was to evaluate the effects of tillage and weeding frequency on growth, yield and yield components of teff varieties.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Fura kebele in Mirab Abaya woreda, Gamo Zone in Southern Ethiopia on a farmer’s field during 2019 short cropping season. The place is located in between 6°11′0″ - 6°13′0″N and 37°37′0″ - 37°45′0″E longitude and latitude, respectively and at an elevation of 1193 masl with the average annual rain fall of 610 mm and average minimum and maximum air temperatures of 19 and 34°C, respectively. The area is located at 30 km away from Arba Minch city in northern direction. Soil properties of the study site are described in Table 1.

Treatments, design and experimental procedures

The experiment consisted of three levels of tillage frequencies (one pass, four passes and six passes), three levels of weeding frequency (no weeding, one time weeding at tillering and two times weeding at tillering and stem elongation stages of the crop) and two varieties of teff (local and improved variety namely Quncho or DZ-Cr-387-RIL 355). Quncho was developed and released by Debrzeit Agricultural Research Centre in 2006, it is a high yielding variety (1.8-2.6 t/ha on-farm), early maturing (86-151 days), and white-

Table 2. Dominant weed species at tillering stage of teff on the clay-loam soil at experimental site.

Weed species	Family	Common name	Population/m^2
Cyperus rotundus L.	Cyperaceae	Purple nutsedge	109.2
Amaranthus retroflexus L.	Amaranthaceae	Redroot pigweed	60
Cynodon dactylon (L.) Pers.	Poaceae	Bermuda grass	58.4
Datura metel L.	Solanaceae	Thorn apple	42.2
Other minor weed species	-	-	168

Table 3. Weed density (plants/m^2) as affected by tillage frequency

Ploughing frequency	Weed population/m^2
One pass	9.0^a
Four passes	4.6^b
Six passes	4.3^b
LSD (0.05)	0.76
CV (%)	19

Mean values followed by the same letter are not significantly different at 5% probability level. LSD= List significant difference; CV= Coefficient of variation.

seeded cultivar adapted to a wide range of altitudes (MoA, 2010). The treatments were laid out in split-split plot design using tillage as a main plot factor, weeding as subplot factor and variety as sub-subplot factor with three replications. Those plots, which received six plowing frequencies, were plowed first at late January, 2020; second up to fifth plows were done within fifteen days interval and the sixth at sowing. The plots, which received four plowing frequencies, were plowed first, at the late February, 2020; second up to third plows were done within fifteen days interval and the fourth at sowing. The plots, which received one plowing, were plowed only at sowing.

After preparing the land as per treatments required, each main plot divided into three sub plots (1.2 m × 4 m) and finally each sub plots divided into two sub sub-plots (1.2 m × 2 m) with net plot size of 1.2 m × 2.0 m^2. A plot size of 1.2 m × 2 m (2.4 m^2) with 10 cm row spacing and a total of 10 rows were used. The net harvestable area was 1.0 m × 1.8 m and adjacent plots and blocks were spaced 0.5 and 1 m apart, respectively. All agronomic practices were conducted as per recommendations except the treatments.

Data collection

Data were collected on weed species composition and density as well on crop parameters including days to 50% panicle emergence, days to 90% physiological maturity, total tiller number/m^2, plant height (cm), number of effective tillers/plant, panicle length (cm), total dry biomass yield (kg/ha), grain yield (kg/ha), harvest index (%) and thousand seed weight (g).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using General Linear Model (GLM) procedures of SAS 9.0 (SAS, 2004). Differences among treatment means were compared using Least Significant Differences test at 5% level of probability.

Economic analysis

The economic analysis was done to determine the economic feasibility of the treatments following the procedure developed by CIMMYT (1988). Costs of tillage, weeding and teff seed were taken as variable costs during the experiment and prevailing market price of the crop was estimated at the time crop harvest as return. Seed yield was also adjusted down by 10%.

RESULTS AND DISCUSSION

Weed species composition

Weed species recorded during the study period were grasses and broad-leaved species of herbs. The dominant weeds were grasses in the experimental site (Table 2).

Effect of tillage frequency on weed density

Weed density was significantly ($P< 0.05$) influenced only by the main effect of tillage frequency (Table 3). Weed density was significantly reduced with increase in the number of tillage passes. 52 and 50% reduction in weed density was observed due to six and four tillage passes, respectively, compared to the value obtained single pass plow. Accordingly, maximum value of weed density (9 plants/m^2) was recorded due to one pass tillage. This result is in agreement with report of ICARDA (1984) which shows that repeated tillage compared to no-till reduced weed infestation by 50%.

Table 4. Days to 50% panicle emergence as affected by tillage and variety.

Treatment	Days to 50% panicle emergence
Ploughing frequency	
One pass	36.0[b]
Four passes	36.9[a]
Six passes	37.0[a]
P-value	**
CV (%)	2.4
Variety	
Local	36.8[a]
Improved (Quncho)	36.4[b]
P-value	*
CV (%)	2.6

Mean values followed by the same letter with in a column for a given treatment level are not significantly different at 5% probability level. ** and * denote significant differences at $P < 0.01$ and $P < 0.05$ probability levels, respectively.

Days to 50% panicle emergence

This parameter was significantly influenced only by the main effects of ploughing frequency and variety (Table 4). Statistically similar and longer durations of days to 50% panicle emergence were recorded due to fourth plowing and six times compared with single pass plowing. The increment in duration of panicle emergence with increase in plowing frequency might be associated with easy and efficient utilization of soil nutrients and moisture from fine seedbed which favored prolonged time for vegetative growth with delayed heading of the crop. Regarding varietal effect, local variety took statistically longer duration to (36.8 days) 50% panicle emergence compared with improved variety (Quncho). This variation might be due to genetic difference between the two varieties as reported by Seyfu (1993).

Days to maturity and growth parameters

Days to maturity, total number of tillers/m^2, number of effective tillers/plant and plant height were significantly affected by the interaction effects of plowing frequency, weeding frequency and variety (Table 5).

Days to 90% physiological maturity

The maximum number of days to maturity (69.67 days) was observed for treatment combination of ploughing six times with two times hand weeding at tillering and stem elongation stage for the local variety, while the lowest value (65 days) was recorded for ploughing once combined with un-weeded control for the improved variety. The prolonged period for maturity with increased ploughing frequency and weed free environment or minimized weed population might be due to prolonged period of vegetative growth at the expense of reproductive development as a result of probably improved nutrient and moisture supply to the plants. Varietal difference in maturity period could be attributed to differences in the genetic makeup. This result was in agreement with the findings of Haftamu et al. (2009) who reported that teff requires 90 to 130 days for growth depending on variety and altitude. In contrast, Evert et al. (2008) reported that little weed competition due to suitable agronomic management practice results in fast growth and earlier maturity of crops.

Total tiller number

The highest total tiller number (182.67) was recorded for the combination of six plows with two times hand weeding for the improved teff variety, followed by the same treatment combination for the local teff type (177.67) (Table 5), while one plow with un-weeded control resulted in lower values for both local (95.67) and improved (122.30) varieties. This might be due to more availability of soil nutrients and other environmental inputs for the crop due to repeated tillage and in the absence of weed competition. The result was in line with the findings of Aleligne (1988) who reported that fine seedbed preparation is to get weed free environment and good crop germination. Similarly, Tenaw (2010) has reported that repeated tillage created conducive environment for better plant establishment, growth and production because of reduced weed competition and possibly better root growth.

Plant height

Maximum plant height (114.3 cm) was recorded for

Table 5. Interaction effect of treatments on days to maturity, tillering and plant height.

Ploughing frequency	Weeding frequency	Variety	Days to maturity	Total tiller number/m^2	Plant height (cm)	Number of effective tillers/plant
	No weeding	Local	67.0^{defg}	95.60^{h}	114.3^{a}	2.6^{d}
One pass	Weeding once	Local	68.6^{abcd}	139.6^{def}	89.3^{f}	5.3^{abc}
	Weeding twice	Local	68.6^{abcd}	135.6^{ef}	92.0ef	6.0^{abc}
	No weeding	Local	67.0^{defg}	139.3^{def}	100.6^{bcdef}	4.6^{bcd}
Four passes	Weeding once	Local	67.6^{bcde}	148.3^{cde}	96.3^{cdef}	6.0^{abc}
	Weeding twice	Local	68.6^{abcd}	160.0^{bc}	112.0^{ab}	7.0^{a}
	No weeding	Local	69.0^{abc}	145.6^{def}	106.3^{abc}	4.3^{cd}
Six passes	Weeding once	Local	69.3^{ab}	177.6^{a}	107.0^{abc}	5.6^{abc}
	Weeding twice	Local	69.6^{a}	177.6^{a}	102.3^{abcdef}	6.0^{abc}
	No weeding	Quncho	65.3^{g}	122.3^{g}	112.3^{ab}	3.0^{d}
One pass	Weeding once	Quncho	65.6^{fg}	141.6^{def}	90.6^{ef}	6.0^{abc}
	Weeding twice	Quncho	66.0^{efg}	146.0^{def}	102.3^{abcdef}	6.3^{abc}
	No weeding	Quncho	67.0^{defg}	133.6^{fg}	106.0^{abcd}	4.3^{cd}
Four passes	Weeding once	Quncho	65.6^{fg}	150.0^{cd}	97.0^{cdef}	6.3^{abc}
	Weeding twice	Quncho	67.3^{cdef}	172.3^{ab}	92.6^{def}	7.0^{a}
	No weeding	Quncho	68.6^{abcd}	145.6^{def}	97.0^{cdef}	4.3^{cd}
Six passes	Weeding once	Quncho	68.0^{abcd}	176.6^{a}	104.0^{abcde}	6.6^{ab}
	Weeding twice	Quncho	67.0^{defg}	182.6^{a}	100.6^{bcdef}	7.3^{a}
P value			***	*	*	**
CV (%)			1.6	5.2	8.1	23.3

Means followed by same letter(s) within a column are not significantly different at P > 0.05. ***, ** and * denote significant differences at P < 0.001, P < 0.01 and P < 0.05 probability levels, respectively.

minimum tillage combined with un-weeded plot for the local variety, while the lowest value (89.3 cm) was recorded for the same variety with one plow pass combined with weeding once at tillering stage (Table 5).
This may be due to fast vertical growth of the crop to successfully compete with weeds for light and space. The result of the present study was in agreement with the findings of Bekele et al. (2018) who reported that increased plant height with the weedy plot might be due to the effect of severe competition among plants which make them elongated in search of light.

Number of fertile tillers

The highest number of effective tillers (7.3) was recorded for six plows with two times weeding for the improved variety, followed by (7) four times plowing combined with two times weeding for the local variety, whereas the lowest value (2.6) was recorded for minimum tillage (one plow pass) with un-weeded treatments for the local teff type (Table 5). Number of fertile tillers increased with increasing tillage and weeding frequency, probably because of increased nutrient and moisture supply and more light to teff plants as a result of reduced inter-specific competition between the crop and weed species. The current result was in agreement with the findings of Gebreyesus (2014) who reported that number of effective tillers significantly increased as tillage frequency increased from minimum tillage up to four times plowing.

Thousand seed weight and harvest index

Thousand seed weight was significantly influenced only by the main effect of weeding frequency whereas harvest index insignificantly responded to main and interaction effects of the treatments (Table 6). Treatments in which weeds were removed resulted in higher and statistically similar values of thousand seed weight compared with control (un-weeded treatment). This might be attributed to better availability of growth resources for the crop in weeded treatment which results in better assimilate production and translocation to sink (seed).

Yield and yield components

Panicle length, above ground biomass and grain yield were significantly influenced by the interaction effects of

Table 6. Effect of ploughing and weeding frequencies on thousand seed weight and harvest index of teff.

Treatment	Thousand seed weight	Harvest index (%)
Ploughing frequency		
One pass	0.28	19.7
Four passes	0.32	18.6
Six passes	0.31	18.6
P-value	NS	NS
CV (%)	21.2	18
Weeding frequency		
No weeding	0.26^{b}	20.0
Weeding once	0.32^{a}	18.2
Weeding twice	0.31^{a}	18.7
P-value	*	NS
CV (%)	20.5	17.7
Variety		
Local	0.29	19.5
Quncho	0.32	18.5
P-value	NS	NS
CV (%)	21.3	17.8

Means followed by same letter(s) within a column are not significantly different at P > 0.05. NS and * denote non-significant difference and significant difference at P < 0.05 probability levels, respectively.

ploughing frequency, weeding frequency and variety (Table 7).

Panicle length

The longest panicle (39.0 cm) due to four times ploughing and twice weeding in local variety whereas the shortest panicle (27.30 cm) was observed due to combined effect of one pass ploughing, un-weeded (control) treatment and local variety (Table 7). The increase in panicle length with repeated tillage and frequent weeding might be attributed to better availability of growth resources for the crop resulting from repeated tillage and weeding which is in agreement with the findings of Reicosky and Allmaras (2003) and the genetic nature of varieties.

Biomass yield

The highest biomass yield (6759.2 kg/ha) was obtained from treatment combination of six times plowing with two times hand weeding at tillering and stem elongation stage for the improved teff variety, while the lowest value was 3888.8 kg/ha followed by 3981.4 kg/ha recorded for one-time plow and un-weeded plot for the improved variety (Quncho) indicating that 74% variation in above ground biomass due to treatment interaction (Table 7). The increase in biomass yield with increasing tillage and weeding frequency could be attributed to increased availability of nutrients and soil moisture for the crop as a result of reduced competition by weeds as reported by Alemu et al. (2016). On the other hand, the lowest biomass yield (3888.9 kg/ha) was recorded from combined effect of one plow and un-weeded plots in improved variety implying the sensitivity of modern varieties to poor agronomic practices.

Grain yield

The highest grain yield (1192.53 kg/ha) was recorded for six times (conventional) plowing combined with twice hand weeding for the improved variety (Quncho) followed by the value (1135.17 kg/ha) obtained from four times plowing with weeding twice at tillering and stem elongation stage for the local variety, which produced the lowest grain yield (775.93 kg/ha) when un-weeded and grown with one plow pass (Table 7). The increase in grain yield with frequent tillage and weeding might be due to enhanced uptake of essential nutrients by teff plants as a result of reduced competition of weeds as reported by Alemayehu et al. (2008). Variation in grain yield (54%) was recorded due to interaction effects of ploughing frequency, weeding frequency and varieties. The current result also evidenced by the findings of Gebreyesus (2014) and Alemayehu et al. (2008) who reported higher grain yield under frequent ploughing and weeding, respectively. Grain yield showed highly significant and positive linear relationship with total tiller number (r= 0.72**), effective number of tillers (r= 0.59**), panicle

Table 7. Interaction effects of treatments on yield and yield components of teff.

Ploughing frequency	Weeding frequency	Variety	Panicle length (cm)	Above ground biomass (kg/ha)	Grain yield (kg/ha)
One pass	No weeding	Local	27.3^{f}	3981.4d^{e}	775.93^{i}
	Weeding once	Local	32.3^{def}	5462.9^{abcde}	964.77^{fg}
	Weeding twice	Local	33.6^{bcd}	5000.0^{bcde}	1087.00^{bcd}
Four passes	No weeding	Local	32.3^{def}	4351.8^{cde}	981.43^{efg}
	Weeding once	Local	34.3^{abcd}	5926.0^{abc}	986.97^{defg}
	Weeding twice	Local	39.0^{a}	6388.8^{ab}	1135.17^{ab}
Six passes	No weeding	Local	34.3^{abcd}	5185.2^{abcde}	1012.63^{def}
	Weeding once	Local	38.6^{ab}	6203.7^{ab}	1079.60^{bcde}
	Weeding twice	Local	36.0^{abcd}	6388.9^{ab}	1118.50^{abc}
One pass	No weeding	Quncho	27.6^{ef}	3888.9^{e}	799.97^{hi}
	Weeding once	Quncho	32.6^{de}	4074.0^{de}	811.13^{hi}
	Weeding twice	Quncho	36.3^{abcd}	5740.7^{abc}	1016.67^{def}
Four passes	No weeding	Quncho	33.3^{cd}	5555.6^{abcd}	890.70^{gh}
	Weeding once	Quncho	36.6^{abcd}	5370.4^{abcde}	1024.00cd^{ef}
	Weeding twice	Quncho	37.0^{abcd}	6203.6^{ab}	1135.17^{ab}
Six passes	No weeding	Quncho	36.6^{abcd}	4999.9^{bcde}	974.00^{fg}
	Weeding once	Quncho	38.0^{abc}	6296.3^{ab}	1062.93^{bcdef}
	Weeding twice	Quncho	35.0^{abcd}	6759.2^{a}	1192.53^{a}
P-value			**	**	***
CV (%)			9.1	17.7	6.0

Values within a column followed by the same letter are not significantly different at P > 0.05. *** and ** denote significant differences at P < 0.001 and P < 0.01 probability levels, respectively.

Table 8. Economic feasibility analysis of interaction of tillage and weeding frequency for teff production in 2019 cropping season.

Treatment	GY (kg/ha)	AGY (kg/ha)	GB (Birr/ha)	TVC (Birr/ha)	NB (Birr/ha)	MRR (%)
T1W0V1	775.93	698.3	21647.3	1100	20547.3	-
T1W0V2	799.97	720.0	23040	1175	21865	1757
T1W1V1	964.77	868.3	26917.3	1900	25017.3	435
T1W2V1	1087	978.3	30327.3	2700	27627.3	861
T4W1V1	986.97	888.3	27537.3	4300	23237.3	161
T4W1V2	1024.03	921.6	29491.2	4375	25116.2	2505
T4W2V1	1135.17	1021.2	31656.27	5100	26557.2	199
T4W2V2	1135.17	1021.2	32678.4	5175	27503.4	5800
T6W1V1	1079.6	971.6	30119.6	5900	24219.6	185
T6W1V2	1062.93	956.6	30611.2	5975	24636.2	555
T6W2V2	1192.53	1073.5	34352	6775	27577	4092

GY: Grain yield, AGY: adjusted grain yield, GB: gross benefit, TVC: total cost, NB: net benefit, MRR: marginal rate of return. Whereas T1, T4 and T6 were tillage frequencies of one plow, four plows and six plows, respectively; V1 and V2 were local and Quncho teff varieties, respectively; W0, W1 and W2 were weeding frequencies of un-weeding (as control), weeding once and weeding twice, respectively.

length (r= 0.57**), thousand seed weight (r= 0.36**), days to physiological maturity (r= 0.35**) and above ground biomass (r= 0.66**) implying that improvement in these parameters is also associated with better grain yield.

Economic analysis

The results of economic analysis indicated that the highest net benefit (Table 8) was obtained from the treatment of one plow pass combined with twice hand weeding at tillering and stem elongation stage on local variety due to its lower cost of production (about 48% lower) but its marginal rate of return (MRR) of 861% was lower than that of four-time plows combined with twice weeding for variety Quncho (5800%). According to the current result, plowing the soil more than once is important for teff production and productivity.

Conclusion

Four times tillage passes showed better yield and yield components as compared to the others. Similarly, twice hand weeding showed significantly higher yield of teff. improved variety (Quncho) resulted in a better yield compared to the local variety for most treatment combinations. In general, four times oxen plowing combined with twice hand weeding (at tillering and stem elongation stage) gave the highest MRR (5800%) and NB (ETB 27503.4 ha^{-1}) for variety Quncho as compared to other treatment combinations. Therefore, four ploughing, twice hand weeding (at tillering and stem elongation) and Quncho variety should be recommended for higher productivity and profitability of teff in Mirab Abaya, Southern Ethiopia.

Hence, this practice was found to be the best option that could be recommended for teff production by using improved variety (Quncho) in the study area and in areas with similar agro-ecology. However, it is advisable to undertake further research across different soil types, seasons and locations to draw a more comprehensive recommendation.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of impurity on the efficiency of a legume threshing machine

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A motorized legume thresher was redesigned to utilize coefficient of friction to separate grains from impurities, the thresher performance was evaluated and best combination of parameters for highest separation efficiency determined. The study was carried out using a randomized design of three batch weights (BW): 100, 150 and 200 g, four surfaces (S) mild steel, plywood, rubber carpet and rug at an angle of inclination of 25° and a height fall of 90 mm, two impurity levels (I) using cowpea (*Vigna unguiculata*) IT84^{S}-2242 and soybean (*Glycine max* L) 1448-2E variety. Sorghum seeds at 10% of each batch weight were mixed in with each test samples as additional impurity. Data obtained was analyzed using SAS and Duncan tests. Results showed, cleaning efficiency varied between 90 and 63.28% for soybean samples, threshing efficiency varied between 87 and 97% with an average minimal damage of 0.78% from using carpet surface. For cowpea threshing and cleaning efficiencies were 97.44 and 97.16%, respectively with average loss 2.60%. Analysis of variance (ANOVA) showed that impurity level and feed rate affected cleaning efficiency at both 1 and 5% significance, type of surface affected threshing percentage and broken seeds at 5% significance. The best combination of batch weight, surface and impurity level to obtain cleaning efficiency, threshing efficiency, low grain damage and grain losses was 100 g batch weight using carpet surface. In conclusion, coefficient of friction could be utilized to increase separation efficiency of thresher, this would aid the development of appropriate technologies for legumes processing.

Key words: Threshing, threshing efficiency (TE), cleaning efficiency (CE), coefficient of friction, percentage impurity.

INTRODUCTION

Agricultural mechanization involves using agricultural machinery to perform farm operations speedily and efficiently. It plays a significant economic role by reducing cost of cultivation, increasing agriculture production and in increasing productivity as well as overall returns to the farmers (Kamboj et al., 2012). Mechanization is critical to meet the increased food demand from global human population explosion (Irtwange, 2009).

Cowpea and soybean are legumes commercially cultivated in more than 35 countries of the world, soybean is the world's leading vegetable oil source and accounts for about 20 to 24% of plant based oils in the

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world (Polat et al., 2006). Soybean is also an important food source in human and animal nutrition with total worldwide production of about 180 million tons per year (Manuwa, 2011). The importance of cowpea lies in its food value as a major source of protein of high biological value, energy, vitamins and roughage, Nigeria cultivates about 40 million hectares of cowpea and produces an estimated 850,000 tons annually (Faleye et al., 2013; Olaoye, 2011).

The post-harvest processing of these legumes into animal and human food poses enormous challenges, because poor harvesting and post-harvest handling methods often leads to the introduction of contaminants such as stones, sticks, chaff and dust which necessitates cleaning into the grains (Ajit et al., 2006). Threshing considered as one of the foremost important post-harvest operation in crop processing (Dhananchezhiyan et al., 2013), is the removal of grains from the plant residues and results in the separation of impurities and contaminants from sound grains, thus reducing the problems that occur during storage and handling (Ajit et al., 2006).

Grain cleaning is the separation and removal of chaff and other debris such as foreign materials, broken kernels and splits from grains (Wang et al., 1994), by using the aerodynamic properties of crops such as terminal velocity and drag coefficient so that desirable products are separated from unwanted materials (Vasundhara et al., 2019). Air is introduced into the stream of crops and foreign material at a velocity lesser than the terminal velocity of crops but greater than the terminal velocity of unwanted materials to achieve separation due to distinct differences between the velocities of individual components in the mixture (Panasiewicz et al., 2012). This separation is conventionally carried out using screen or pneumatic separators, this results in the separation of those contaminants from sound grains and reduces the problems that occur during storage and handling and is (Wang et al., 1994). Some of the factors that influence the precision of segregation of the components in a mixture are evenness of feeding the input material, initial velocity of the input material, velocity and turbulence of the stream of air, and the time the input mixture stays in the stream of air (Panasiewicz et al., 2012). Mechanization of cleaning operation saves effort, reduces crop losses and reduces operation time. A good cleaning machine should therefore remove all chaff straws and plant debris with very little grain loss (Muhammad et al., 2013).

Processing factors affect separation, for instance El-Khateeb et al. (2008) showed that the combination of airstream velocity and specific feed rate significantly affected cleaning efficiency of sunflower heads, while increasing feed rate from 2.5 to 10 kg/min caused 1.95% decrease in cleaning efficiency (Hemmat et al., 2007). Also, machine factors affects separation efficiency, an increase in threshing drum speed in sunflower processing from 300 to 600 rpm, increased the cleaning efficiency by 4.82 and 6.51% at 2.5 and 10 kg/min feed rates (Chimchana et al., 2008). This is because high drum speed increased the velocity of cleaning air which resulted in higher capability of air to carry the foreign material and residual of sunflower heads from seeds consequently increased cleaning efficiency (Chimchana et al., 2008). Awady and Sayed (1994) reported mean values of peanut grains terminal velocity were 4.3, 6.5, 6.8, and 7.2 m/s for shells, unshelled, split and intact seeds, respectively, so that 7.5 m/s air speed separated 96% of shells with losses of 3% of unshelled seeds. Aderinlewo et al. (2011) reported that at 8.2 to 18.2% moisture content, the terminal velocity for four cowpea varieties was 13.72-14.04 m/s (Ife brown), 14.14-14.47 m/s (Drum), 13.80-14.30 m/s (Ife 98-12) and 13.35-14.30 m/s (IT90K-277-2) and reported higher terminal velocity as cowpea seed mass increases. Owolarafe et al. (2007) applied static COF to separate cocoa husks and beans mixture, by sliding test samples on an inclined plane covered with rubber or carpet material. The separation efficiency at angle of inclination 35° was 50 to 86%, while separation efficiency at 25° was 80 to 99%, at separation efficiency at angle of inclination 20° was 74 to 97%. This shows that increasing the angle of inclination reduces the separation efficiency, because increased angles encourages quick bulk flow of materials, while small angle of inclinations causes materials to slide down the incline slowly. Thus differences in the static COF of cocoa seeds and its husks could be used to separate them (Owolarafe et al., 2007). Ibrahim (2007) reported COF of cowpea grains on four surfaces as rubber (0.37 - 0.40), aluminium (0.32 - 0.35), stainless steel (0.20 - 0.24) and galvanized iron (0.17 - 0.21), while Polat et al. (2006) stated the static COF of soybean on three surfaces as plywood (0.22 - 0.35), glass (0.19 - 0.33), and galvanized steel (0.21 - 0.34).

This study redesigned a motorized thresher to incorporate different separation surfaces, evaluation tests were carried out to determine the performance of the modified legume thresher considering feed rate and level of impurity, so that the best combination of parameters for achieving optimal separation efficiency could be determined.

Machine description

The original threshing machine was designed to process cowpea only and is described in Ige (1978); it used a square cross-section threshing drum that gave it extra air separation effect. The thresher centrifugal fan is straight bladed and is capable of operating satisfactorily in an environment containing dust particles such as that which occurs during threshing (Ige, 1978). The reported threshing efficiency of the machine varied between 81 and 99% for the different cowpea varieties considered. Grain damage of 1 to 8% was recorded and chaff content between 11.5 and 18.3% were obtained (Ige, 1978). The thresher was redesigned to modify the cowpea thresher into a multi-crop thresher for legumes, this became necessary because soybean contains a higher amount of foreign materials and impurities as it is harvested with most of its stem and stalks intact. The modification were done to increase the cleaning efficiency of the machine at higher feed rates, ensure the machine efficiency is maintained for poorly harvested samples containing stones and to minimize cleaning loss when threshing soybean samples. This present study exploited the difference in COF of legume grains and chaff by introducing a slanted collection trough and introduced another centrifugal fan at the base of the collection trough.

MATERIALS AND METHODS

Plate 1 shows the modified threshing machine in operation. The AUTOCAD 3D isometric and orthographic drawings of the modified thresher are as shown in Figures 1 and 2, respectively. This inclined trough was attached beneath the threshing chamber and collected threshed materials which it discharged into a suitably attached blower. The trough was shaped as chute; its base rectangular dimension was 25 × 75 cm and was made of a 1.5 mm thick mild steel inclined at an angle 25° from the vertical. Four different materials mild steel (MSTEEL), plywood (PLYWD), rug (RUG) and carpet (CARPT) were utilized in the study. It was assumed that friction between chaff and the rug or carpet surface will reduce the velocity of chaff compared to grains, also plywood or mild steel should impart a higher rebound energy to grains as they land on the hard surface propelling them more quickly into the centrifugal fan. The trough base was then covered with the strips of each material. Combining an additional centrifugal fan and COF of crops should result in higher separate efficiencies; this assumption was verified by mixing in 10, 15 and 20% weight of sorghum seeds for each feed-rate into each sample experimental run. Threshed materials from each experimental run were collected in a well-labeled transparent polyethylene bag for analysis. The non-grain materials such as stones, stalks, sticks and chaff are referred to as materials-other than grain (MOG). Three replications were carried out for each treatment level combination for soybean and two replications for cowpea samples.

Performance evaluation of modified threshing machine

The moisture content (wet basis) of the soybean grain from oven drying method was 7.63 and the cowpea moisture content was 11.43%. Using the digital anemometer, air velocity of the blower was determined between 7.5 and 10.0 m/s at blower exit and 1.0 and 2.0 m/s at blower inlet. The COF of the cowpea variety on the four surfaces are: plywood (0.22-0.35), (0.37-0.40) on the rubber carpet made from mixture of polystreylene and polypropylene (CARPT) COF, rug (RUG) COF 0.33-0.35, and the unlined trough of mild steel material (MSTEEL) COF 0.17-0.28) were considered. Sorghum seeds were mixed with each test samples for threshing, at 10% weight of each batch (Aderinlewo et al., 2011). A digital air manometer (*AM-4812*) was used to record the air velocity produced by the blower. Also, a digital Tachometer (*DT-2236B*) was used to determine shaft speed. Preliminary tests resulted in carrying out performance evaluation with a reduced speed of between 1350 and 1550 rpm for the blower shaft and 800 to 900 rpm for the threshing shaft to avoid excessive grain damage. The evaluation was carried out in two phases using a completely randomized design. In the first phase, three batch weights 100, 150 and 200 g (corresponding to 10, 15 and 20 g/s feed rate, respectively) of two legumes cowpea (*Vigna unguiculata*) and soybean (*Glycine max* L) were used, to avoid chocking of the threshing drum. The batches were fed over a 10 s period giving 10, 15 and 20 g/s feed rate, respectively of the crop samples. The seeds were collected from Oyo State Agricultural Development Programme (OYSADEP) Iwo, Osun State and a private farm in Saki, Oyo State all in South West Nigeria. The following are the evaluated machine performance parameters.

Threshing efficiency (TE)

This is the ratio of the mass of threshed grains to the mass of the whole panicle, and is expressed in percentage as:

$$TE = \frac{M_t}{M_u + M_t} \times 100\% \quad (1)$$

where TE is the threshing efficiency (%), M_t is the mass of threshed grains (g) and M_u is the mass of unthreshed grains (g) (Gbabo et al., 2013).

Cleaning efficiency (CE)

This is the ratio of the mass of separated impurities to the total mass of impurities in a crop sample, cleaning efficiency is the effectiveness of threshing machine in separating chaff from grain kernels. According to Oduma et al. (2014) as:

$$CE = \frac{W_t - W_c}{W_t} \times 100\% \quad (2)$$

where W_t is the total weight of mixture of grain and chaff received at grain outlet, and W_c is the weight of chaff at chaff outlet of the thresher (g).

Percentage loss (PL)

It is the ratio of quantity of seed loss to the total quantity of the millet panicle expressed as a percentage and is given by Gbabo et al. (2013) as:

$$PL = \frac{M_l}{M_r + M_l} \times 100\% \quad (3)$$

where PL is the percentage loss (%), M_r is the mass of recovered seed (g) and M_l is the mass of seed losses (unthreshed loss + separation loss scattering + blower loss) (g).

Grain throughput capacity (T)

This is the capacity of the thresher in terms of the total quantity of threshed materials in sample per unit time. Grain throughput capacity was calculated as done by Amadu (2012) as:

Plate 1. Modified threshing machine.

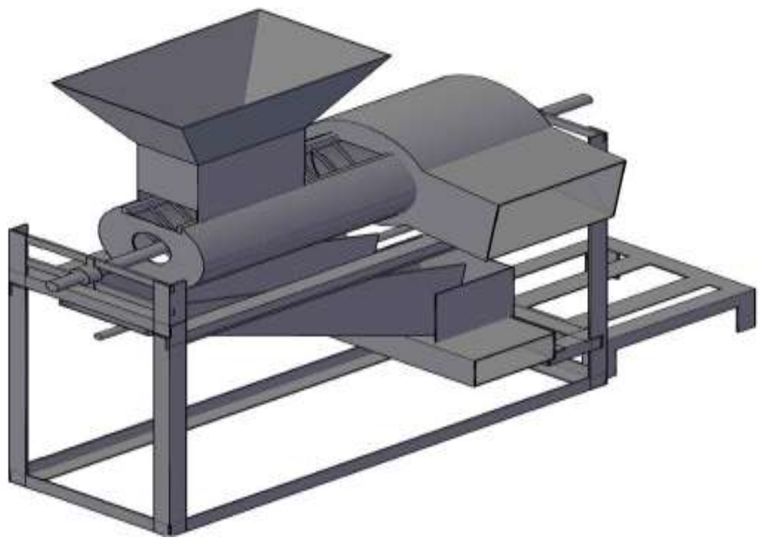

Figure 1. Isometric drawing of the modified legume threshing machine.

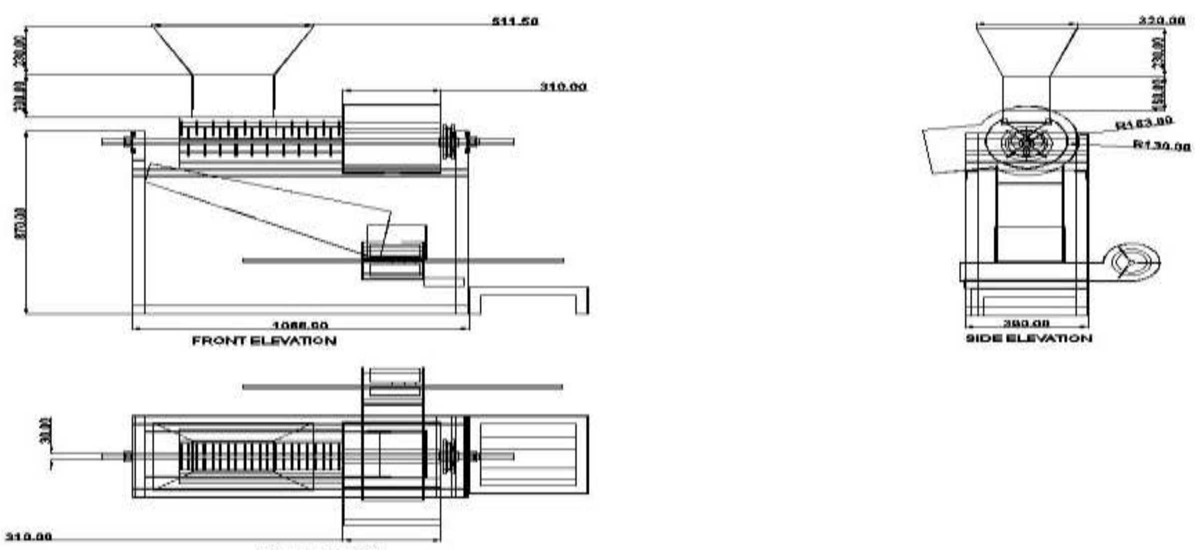

Figure 2. Orthographic projection of the modified legume threshing machine.

$$T = \frac{Q}{t} \times 100\% \quad (4)$$

where Q is the quantity of threshed grain collected after a threshing operation (kg) and t is the time taken for a complete threshing operation (h).

RESULTS AND DISCUSSION

Threshing efficiency of the machine for cowpea samples

Threshing efficiency obtained for cowpea samples on various surfaces is as shown in Figure 3. Maximum TE values for MSTEEL, CARPT, RUG and PLYWD surfaces are: 92.62% at FR2 (150 g/batch), 97.44% at FR1 (100 g/batch), 95.33% at FR1 (100 g/batch) and 95.43% at FR1 (100 g/batch), respectively, this shows that the highest TE values are obtained at FR1 (100 g/batch) for all surfaces except MSTEEL. Furthermore, TE reduces with increase in feed rate. Also, irrespective of the feed-rates, all surfaces except MSTEEL had TE values of at least 95%. In comparison, Oriaku et al. (2014) reported 77.0 to 80.0% TE for maize as sample weight decreased from 8 to 2 kg (Olaoye, 2011) reported TE between 80.0 and 98.0% as threshing speed increased from 50 to 125 rpm (Olaoye, 2011). Oduma et al. (2014) reported that TE reduced from 100.0 to 98.0% as feed-rate increased from 1 to 4 kg/min. Thus the TE values reported for the modified thresher is comparable to those reported in literature. Osueke (2011) explains the increase in threshing loss with increase in feed rate by observing that:

Threshing efficiency = 1 – losses (5a)

Losses = 1 – Threshing efficiency (5b)

This equation implies that here is a biphasic nature between threshing efficiency and threshing loss; as feed-rate is increased, threshing efficiency reduces and losses increase. This is because when grains are fed into threshing machine at high feed rate, threshing energy applied to each grain is reduced; these cushions grains from the threshing impact, the unthreshed grains are added to impurity which reduces TE in Equation 1.

Cleaning efficiency of the machine for soybean samples

The cleaning efficiency of the threshing machine for soybean with various weight percentage of Sorghum crop added for the four surfaces is as shown in Figure 4. It shows that CE reduced for all the surfaces as feed-rate was increased from FR1 (100 g soybean + 10 g sorghum) to FR3 (200 g soybean + 20 g sorghum). Some values of CE obtained are 90% (RUG), 87% (PLYWD), 84% (CARPT) and 82% (MSTEEL) and were all obtained at FR1 (100 g soybean + 10 g sorghum). It can be inferred from Table 1 that mixing sorghum seeds with samples to increase sample impurity caused CE to reduce by 9 to 13% for PLYWD surface, while CE values were between 95.94% (FR1) and 94.44 (FR3) without sorghum addition. Furthermore, CE decreased for all surfaces as feed-rate was increased which is in line with the results of an earlier study by Gbabo et al. (2013), where increased material flow, feed rate and seed moisture content caused reduction in CE. The obtained values are lower than those reported by Rouzegar et al. (2013) 98.99 to 99.44%, but comparable to 82, 85 and 72% for sorghum, soybean and millet reported by Muhammad et al. (2013). Oriaku et al. (2014) reported CE values for maize as being higher than 68.1%, while Gbabo et al. (2013) reported between 56.3 and 62.7% CE for millet. CE decreased with increase in feed rate at the rate of 2.712, 1.116, 1.11 and 0.78 kg/s for rug, plywood, mild steel and carpet surfaces, respectively. Gbabo et al. (2013), explained that the reduction in CE with increasing feed rate is because at increased feed rates, constant high stripping and impacting forces applied to seed materials increase threshed material output and impurity which often times leads to clogging and reduced cleaning efficiency. Also, according to Simonyan and Yiljep (2008), the reduction of CE with feed rate may be due to increasing load intensity on the blower, the authors stated that multiple particles act as obstructions to airflow, increase number of particles and cause turbulence all of which reduces CE.

Cleaning efficiency of the machine for cowpea samples

The cleaning efficiency of cowpea mixed with sorghum grains for various is presented in Figure 5. It shows that as feed-rate was increased from FR1 (100 g soybean + 10 g sorghum) to FR3 (200 g soybean + 20 g sorghum), the CE reduced for PLYWD and MSTEEL surfaces. Some of the values obtained for cleaning efficiencies are 88.2 (MSTEEL), 85.6 (CARPT), 87.1 (RUG) and 87.3% (PLYWD), respectively. At FR3 (200 g cowpea + 20 g sorghum), the corresponding CE reduced to 76.8 (MSTEEL), 85.1 (CARPT), 86.9 (RUG) and 81.1% (PLYWD), respectively. The graph lines for (CARPT) and (RUG) showed little variation as f_ed-rate was increased, this suggests that CE from the surfaces were independent of feed rate. Furthermore, the observed clustering at FR1 suggests that the utilization of COF using the inclined trough as an intermediate stage had more effect than the differences in separation ability of the various surfaces. Cleaning efficiency is in line with CE value of 83.55 to 100%, reported by Ilori et al. (2013) and CE 92.96 to 97.65% reported for pigeon pea (Oduma et al., 2014). Also, the results are lesser than with the CE of

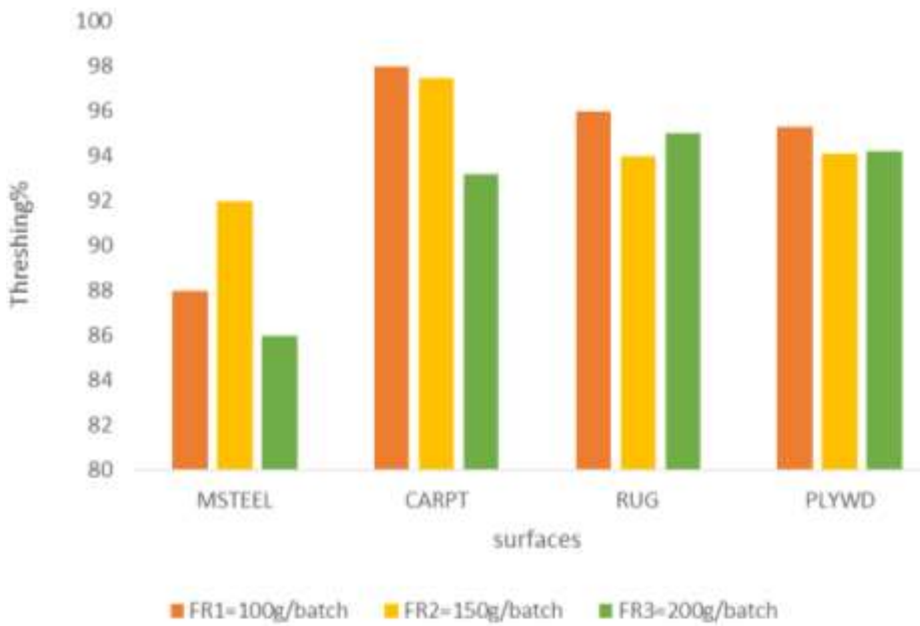

Figure 3. Threshing efficiency of cowpea with additional impurity for various surfaces.

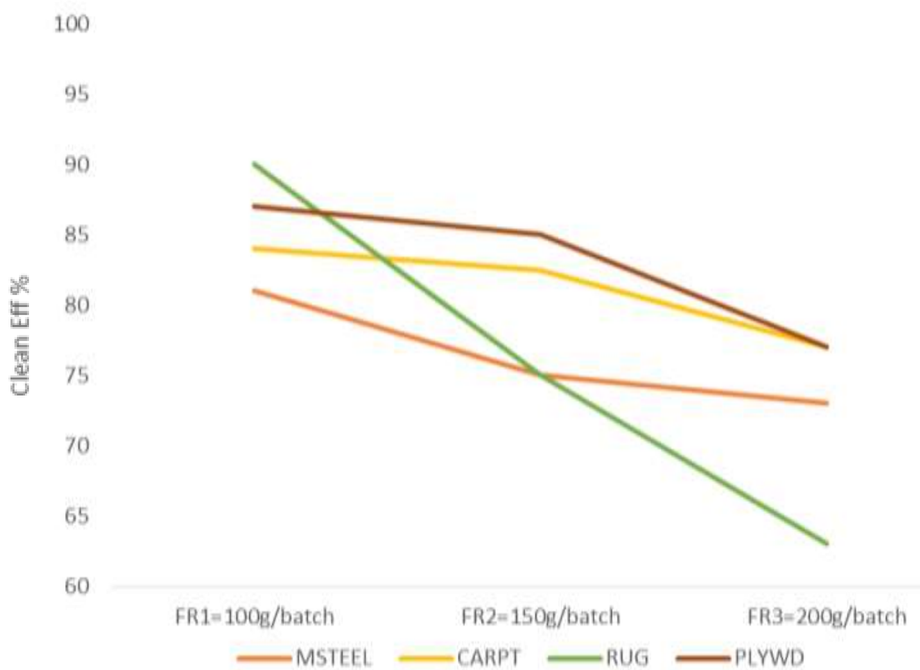

Figure 4. Cleaning efficiency of soybean for various surfaces.

97.44 and 98.18% reported by Ahmad et al. (2013). The CE decreased with increase in feed rate at the rate of 1.141, 0.613, 0.052 and 0.018 kg/s for MSTEEL, PLYWD, CARPT and RUG surfaces, respectively. This showed that MSTEEL has the highest response to change in feed rate, followed by PLYWD and CARPT and that RUG has the least decrease in rates. Regression modelling for cowpea grains showing the correlation between the different surfaces and feed-rate is:

$$CE\ (MS) = 0.585FR + 92.0$$
$$R^2 = 0.92 \quad (6a)$$

Table 1. Machine performance results for soybean using plywood surface.

Feed rate (g/s)	Seed weight (g)	Unthreshed grain weight (g)	Cleaning efficiency (CEa, %)	Cleaning efficiency (CEb, %)	Threshing efficiency (Tea, %)	Threshing efficiency (TEb, %)
10	59.59	3.33	95.94	87.26	94.4	93.43
15	78.47	4.30	94.54	84.66	94.52	94.44
20	114.63	6.45	94.44	81.13	94.37	95.43

*Each value represents mean of three replicates. CEa% = CE of samples without sorghum impurity, CEb%= CE of samples with 10% sorghum impurity, TE a% = TE of samples without sorghum impurity, and TE b% = TE of samples with 10% sorghum impurity.

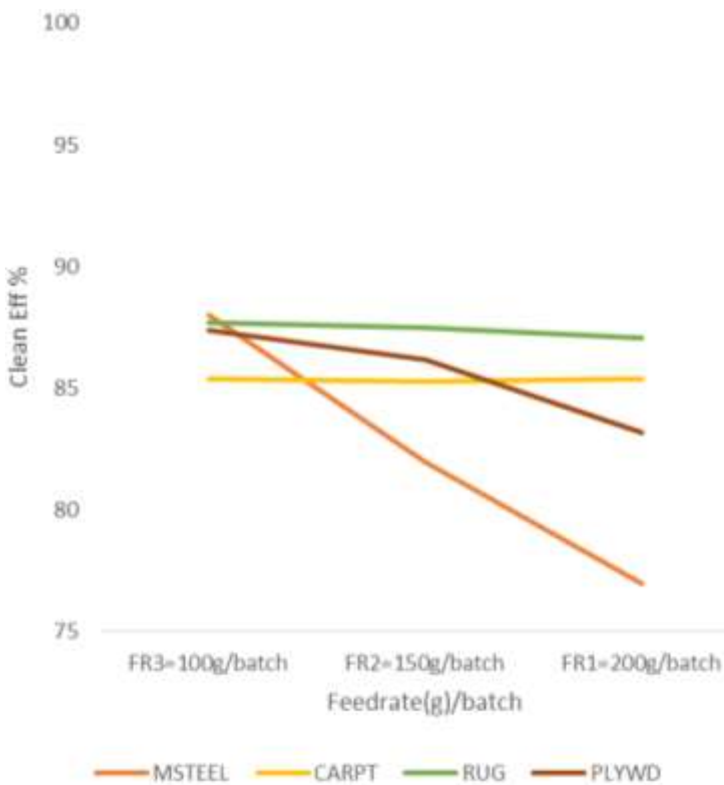

Figure 5. Cleaning efficiency of cowpea with additional impurity for various surfaces.

CE (RUG) =0.594*FR* + 87.1
R^2 = 0.93 (6b)

C.E. (CARPT) =0.594*FR* + 87.1
R^2= 0.93 (6c)

C.E. (PLYWD) = 0.589*FR* + 89.9
R^2= 0.93 (6d)

This could be compared with 2.86% damage reported by Oforka (2004), at moisture content of 10%. Simonyan and Yiljep (2008) obtained cleaning loss values of 9.73 to 54% for sorghum at 6 to 12 sieve oscillations per second, while Osueke (2013) obtained grain loss values of 5 to 35% at 0.1 to 0.25 kg/s for a cereal thresher (Figure 6).

Considering a ceiling value of 3% separation losses as per ASABE Standards (1997), it is evident that at FR1, all surfaces had acceptable losses except RUG. At FR2 only CARPT and PLYWD surfaces had minimal losses. Also CARPT surface only had excessive losses except at FR3 where the losses were minimal. At FR2, MSTEEL loss was 7.7% and RUG 9.99%, also at FR3, mild steel loss was 9.57 and carpet surface loss was 14.9% (Chimchana et al., 2008). The percentage reduction in broken grains is due to the fact that increased crop material from samples with added impurity gave a cushion to the crops,

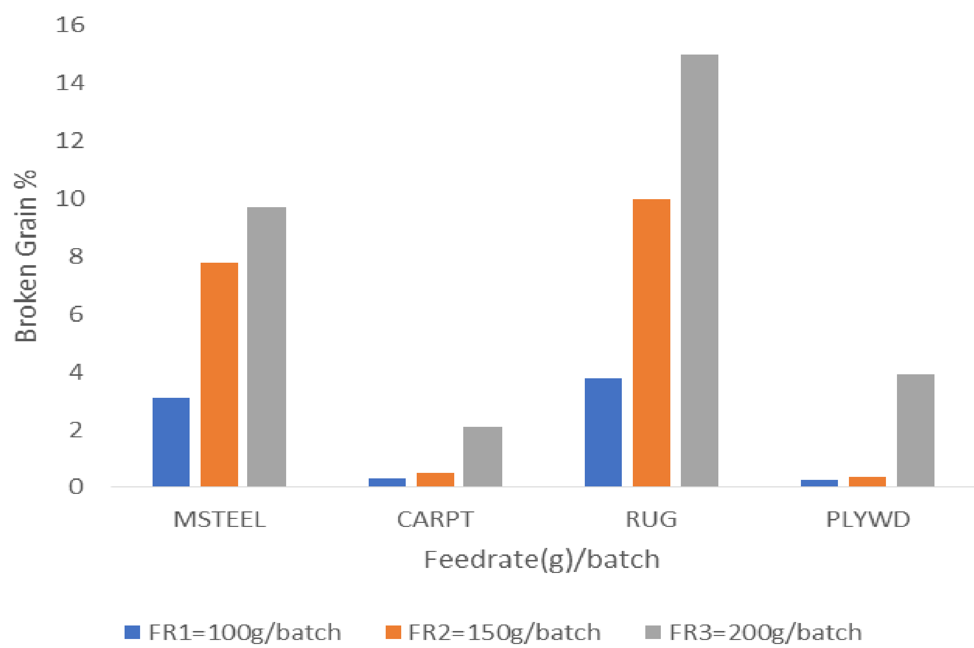

Figure 6. Percentage of broken soybean with additional impurity for various surface.

Table 2. Grain output (g) of soybean (SOY) and cowpea (COW) for various surfaces.

Crop type	SOY^a	SOY^b	COW_a	COW^b
MS	191.40	220.16	420.58	456.20
CAR	241.34	252.48	465.56	480.69
RUG	168.96	191.40	444.45	493.60
PLY	169.23	218.01	480.02	507.96

thus minimizing the amount of energy impacted on the threshed material.

Machine grain output

Table 2 shows grain output from which it is found that yields for cowpea were higher than the yield for soybean, this is because the percentage of grains in the cowpea panicle samples was approximately 54% which was higher than 24% for soybean. The total amount of cowpea ranged between 420.58 g for mild steel at FR1 and 480.02 g for plywood, at FR2 the lowest output was 456.20 g occurred for mild steel and the output was 507.96 g for plywood. Samples with impurity had higher amount of yield compared to samples without added impurity.

Statistical analysis and ANOVA results of the experiment

The analysis of variance (ANOVA) of the main effects for soybean is as shown in Table 3. Test results showed that the effect of impurity (I) and batch weight (BW) were the most significant at 1 and 5% for CE and collected seed weight. Among the first order interactions, surface and batch weight, impurity and batch weight showed high significance at 1 and 5% on broken weight and seed weight, respectively. Test results showed that only the main effects were significant. CE and seed weight impurity (I) was highly significant; surface was highly significant for TE and unthreshed weight while it is observable that batch weight (BW) was highly significant for collected seed weight and unthreshed weight.

Conclusions

Based on the performance evaluation of the modified thresher, the following conclusions were drawn from the results obtained earlier; the separation surfaces showedability to maintain high cleaning efficiencies if the impurity consists of light weight chaff materials, but some moderate weight materials such as sticks, added sorghum and small stones remained in the collected crop

Table 3. ANOVA results of the threshing unit performances for cowpea.

Source of Variation	d.f.	F-Value				
		CE	UNTSHWT	SEDWT	THRSPER	SOGHPER
Replication	1					
Impurity (I)	1	82.32**	1.35^{ns}	37.73**	0.00 ns	748.20**
Surface (S)	3	1.78^{ns}	11.89**	2.61^{ns}	17.60**	1.76^{ns}
Batch weight (BW)	2	3.27*	10.63**	188.90**	0.23^{ns}	0.21^{ns}
I × S	3	0.70^{ns}	0.90^{ns}	0.69^{ns}	0.95^{ns}	1.76^{ns}
I × BW	2	0.62^{ns}	1.06^{ns}	0.25^{ns}	1.08^{ns}	0.21^{ns}
S × BW	6	0.53^{ns}	0.80^{ns}	4.97 **	0.30^{ns}	0.23^{ns}
I × S × BW	6	0.27^{ns}	1.70^{ns}	1.77^{ns}	1.68^{ns}	0.23^{ns}

**Highly significant at 1% level;*significant at 5% level; ns, non-significant; df, degrees of freedom. CE= cleaning efficiency; UNTSHWT= unthreshed seed weight; SEDWT= seed weight; THRSPER= threshed seed percentage; SOGHPER= sorghum percentage.

samples. The sorghum samples in the collected crops increased as added sorghum increased from 10 to 20 g. Threshing efficiency of cowpea increases with increase in feed rate, attains a maximum of about 97% at FR2 before reducing at further increase of feed rate. Also, cleaning efficiency reduced significantly at 1 and 5% with increase in feed rate for all surfaces, from about 97 to 63.28% by the introduction of MOG; the effect of additional impurity clearly outweighed the increase in feed rate. Based on the foregoing, the following recommendations can be considered in future works.

(i) To separate impurities with similar weights as the grains, alternative methods of separation such as the use of screens can be considered.
(ii) Other factors such as blower speed, moisture content and different crop varieties can be considered in further performance evaluations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of genotypes and sowing time on phenology and yield performance traits of tef [Eragrostis tef (Zucc.) Trotter] in low moisture stress environments

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Tef *(Eragrostis tef (*Zucc.) Trotter) is among the most important cereals in Ethiopia in terms of both acreage and production. However, its productivity is relatively low as compared to other cereals mainly due to drought and climate variability coupled with lack of drought tolerant varieties. A field experiment was conducted with the objectives of assessing the effects of genotypes and sowing dates on the growth and yield performances of tef. The experiment consisted of 36 entire factorial combinations of three sowing dates, and 12 tef genotypes including two standards checks with three replications laid out in split plot design of sowing dates as the main plots and genotypes as sub-plots were planted at Melkassa and Alme Tena during 2017/2018 main season. The combined analysis of variance over locations revealed highly significant (P≤0.01) variations for both sowing dates, and genotypes for most of the traits evaluated. The dates that ranged from July 15 to July 20 would be recommendable as appropriate time for sowing and the genotype Dtt2XDtt13 (RIL No.37) mean grain yield of 1345 kg ha^{-1} over locations out-performed the rest of the genotypes including the standard checks. Hence, this genotype can be used for further testing. Since this is a mono-season experiment, further studies over multiple seasons and locations are required to make comprehensive conclusions and recommendations.

Key words: Genotypes, grain yield, growth parameters, phenology, sowing dates, tef.

INTRODUCTION

Tef [*Eragrostis tef* (Zucc.) Trotter] is an ancient crop in Ethiopia, and the country is considered to be center of both origin and diversity for the species (Vavilov, 1951). Tef belongs to the grass family Poaceae. It is a C4; self-pollinated chasmogamous annual cereal (Ketema, 1993). It is an allotetraploid species with a chromosome number

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of 2n = 4X = 40 (Tavassoli, 1986).

Tef plays an essential role in the Ethiopian agriculture in general and the food crop production system in particular. Nearly seven million farmers grow the crop that occupies 22% of the total cultivated area (CSA, 2018). With annual acreage of 3.02 million ha from which 1 about 5.283 million tons are harvested, tef accounts for about 30% of the total acreage and 20% of the gross grain production of all cereals cultivated in Ethiopia (CSA, 2018). It is second only to maize in terms of production. Being produced by about 6.77 million Ethiopian smallholder farmers that constitute over 43% of all the country's farmers' households (CSA, 2018), tef mainly serves as the major staple cereal for over 70% of the estimated 110 million Ethiopian population. In spite of its supreme agricultural sand economic significance, the productivity of tef is very low compared to other cereals mainly due to lack of adequate scientific improvement on the crop, widespread use of local varieties and lack of drought tolerant varieties. In South Africa, India, Pakistan, Uganda, Kenya and Mozambique tef is mainly grown as forage or pasture crop (Assefa et al., 2011a,b).

In recent years, tef has been gaining enormous global popularity and various products are available in Europe and North America as health foods especially for persons with gluten in tolerance (Saturni et al., 2010). Besides, the crop is currently increasing receiving global attention as "health and performance food" for its nutritional advantages because it is rich in nutrients. In Ethiopia, consumers prefer tef not only because it makes good quality "*injera*", pancake-like soft bread, but it is also nutritious due to its high protein and mineral contents (Bultosa et al., 2002). Furthermore, tef is a good flour source for segments of the population suffering from gluten intolerance (Spaenij-Dekking et al., 2005). Notwithstanding its numerous relative advantages and economic importance, the productivity of tef in Ethiopia is low amounting 1.75 tons ha^{-1} (CSA, 2018). Among the major yield limiting factors in tef are lack of cultivars tolerant to lodging and drought (Assefa et al., 2011). Yield losses are estimated to reach up to 40% during severe moisture stress (Ayele, 1993). Further, yield reduction of 69 to 77% has been reported to have occurred as a result of drought at the anthesis stage of tef (Tekele, 2001). Nevertheless, drought is one of the most important factors that limit crop production in the moisture deficit environment. The best option for crop production under drought stress environments is to develop tolerant varieties which will reduce yield loss due to drought stress (Richards et al., 2002). Furthermore, about 75% Ethiopia landmass is categorized as, experiencing moisture stress during most months of the year and having between 45 and 120 days of growing season per year (Giorgis et al., 2018). Therefore, this study was initiated to examine the phenologic plasticity, and evaluate the effects of different tef genotype characters and planting time on tef growth performance in the low moisture stress tef growing areas in central Ethiopia.

MATERIALS AND METHODS

Plant materials

Ten selected recombinant inbred lines (RILs) and two early maturing standard checks were used for the study (Table 1). The RILs have been developed at DZARC by the National Tef Improvement Program, and they were relatively early maturing types and selected based on their high grain and biomass yield in the moisture stress environments of the rift valley areas of Ethiopia in earlier observation nurseries. The parents of the RILS were developed through Target Induced Local Lesions in Genomes (TILLING). The seed colors of all the test genotypes were white.

Experimental sites and season

The field experiment was carried out at two terminal drought-prone locations (*viz.* Melkassa Agricultural Research Center and Alem Tena sub-station of Debre Zeit Agricultural Research Center) during the 2017/2018 main cropping season. Melkassa is located in East Shewa Zone of Oromiya, about 115 km South East of Addis Ababa. Alem Tena is also located in East Shewa Zone of Oromiya about 112 km south-south east of Addis Ababa. Detail description of the two locations during the growing period is summarized and presented in Table 2.

Experimental design and management

The field experiment consisted of 36 entire factorial treatment combinations of three sowing dates and twelve tef genotypes including two standards checks. It was carried out in three replications of split plot design with sowing dates as main plots and genotypes as sub-plots. The size of the main plots was 23 m × 27 m and the sub-plots 2 m × 1 m (2 m^2). The total number of rows per sub plot was five and the spacings were 0.2 m between rows, and 1.5 and 1 m between blocks and plots, respectively. As per the research recommendations 15 kg ha^{-1} (3 g $plot^{-1}$) of seeds was hand broadcast on the surfaces of each row.

Fertilizers used were 40 kg N and 60 kg P_2O_5 per hectare as recommended for "Nitosols" (light soils) (Mamo et al., 2002). DAP was applied in all planting stages, while urea was applied three weeks after sowing and top dressed at tillering stage. Hand weeding was made two times during the crop growth stages (early and late tillering) depending on the weed infestation. All other pre- and post-stand establishment management practices were performed as per the recommended cultural practices of the specific test locations.

Data collection

Data on days to panicle emergence, maturity, lodging index (Caldicott and Nuttall, 1979), 100-kernel weight, biomass yield, grain yield and harvest index were taken on whole plot basis, while plant height, panicle length, panicle weight and grain yield per panicle were taken from five random samples of plants from the three central rows of each plot; and the averages of the five samples of plants were used for statistical analysis.

Table 1. Description of the tef genotypes used in the field experiment.

S/N	Genotype	Panicle form	Lemma Color (Immature)	Phenology	
				Days to heading	Days to maturity
1	Dtt_2 X Dtt_{13}/RIL182	Loose	Variegated (purple and yellow)	35	78
2	Dtt_2 X Dtt_{13}/RIL78	Loose	Yellowish green	34	78
3	Dtt_2 X Dtt_{13}/RIL270	Very loose	Yellowish green	35	77
4	Dtt_2 X Dtt_{13}/RIL128	Very loose	Variegated (purple and yellow)	35	76
5	Dtt_2 X Dtt_{13}/RIL96	Loose	Yellowish green	33	78
6	Dtt_2 X Dtt_{13}/RIL37	Loose	Yellowish green	38	78
7	Dtt_2 X Dtt_{13}/RIL101	Very Loose	Yellowish green	35	79
8	Dtt_2 X Dtt_{13}/RIL70	Loose	Yellowish green	34	78
9	Dtt_2 (Parental line)	Very loose	Yellowish green	35	76
10	Dtt_{13} (Parental line)	Very loose	Yellowish green	35	78
11	Boset (DZ-Cr-409) (Standard check)	Fairly loose	Variegated (red and yellowish)	37	78
12	Simada (DZ-Cr-385) (Standard check)	Fairly loose	Yellowish green	34	76

Dtt refers to "drought tolerant".

Table 2. Geographical coordinates and weather data of the test locations.

Location	Latitude(N)	Longitude(E)	Altitude(masl)	Rainfall (mm)	Mean temperature (°C)		Soil type
					Maximum	Minimum	
Melkassa	8°23'52"	39°20'6"	1539	591	28.56	16.07	"*Nitosols*"
Alem Tena	8°18'27"	38°20'6"	1575	589	29.49	15.29	"*Nitosols*"

Data analyses

All measured variables were subjected to analyses of variance (ANOVA) on individual location basis using the standard procedure for split plot design in randomized complete blocks as described by Gomez and Gomez (1984). Homogeneity of error variance was checked using the method of F-max test method of Hartley (1950), which is based on the ratio of the larger mean square of error (MSE) from the separate analysis of variance to the smaller mean square of error. Combined analysis of variance over locations was done after getting positive results from the testing for homogeneity of error variances using SAS statistical package (SAS, 2002).

RESULTS AND DISCUSSION

Phenology

Days to seedling emergence

The analyses of variance showed highly (P<0.01) significant variation of sowing dates on days to seedling emergence both at Alem Tena and Melkassa, whereas the interaction effects of sowing dates and genotypes on days to seedling emergence were statistically not significant at both locations (Appendix Table 1). On the other hand, the combined analysis of variance over locations revealed highly significant effects of locations, sowing dates, and interaction of sowing dates, whereas some trait was not significantly affected by genotypes, and the interactions of sowing dates and genotypes, locations and genotypes as well as locations, sowing dates and genotypes (Appendix Table 1). The differential responses of the tef genotypes in terms of number of days to seedling emergence can be attributed to the inherent genetic differences among the genotypes, which were expressed only at Alem Tena but not at Melkassa and on the average over the two locations.

Days to heading

The analyses of variance for days to heading showed significant main effects of sowing dates and genotypes at both locations as well as in the combined analysis over the two locations; while the interaction effect of the two factors was significant only at Melkassa, but not at Alem Tena and in the combined analysis across the two locations (Appendix Table 1). At Alem Tena, the standard check variety Boset (DZ-Cr-409) coupled with the genotype

Table 3. Means of number of days to seedling emergence, heading and maturity of tef as affected by sowing dates and genotypes at two locations.

Treatments	Days to seedling emergence*			Days to heading*			Days to maturity*		
	Alem Tena	Melk-assa	Mean	Alem Tena	Melk-assa	Mean	Alem Tena	Melk-assa	Mean
Means of sowing dates (Over all genotypes)									
D1	12.19^{a}	12.36^{a}	12.27^{a}	36.36^{a}	39.77^{a}	38.06^{a}	79.16^{a}	86.41^{a}	82.79^{a}
D2	6.36^{b}	4.72^{b}	5.54^{b}	33.80^{b}	37.02^{b}	35.41^{b}	78.19^{a}	82.08^{b}	80.13^{a}
D3	6.94^{b}	4.00^{c}	5.47^{b}	31.63^{c}	33.97^{c}	32.80^{c}	69.63^{b}	72.88^{c}	71.26^{b}
LSD (0.05)	0.74	0.64	4.75	1.04	1.12	1.75	1.49	1.84	6.53
Means of genotypes (over all sowing dates)									
Dtt2XDtt13 (RIL No.182)	8.33	7.44	7.88^{abc}	33.00^{b}	37.22^{abc}	35.11^{c}	76.77	79.44	78.11
Dtt2XDtt13 (RIL No.78)	9.11	7.33	8.22^{ab}	32.44^{b}	36.77^{bc}	34.61^{c}	76.67	80.66	78.66
Dtt2XDtt13 (RIL No. 27-0)	8.88	7.55	8.22^{ab}	32.66^{b}	37.77^{ab}	35.22^{c}	75.22	79.44	77.33
Dtt2XDtt13 (RILNo.128)	9.55	7.22	8.38^{a}	34.33^{b}	36.88^{abc}	35.61^{bc}	74.44	79.22	76.83
Dtt2XDtt13 (RIL No.96)	8.44	7.00	7.72^{abc}	32.44^{b}	35.22^{c}	33.83^{c}	75.77	80.88	78.33
Dtt2 XDtt13 (RIL No.37)	8.88	6.67	7.77^{abc}	37.44^{a}	39.11^{a}	38.27^{a}	76.33	80.00	78.33
Dtt2 XDtt13 (RIL No.101)	8.44	7.11	7.77^{abc}	33.44^{b}	37.11^{abc}	35.27^{c}	77.67	81.77	79.72
Dtt2 XDtt13 (RIL No.70)	8.55	6.67	7.61^{bc}	33.44^{b}	35.77^{bc}	34.61^{c}	75.88	8188	78.88
Dtt2 (Parental line)	8.33	7.22	7.77^{abc}	34.00^{b}	36.11^{bc}	35.05^{c}	73.67	79.77	76.72
Dtt13 (Parental line)	7.55	6.11	6.83^{d}	33.22^{b}	37.33^{abc}	35.27^{c}	76.00	80.67	78.33
Boset (DZ-Cr-409) (Stan. check)	8.00	7.11	7.55^{bcd}	37.88^{a}	37.55^{ab}	37.72^{ab}	75.22	82.44	78.83
Simada (DZ-Cr-385) (Stan. check)	7.88	6.89	7.38	32.88^{b}	36.22^{bc}	34.55^{c}	74.33	79.00	76.66
LSD	NS	NS	NS	2.08	2.25	2.29	NS	NS	NS
Overall mean$^{\delta}$	8.50a	7.02b	7.76	33.93b	36.92^{a}	35.43	75.67^{b}	80.46^{a}	78.06
(Pr >F)	0.3861	0.6681	0.1737	<.0001	<.0001	<.0001	0.3180	0.6843	0.2111
CV (%)	18.75	19.43	19.12	6.54	6.49	6.52	4.19	4.88	4.57

*Means in the same column and same treatment category followed by the same letter are not significantly different as judged by LSD at P≤0.05; NS=not significant at P≤0.05. $^{\delta}$Overall means of the two locations followed by different letters indicate significant differences at P≤0.05.

(Dtt2 XDtt13 RIL.37) depicted significantly higher mean number of days to heading (37.44 -37.88) than all of the remaining genotypes which showed statistically comparable lower means (Table 3). Likewise, at Melkassa and on the average across the two locations, the highest means for number of days to heading occurred for the genotype Dtt2 XDtt13 (RIL No.37) (Table 3). The statistically significant interaction effects of sowing dates and genotypes on days to heading at Melkassa indicated differential heading date responses of the tef genotypes to sowing dates at this particular location. Generally, low mean for number of days to heading were noted for the standard check variety Simada (DZ-Cr-385).

Days to physiological maturity

The main effects of sowing dates on number of days to maturity were significant at both locations and in the combined analysis over the two locations, while the main effects of genotypes were not on days to maturity were not statistically significant at both locations (Appendix Table 1). On the other hand, the interaction effect of sowing dates and genotypes on days to maturity were statistically significant (P≤0.05) only in the combined analysis over the two locations, but it was not significant at each of the individual test locations.

In the present study, the test tef genotypes did not show significant variations in number of days to maturity (Table 3). In contrast, studies on tef germplasm populations collected from different altitudinal zones showed significant genetic diversity in the range of 82-113 for days to maturity (Kebebew et al., 2001a). Such variations are very essential to augment the efforts to develop varieties fitting to various agro-ecologies and cropping systems to increase tef production and productivity. Thus, it enables breeders to develop variety that can escape late season drought by focusing on traits related to earliness.

Plant height and its components

Except for the main effects of sowing date at Alem Tena, plant was significantly (P≤0.05) affected by the main effects of both sowing dates and genotypes, and the interaction of sowing dates and genotypes at Melkassa, Alem Tena and the combined analysis over the two locations (Table 4). The test tef genotypes showed significant variations in plant height ranging from 82.55-94.46 cm at Alem Tena, 83.71-95.73 at Melkassa, and 84.10-94.51 cm on the average over the two locations (Table 4). In line with the present results, previous studies of Tefera et al. (1992), van de Wouw et al. (2010) revealed substantial genotype differences in tef plant height ranging from 73.6 -123 cm. Compared to the present findings, the total height of tef plant based on review of studies made using different genotypes at different locations was characterized with a much wider range of 20-156 cm (Assefa et al., 2001; Chanyalew et al., 2013; Degu, 2010). Key Murri consistently showed larger plant height and longer primary root than E.pilosa under drought Quantitative trait loci (QTLs).

The analysis of variance showed that at Alem Tena culm length was significantly affected by only sowing dates, while neither the main effects nor the interaction effects of sowing dates and genotypes exerted any statistically significant effects on culm length at Melkassa (Appendix Table 1). On the other hand, the combined analysis of variance over the two locations revealed that culm length was significantly affected by location and genotypes (Appendix Table 1). At Alem Tena, the means of culm length depicted that the tef plants were significantly taller for the latest sowing date than that of the two earlier sowing dates showing statistically similar means (Table 6). Generally, the mean culm length of the tef test genotypes over the two locations ranged from 18.17 cm for the parental line Dtt13 to 20.96 for the genotype Dtt2 X Dtt13 (RIL No.128).

The analysis of variance for panicle length showed significant main effects of both sowing dates and genotypes at Alem Tena, and significant main effects of only genotypes at Melkassa, while the interaction effects were not significant at both locations (data not shown). The panicle length means of the tef genotypes showed substantial variation at each of the two locations as well as on the average across the two test locations (Table 4). Both at each location as well as on average across the two locations, the highest mean panicle length of 42-44 cm was noted for the genotype (Dtt2 X Dtt13 RIL101). The substantial variation in panicle length of the genotypes can be attributed to their inherent genetic variation. Averaged over the two locations, the mean panicle length of the test tef genotypes which are in the loose panicle form types ranged from 33.74-42.14 cm. In comparison to this, Ebba (1975). in characterizing tef cultivars, described tef panicles with length of 7-65 cm; while the inflorescence takes one of four forms, namely: very loose; fairly loose; semi-compact (fairly loose and pyramidal); and very compact. Likewise, the length of the panicles of tef plants based on review of various studies made using diverse genotypes at different locations has been described as ranging from 10-65 cm (Assefa et al., 2001; Chanyalew et al., 2013). Descriptive statistical values of the plant are phenological traits, components of height, shoot biomass, harvest, index, flag leaf area and culm thickness for 2255 pure line accessions of tef (Ketema, 1993).

Yield and yield related traits

Number of fertile tillers per plant (NFT)

At Melkassa, the mean of fertile tillers per plant for the first and third sowing dates was greater than that of the second sowing date (Table 4). The means for number of fertile tillers per plant combined across locations showed significant differences among the genotypes and locations. However, it was not significant at each individual location (Table 4). Mean performance across locations and sowing dates, substantially the highest mean number of fertile tillers per plant out of all the genotypes was noted for the genotype Dtt2 X Dtt13 (RIL37), while a number of genotypes scored statistically comparable lower means (Table 4).

Similar to the present findings significant effects of locations and genotypes on the number of fertile tillers per plant were also noted in previous other studies on tef by Legesse (2004) and Gebretsadik et al. (2009). As most of the quantitative traits including yield are

Table 4. Means of plant height, culm length, and panicle length of tef as affected by sowing dates and genotypes at two locations.

Treatments	Plant height (cm)*			Culm length (cm)*			Panicle length (cm)*		
	Alem Tena	Melk-Assa	Mean	Alem Tena	Melk-Assa	Mean	Alem Tena	Melk-assa	Mean
Means of sowing dates (Over all genotypes)									
D1	86.56	84.17^{b}	85.48^{b}	19.61^{b}	18.30	18.95	38.93^{a}	34.44	36.68
D2	88.05	87.45^{a}	87.53^{ab}	20.13^{b}	19.70	19.91	36.65^{b}	35.66	36.15
D3	87.65	88.80^{a}	88.80^{a}	21.91^{a}	19.12	18.95	36.00^{b}	36.20	36.10
LSD (0.05)	NS	2.75	2.47	1.04	NS	NS	2.13	NS	NS
Means of genotypes (over all sowing dates)									
Dtt_2XDtt_{13} (RIL182)	89.64^{a-d}	86.46^{cd}	88.05^{bc}	20.84	20.82	20.83^{a}	35.68^{c}	34.81^{cd}	34.81^{cd}
Dtt_2XDtt_{13} (RIL78)	82.55^{d}	85.64^{cd}	84.10^{d}	21.22	19.73	20.47^{ab}	34.97^{c}	34.77^{cd}	34.77^{cd}
Dtt_2XDtt_{13} (RIL27-0)	84.86^{bcd}	86.20^{cd}	85.53^{cd}	20.80	18.86	19.83^{a-d}	34.97^{c}	34.74^{cd}	34.74^{cd}
Dtt_2XDtt_{13} (RIL128)	87.62^{a-d}	88.80^{bc}	88.21^{bc}	20.95	20.97	20.96^{a}	35.00^{c}	36.28^{cd}	36.28^{cd}
Dtt_2XDtt_{13} (RIL96)	86.80^{a-d}	84.53^{cd}	85.66^{cd}	20.26	18.77	19.52^{a-d}	35.73^{c}	34.76^{cd}	34.76^{cd}
Dtt_2 X Dtt_{13} (RIL37)	94.46^{a}	93.73^{ab}	94.10^{a}	21.13	18.60	19.86^{a-d}	43.68^{a}	42.14^{a}	42.14^{a}
Dtt_2 X Dtt_{13} (RIL101)	93.68^{ab}	95.33^{a}	94.51^{a}	21.35	20.17	20.76^{a}	40.33^{ab}	39.71^{ab}	39.71^{ab}
Dtt_2 X Dtt_{13} (RIL70)	84.60^{cd}	81.93^{d}	83.26^{d}	20.71	19.84	20.27^{abc}	37.40^{bc}	39.71^{ab}	35.48^{cd}
Dtt_2 (Parent line)	84.68^{cd}	81.15^{d}	82.92^{d}	19.68	17.60	18.64^{cd}	37.35^{bc}	35.48^{cd}	36.70b^{cd}
Dtt_{13} (Paren line)	86.60^{a-d}	85.84^{cd}	86.22^{dc}	19.66	16.68	18.17^{d}	36.53^{bc}	36.70^{cd}	35.08^{cd}
Boset (DZ-Cr-409) (check)	92.73^{abc}	89.28^{bc}	91.01^{ab}	20.62	17.22	18.92^{bcd}	40.44^{ab}	35.08^{cd}	37.52^{bc}
Simada (DZ-Cr-385) (check)	84.66^{cd}	82.75^{d}	83.71^{d}	19.37	19.17	19.27^{a-d}	34.22^{c}	37.52^{bc}	33.74^{d}
LSD (0.05)	3.67	5.51	3.52	NS	NS	1.76	4.27	3.23	3.23
Overall mean$^{\delta}$	87.42	86.80	87.27	20.53a	19.04b	19.79	37.19a	36.31b	35.43
CV (%)	10.76	6.75	9.00	11.11	17.30	14.32	12.22	12.46	12.71

*Means in the same column and same treatment category followed by the same letter are not significantly different as judged by LSD at P≤0.05; NS=not significant at P≤0.05. $^{\delta}$Overall means of the two locations followed by different letters indicate significant differences at P≤0.05.

polygenically controlled and are much influenced by environmental factors, an understanding of inheritance and study of association between yield and its components are necessary for planning an effective selection program in identifying high yielding varieties

Harvest index

At Alem Tena, harvest index was significantly (P≤0.05) affected by sowing dates as well as genotypes, but the interaction effect of sowing date and genotypes was not statistically significant (Appendix Table 1). Likewise harvest index at Melkassa was highly significantly (≤0.01) affected by both sowing dates and genotypes, while the interaction effect of the two factors was not statistically significant (Appendix Table 1).

In addition, the combined analysis of variance over locations depicted that harvest index of tef was highly significantly (P≤0.05) affected by sowing dates, locations and genotypes, and significantly (P≤0.05) by the interaction of sowing dates and locations. While the interaction effects of sowing dates and genotypes and that of sowing dates, location and genotypes were not statistically significant (Appendix Table 1).

Lodging index

The analysis of variance showed that at Alem Tena lodging index was significantly affected by both sowing dates and genotypes, whereas the interaction effects of sowing dates and genotypes were not statistically significant (Table 5) Likewise, lodging index at Melkassa did not show statistically significant effects of both sowing dates and genotypes as well as the interaction of these two factors (Appendix Table 1).

At Alem Tena, the first two sowing dates gave statistically comparable lodging index means that were significantly higher than that of the last sowing date (Table 5). At this location the genotype (Dtt2 X Dtt13 RIL No.27-0) exhibited the highest mean lodging index (74.88), while the lowest mean (63.22) was recorded for the standard check variety Boset (Table 5). Averaged over the two locations and all sowing dates, the maximum mean lodging index was recorded for the standard check variety Simada, and the least mean lodging index (63.05) occurred for the genotype (Dtt2 X Dtt13 RIL 128). Regarding the locations, the mean lodging index was higher for Alem Tana than for Melkassa (Table 5). Similar to the present study, studies of genetic gain in tef breeding using varieties released until 2013 revealed significant lodging index differences among tef varieties at both test locations of Debre Zeit and Melkassa (Dargo et al., 2016).This study also showed that the mean lodging indices were 66 and 63 at Debre Zeit and Melkassa, respectively.

Number of fertile florets per central primary panicle branch

At Alem Tena, the highest mean number of fertile florets per central primary panicle branch occurred for the last sowing date, and this significantly excelled only the lowest mean noted for the earliest sowing date (Table 4). Averaged over the two locations and sowing dates, the highest mean number of fertile florets per central primary panicle branch was observed for the genotype (Dtt2 X Dtt13 9RIL37) followed by the genotypes (Dtt2 X Dtt13 RIL96) and the standard check variety Boset (Table 4). The two standard check varieties showed the lowest means for number of fertile florets per central primary panicle branch. Variability of some agronomic characters of tef germplasm was found to be high variations in some agronomic and morphologic characters of 506 tef accessions (Ayele and Ketema, 1995; Ketema, 1997). Its spikelet's have 2-12 florets. Each floret has a lemma, palea, three stamens, an ovary and mostly two, in exceptional cases three, feathery stigmas. These studies investigated that no significant differences were obtained among diverse altitude zones for parameters like days to panicle emergence, culm and panicle length, number of panicle branches, counts of fertile florets/spikelet, and shoot biomass (Assefa et al., 2001a,b).

Above-ground dry biomass yield

The combined analysis of variance over locations revealed that above-ground dry shoot biomass yield of tef was highly and significantly (P≤0.05) affected by sowing dates, locations, and genotypes; while none of the first order or the second order interactions of these factors exerted statistically significant effects on above-ground tef shoot biomass yield (Appendix Table 1).

At each of the individual test locations of Alem Tena as well as on the average over these two locations, the means for above-ground dry tef shoot biomass yield for the first sowing date were significantly lower than those of the second and third sowing dates which exhibited statistically comparable means; substantial genotype differences in above-ground dry shoot biomass yield were also noted at both test locations as well as on the average over the two locations (Table 6). Accordingly, both at Alem Tena and Melkassa and when averaged over these two locations, the highest means of above-ground shoot biomass yield were recorded for the genotype Dtt2 X Dtt13 (RIL No. 37) and the standard check variety Boset which showed statistically comparable means (Table 6). On the other hand, the lowest means of above-ground dry shoot biomass yield was recorded for the genotype Dtt2 X Dtt13 (RIL No. 27-0) at Alem Tena and on the average of over the two locations, and for the genotype Dtt2 X Dtt13 (RIL No. 182) at Melkassa. Of the two locations, statistically higher mean above-ground shoot biomass yield was noted for Alem Tena (7026 kg/ha) than for Melkassa (5039 kg/ha). Similar to the present results, former studies also showed that above-ground biomass yield showed a similar trend to that of plant height and tillering capacity and shoot biomass (Birhanu et al., 2020).

Grain yield

The analysis of variance showed that both at Alem Tena and Melkassa Grain yield was highly significantly (P≤0.05) affected by sowing date as well as genotypes, while sowing dates and genotypes did not significantly interact

Table 5. Means of number of fertile tillers/plant and fertile florets per central primary panicle branch, and lodging index of tef as affected by sowing dates and genotypes at Two Locations

Treatments	No. fertile tillers/plant*			No. fertile florets/central primary panicle branch*			Logging Index*		
	Alem Tena	Melk-Assa	Mean	Alem Tena	Melk-Assa	Mean	Alem Tena	Melk-assa	Mean
Means of sowing dates (Over all genotypes)									
D1	8.07	10.37[a]	9.64[a]	157.08[b]	146.31	157.08	72.94a	62.42	67.68
D2	7.43	8.83[b]	9.45[a]	163.27[ab]	156.93	163.27	70.75a	65.31	68.02
D3	7.12	9.88[a]	10.45[a]	173.64[a]	153.63	173.64	64.05b	63.83	63.94
LSD (0.05)	NS	0.99	NS	13.04	NS	NS	4.39	NS	NS
Means of genotypes (over all sowing dates)									
Dtt_2 X Dtt_{13} (RIL182)	7.77	10.11	9.58[d]	158.96	173.67	151.52[ab]	73.88[abc]	65.00	69.44[abc]
Dtt_2 X Dtt_{13} (RIL78)	7.11	9.06	9.43[d]	159.11	154.69	156.90[ab]	67.77[a-e]	62.33	65.05[abc]
Dtt_2 X Dtt_{13} (RIL270)	8.04	8.80	8.77[d]	159.40	151.82	155.61[ab]	74.88[a]	60.33	67.61[abc]
Dtt_2 X Dtt_{13} (RIL128)	7.75	9.53	9.48[d]	178.64	147.51	163.08[ab]	64.77[de]	67.44	66.11[abc]
Dtt_2 X Dtt_{13} (RIL96)	8.51	9.04	9.75[d]	157.58	130.69	144.13[b]	71.22[a-e]	54.93	63.05[c]
Dtt_2 X Dtt_{13} (RIL37)	7.17	10.82	12.77[a]	183.62	170.94	183.86[a]	65.46[cde]	60.91	63.17[bc]
Dtt_2 X Dtt_{13} (RIL101)	6.57	9.26	9.22[d]	170.13	160.69	165.41[ab]	65.55[b-e]	61.00	63.27[abc]
Dtt_2 X Dtt_{13} (RIL70)	7.13	9.60	8.95[d]	168.71	142.58	155.65[ab]	67.51[a-e]	60.33	63.92[abc]
Dtt_2 (Parental line)	7.60	9.77	8.63[d]	155.78	144.53	150.16[ab]	69.55[a-e]	65.33	67.44[abc]
Dtt_{13} (Parental line)	8.13	9.53	9.22[d]	160.22	148.19	154.21[ab]	72.88[a-e]	71.66	72.27[ab]
Boset (DZ-Cr-409) (Stan. check)	6.36	10.51	11.26[b]	158.69	156.13	173.16[bc]	63.22[e]	66.44	64.83[abc]
Simada (DZ-Cr-385) (Stan. check)	8.35	10.33	11.08[bc]	165.18	146.09	155.63[ab]	74.22[ab]	70.56	72.38[a]
LSD (0.05)	NS	NS	1.39	NS	NS	34.85	8.78	NS	9.16
Overall mean$^{\delta}$	7.54b	9.70a	9.85	164.66	152.29	159.10	69.24a	63.85b	66.55
CV (%)	24.51	21.87	24.00	16.85	21.23	19.15	13.39	16.60	14.98

*Means in the same column and same treatment category followed by the same letter are not significantly different as judged by LSD at P≤0.05; NS=not significant at P≤0.05. . $^{\delta}$Overall means of the two locations followed by different letters indicate significant differences at P≤0.05.

on this parameter at either of the two locations. The combined analysis of variance over locations revealed that grain yield of tef was highly significantly (P≤0.05) affected by sowing dates, locations, and genotypes while neither the first order nor the second order interactions of these factors exerted statistically significant effects on grain yield (Appendix Table 1).

At Alem Tena, the highest mean grain yield (1521 kgha^{-1}) was recorded for the latest 3rd sowing date, and this was statistically and significantly greater than only the mean (1302 kg/ha) of the second sowing date (10 July 2017); while the mean yield (1380 kg/ha) of the first sowing date (01 July 2017) was not statistically different from either the lowest or the highest means of the second and third sowing dates, respectively (Table 6). Likewise, at Melkassa, the two later sowing dates with statistically comparable mean grain yields (831-884 kg/ha) significantly excelled the earliest sowing date which exhibited

Table 6. Means of above-ground shoot biomass, and grain yield of tef as affected by sowing dates at two locations.

Treatments	Above-ground shoot biomass (kg ha^{-1})			Grain yield (kg ha^{-1})*		
	Alem Tena	Melk-Assa	Mean	Alem Tena	Melk-Assa	Mean
Means of sowing dates (Over all genotypes)						
D1	6625.0^{b}	4520.8^{b}	5572.92^{b}	1380.35^{ab}	710.83^{b}	1045.6
D2	7138.9^{a}	5208.3^{a}	6173.61^{a}	1302.22^{b}	830.83^{a}	1066.5
D3	7312.5^{a}	5388.9^{a}	6350.69^{a}	1520.76^{a}	883.75^{a}	1202.2
LSD (0.05)	594.06	313.88	311.24	146.65	75.44	NS
Means of genotypes (over all sowing dates)						
Dtt_2 X Dtt_{13} (RIL No.182)	6500.0^{cde}	4305.6^{e}	5402.8^{de}	1206.1^{d}	667.78^{c}	936.94^{d}
Dtt_2 X Dtt_{13} (RIL No.78)	6611.1^{cde}	4638.9^{ed}	5625.0^{cde}	1509.0^{abc}	775.00^{bc}	1142.22^{bc}
Dtt_2 X Dtt_{13} (RIL No. 27-0)	6083.3^{e}	4388.9^{ed}	5236.1^{e}	1374.4^{bcd}	665.56^{c}	1020.00^{cd}
Dtt_2 X Dtt_{13} (RILNo.128)	6833.3^{cde}	4916.7^{bcde}	5875.0^{bcde}	1502.5^{abc}	740.00^{bc}	1121.28^{bcd}
Dtt_2 XDtt_{13} (RIL No.96)	6750.0^{cde}	4750.0^{cbde}	5750.0^{cde}	1290.6^{cd}	751.11^{bc}	1020.83^{cd}
Dtt_2 XDtt_{13} (RIL No.37)	9055.6^{a}	6111.1^{a}	7583.3^{a}	1691.7^{a}	998.33^{a}	1344.72^{a}
Dtt_2 XDtt_{13} (RIL No.101)	6250.0^{de}	4861.1^{bcde}	5555.6^{cde}	1292.2^{cd}	822.78^{b}	1057.50^{cd}
Dtt_2 XDtt_{13} (RIL No.70)	6305.6^{ed}	4861.1^{bcde}	5583.3^{cde}	1314.4^{cd}	855.00^{ab}	1084.72^{cd}
Dtt_2 (Parental line)	7277.8^{bcd}	4972.2^{bcd}	6125.0^{bc}	1404.7^{abcd}	757.78^{bc}	1081.28^{cd}
Dtt_{13} (Parental line)	6805.6^{cde}	5083.3^{bc}	5944.4^{bcd}	1230.6^{cd}	863.89^{ab}	1047.22^{cd}
Boset (DZ-Cr-409) (Stan. check)	8222.2^{ba}	6250.0^{a}	7236.1^{a}	1607.8^{ab}	982.22^{a}	1295.00^{ab}
Simada (DZ-Cr-385) (Stan. check)	7611.1^{bc}	5333.3^{b}	6472.2^{b}	1388.9^{bcd}	822.22^{b}	1105.56^{cd}
LSD	1188.1	627.76	656.8	293.3	150.89	191.89
LSD	594.06	313.88	311.24	146.65	75.44	323.39
Overall mean$^{\delta}$	7025.46^{a}	5039.35^{b}	6032.40	1401.11^{a}	808.47^{b}	1104.77
(Pr >F)	<.0001	<.0001	<.0001	0.0322	0.0002	<.0001
CV (%)	17.98	13.24	16.75	22.26	19.85	22.45

*Means in the same column and same treatment category followed by the same letter are not significantly different as judged by LSD at P≤0.05; NS=not significant at P≤0.05. $^{\delta}$Overall means of the two locations followed by different letters indicate significant differences at P≤0.05.

mean grain yield of 711 kg/ha (Table 5). Both at Alem Tena and Melkassa and the average over the two locations, the highest means of grain yield was recorded for the genotype Dtt2 X Dtt13 (RIL37) followed by the standard check variety Boset; while the lowest means of grain yield was recorded for the genotype Dtt2 X Dtt13 (RIL182) (Table 6). Comparing the two locations, the higher mean tef grain yield was obtained at Alem Tena (1401 kg/ha) than at Melkassa 998 kg/ha) (Table 6).

CONCLUSION AND RECOMMENDATIONS

The location-wise as well as the combined analysis of variance over locations revealed highly (P≤0.01) significant differences among sowing dates and genotypes for most of the traits evaluated including dry shoot biomass yield and grain yield; while the sowing date by genotype interaction effect was also significant for some of the traits. The very good rainfall distribution in the test season with about 79.2 and 55.83% of the total yearly rainfall received at Alem Tena and Melkssa, respectively generally resulted in good performance of the experimental tef crops at both locations.Hence the test tef genotypes, the recombinant inbred lines appeared to have displayed transgressive segregation as some of them excelled both of the parental lines in many of the evaluated traits including above-ground dry shoot biomass yield, grain yield and harvest index. Overall, the range of sowing date from 15-20 July has proved superior and recommendable, in- general, the genotype Dtt2 X Dtt13 (RIL No.37), gave highest shoot biomass and grain yield at both locations and over all the two locations. This genotype should be used in the future tef breeding program for further evaluation at multiple environments involving many and diverse locations over several seasons in the variety development process for terminal drought-prone areas of Ethiopia. For conclusive recommendations on planting time and suitable genotypes, it would be worth repeating the experiment over many locations and several seasons

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix Table 1. Mean squares from the combined analyses of variance of data on different traits of tef in a sowing date by genotype experiment over two locations

Traits	Mean squares								
	Sowing dates (df =2)	Loc. (df=1)	Sowing date X location interaction (df = 2)	Error(a) (df = 4)	Genotypes (df = 11)	Sowing date x genotype interaction (df = 22)	Genotype x location interaction (df = 11)	Sowing date X location X genotype interaction (df = 22)	Error(b)
DSE	1100.34**	117.04**	43.93	19.36	3.11NS	1.61NS	1.08NS	1.39NS	1.34
DTH	498.76**	483.00**	6.00NS	10.12	30.08**	6.27NS	9.77*	3.24NS	3.24
DTM	2624.31**	1242.24**	83.11*	182.19	17.02NS	7.37*	6.42NS	10.88NS	0.65
GFP	982.34**	161.89*	3.84NS	108.00	43.26*	8.97NS	27.68*	17.59NS	17.59
PH (cm)	201.07*	47.41NS	38.98NS	259.93	292.76**	71.71NS	23.04NS	111.75*	111.75
CUL (cm)	44.79*	123.60*	106.87*	31.74	14.96*	9.93NS	5.76NS	8.98NS	8.98
PAL (cm)	7.58ns	167.48*	44.06*	55.71	107.02**	24.07NS	19.41NS	17.06*	17.06
NFT	3.84NS	88.93**	1194.31NS	25.42	27.34**	7.28NS	3.69NS	5.15NS	5.15
NFF	1475.75NS	2068.63NS	478.46*	19574.63	2114.80*	990.25NS	2256.51*	1378.84*	1378.84
LI (%)	369.55*	1572.48**	0.0007NS	427.17	202.53*	77.42NS	156.00NS	71.35NS	71.35
TSW (mg)	0.00047NS	0.06303**	25147.53NS	0.00139	0.00105*	0.0009*	0.0007NS	0.00057NS	0.0005
HI (%)	138.95**	1677.57**	25.36*	102.19	112.80**	10.00NS	106190.08NS	139161.77NS	13.91
SHB(kg/ha) ($\times 10^3$)	11965.57**	213010.42**	188368.1NS	4991.03	9481.27**	629.08NS	801452.0NS	1104403.4NS	1104.40
GY (kg/ha) ($\times 10^3$)	520.40NS	18969.48**	203365.60*	123.68	236.19**	54.31NS	68407.81NS	83524.08NS	83.52
DDSE	148723.02*	16651.44**	6252.06**	2654.5	426.76NS	204.57(ns)	142.51NS	177.26NS	142.51
DDH	85548.33**	79166.10**	1522.60NS	1303.13	5174.16**	1084.77NS	1997.56NS	455.16NS	1997.56
DDM	335839.57**	160922.58**	13863.87*	18781.54	1820.41NS	898.49NS	1206.74NS	1218.20NS	1206.74

[a]df = degrees of freedom ; NS= not significant,* and ** significant at P≤0.05 and P≤0.01, respectively

DSE= Days to seedling emergence, DTH. Days to heading, DTM = days to maturity, PH = plant height, CUL=Culm length, PAL = panicle length NFT = No. of fertile tillers, NFF=No of fertile florets, LI= lodging index, TSW=thousand seed weight, HI= harvest index, SHB =shoot biomass, GY=grain yield, DDSE = Degree days for seedling emergence, DDH = Degree days for heading, DDM = Degree days for maturity.

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Full Length Research Paper

Foliar and time of application of silicon and the effect on rice yield components, productivity and seed quality

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The application of silicon (Si) has been beneficial to several cultures, mainly those considered Si accumulators, such as rice. Thus, in most cases Si is supplied via soil. However, it has been found that foliar application and small amounts of the element can be a viable alternative for its supply to plants. The objective of the present work was to evaluate the foliar application times of different silicon sources on yield components, productivity and seed quality of irrigated rice. The experiment was conducted in a greenhouse at the Federal University of Pelotas. The experimental design was completely randomized with four replicates. The treatments were: two sources of Si (Caulim®, 50 kg ha^{-1}; and Sifol®, 3 L ha^{-1}), three times of foliar application (tillering, bottling and flowering). Two distinct rice cultivars (IRGA 424 and Puitá Inta CL) were used. The production components evaluated were: Seed yield, number of panicles, and number of seeds per plant. For the evaluation of the physiological quality, the seeds were submitted to the tests of germination and first germination count. Seed health test was performed. To cultivate IRGA 424, the use of Caulim® and Sifol® in the drilling phase increased the number of seeds per panicle. Therefore, for both cultivars the use of silicon provided improvements in seed vigor. We conclude that the application of silica via foliar, through the Caulim® and Sifol®, to the cultivar IRGA 424 in the drilling phase, increases the number of seeds per panicle, and for both cultivars, it improves the vigor of the seeds, in addition to reducing the incidence of fungi.

Key words: *Oryza sativa* L., vigor, germination.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the main cereals produced and consumed worldwide. In Brazil, the national harvest was estimated at 10.8 million tons in 2019/2020 of which about 9.9 million correspond to irrigated cropping areas (CONAB, 2020). Southern Brazil accounts for the highest rice yield, mainly due to the flood

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irrigation system used in 100% of the cultivated commercial crop areas. This system allows efficient weed control, eliminates crop water deficit, and promotes electrochemical changes in the soil that increase nutrient availability for the crop (SOSBAI, 2016). Besides yield, a constant demand of this crop is the search for sustainable management techniques. In this sense, the use of silicon-based products has increased interest from researchers, technicians, and farmers, as it has potential for the protection of grasses (Pereira et al., 2009; Silva et al., 2013; Oliveira et al., 2016; Oliveira, 2016).

Silicon (Si) is an immobile element in the plant, being deposited on leaf blades and sheaths, stems, bark, and roots (Yoshida et al., 1962). This element participates in the biosynthesis of cell wall components that reduce enzymatic degradation. According to Dayanandam et al. (1983), silicon-accumulating plants such as rice tend to have greater resistance to the action of fungi and insects. These benefits may contribute to increased rice yield, as shown by the studies of Tokura et al. (2007), improved seed vigor (Tunes et al., 2014; Oliveira et al., 2016), and decreased use of toxic products in crop management (Vieira et al., 2011), which may assist in practices as integrated pest and disease control.

Some marketed products can be used to supply this element. Caulim® (79% SiO_2) and Sifol® (12% Si), for example, can be used for seed treatment aiming at increased plant yield. Moreover, it is known that the efficiency of agricultural technologies depends on the time of their application (SOSBAI, 2016). It is know that Si is a major constituent in higher plants and the value of Si in crop productivity also has been demonstrated. Nowadays, in many diferent countries, the application of Si fertilizers is very common in crop production systems. Silicon is already recognized as a beneficial element for several crops and may be classified as a quasi-essential element. We need to know if the application in three different moments can also bring benefits to rice plants. These stages of plant development are equally important for rice plants; however, we need to know the total amount of silicon needed and which phases require more of this nutrient. Despite research on the effects of Si on plant growth, much information is still incipient. In this context, further research is needed to address this topic. Thereby, research is needed to study Si application at different times, sources, and doses, exploring all the benefits that this nutrient can bring to rice crop. Given the above, this study evaluated silicon sources and the times for their foliar application in irrigated rice seeds.

MATERIALS AND METHODS

The study was carried out under greenhouse conditions. Seeds of rice cultivars (cv) IRGA 424 (medium cycle) and Puitá Inta CL (medium cycle) were used. These cultivars were selected because they are recommended for all rice-growing regions of Rio Grande do Sul and also for showing higher yield among the cultivars. The seeds were sown in the first 20 days of November and the experimental units were harvested on March 23. They were placed in 10 L buckets filled with sieved and homogenized soil collected from the A1 horizon of a Soil Eutrophic Haplic Planosol (EMBRAPA, 2006) belonging to the Pelotas mapping unit, in Rio Grande do Sul State (RS), Brazil. Nitrogen, phosphate, and potassium fertilization were performed according to the recommended doses for irrigated rice (CQFS – Soil fertility and chemistry commission- RS/SC, 2016). Flooding was carried out when plants had four leaves, maintaining a 10 cm water depth during the experiment. Cultural treatments were performed according to the technical recommendations for irrigated rice cultivation in RS (SOSBAI, 2016). The experimental design was bifactorial, consisting of Si application times (Stage I - full tillering, Stage II - booting, and Stage III - full bloom) and its different sources (Caulim®, at 50 Kg ha^{-1}, and Sifol®, at 3 L ha^{-1}). Four replicates were used for each treatment. Foliar applications were performed with a CO_2-pressurized back-pack sprayer.

Harvesting was carried out and panicles of each plant packed in brown paper envelopes separately and threshed manually. Harvested seeds were dried at the temperature of 42°C. The following characteristics were analyzed: number of seeds per plant, determined by counting the seeds in each plant; number of panicles per plant; and seed yield per plant (g $plant^{-1}$), where seeds were cleaned and moisture was standardized to 13% by drying in an oven with forced air circulation at 32 ± 2°C, followed by weighing on a precision scale. Physiological quality was determined by the germination and first germination count tests, using four replicates of 50 seeds. Seeds were placed between paper sheets and germinated at a temperature of 25 ± 2°C. Counts were performed at 5 and 14 days after sowing (Brasil, 2009). The filter paper method or Blotter Test was used to evaluate seed health. The sample consisted of 200 seeds subdivided into eight subsamples of 25 seeds. The seeds were placed in plastic boxes (gerbox) and incubated at 20°C for seven days (Brasil, 2009). Subsequently, the percentage of fungi-contaminated seeds was determined. Data were subjected to analysis of variance by the F test, and means were compared by the Tukey test at 5% probability, using the Winstat 1.0 software (Machado and Conceição, 2003).

RESULTS AND DISCUSSION

Cultivar IRGA 424 showed no effect between phonological stages and foliarly applied silicon sources for seed yield and number of panicles per plant. For number of seeds per plant, however, there was interaction between treatments (Table 1). The use of Caulim® in the tillering stage differed only from the control, with an average about 20.3% higher. In the booting stage, this source differed from the control and the use of Sifol®. In the flowering stage, Sifol® showed better results for number of seeds per plant, differing from the control and the treatment with Caulim® (Table 1). For cultivar Puitá Inta CL, the application times and silicon sources tested did not affect the number of panicles. Notwithstanding, there was a simple effect of silicon sources for seed yield and number of seeds per plant in the lot with application during the tillering stage, and the use of Sifol® was superior to Caulim® for both variables analyzed (Table 1).

Silicon participates in the biosynthesis of cell wall components. Silicon-accumulating plants tend to have a more rigid leaf architecture and thicker cuticle, which enables greater use of solar energy and helps to reduce

Table 1. IRGA 424 and Puitá Inta CL cultivar- Seed yield per plant (g), number of panicles per plant and number of seeds per plant as a function of foliar application with silicon sources (Caulim® and Sifol®) at tillering, booting and flowering.

Silicon sources	**Seed yield per plant (g) – IRGA 424**			
	Leaf application stages			**Average**
	Tillering	**Booting**	**Flowering**	
Control	28.47 ^{ns}	29.43 ^{ns}	29.87 ^{ns}	29.47
Caulim®	29.22	31.34	29.03	29.85
Sifol®	27.85	31.26	29.89	29.66
Average	28.84	30.67	29.46	
C.V. (%)	9.6	7.7	7.3	
	Number of panicles per plant			Average
Control	15.6 ^{ns}	15.1 ^{ns}	15.3 ^{ns}	15.6
Caulim®	17.0	16.1	16.3	16.7
Sifol®	16.6	16	17.3	16.3
Average	16.5	16.2	16.4	
C.V. (%)	9.4	11.8	8.6	
	Number os seeds per plant			Average
Control	182 bA	189^{bA}	178^{bA}	182
Caulim®	218 aA	232^{aA}	183^{bB}	211
Sifol®	219 aA	177^{bB}	217^{aA}	201
Average	206	197	194	
C.V. (%)	9.1	13.7	7.4	
Silicon Sources	Seed yield per plant (g) – Puit Inta CL			
	Foliar silicon application times			Average
	Tillering	Booting	Flowering	
Control	22.31 b	24.36 ^{ns}	24.02 ^{ns}	24.36
Caulim®	20.36 b	24.46	28.42	24.41
Sifol®	30.24 a	24.52	26.64	27.13
Média	24.98	24.44	26.47	
C.V. (%)	7.1	14.1	10.5	
	Number of panicles per plant			Average
Control	13.0 ^{ns}	13.7 ^{ns}	13.2 ^{ns}	13.0
Caulim®	15.2	15.6	15.4	15.4
Sifol®	15.5	15.4	13.5	14.5
Média	14.3	14.3	13.6	
C.V. (%)	7.3	6.7	8.9	
	Number of seeds per plant			Average
Control	245 a	239 ^{ns}	235 ^{ns}	245
Caulim®	164 b	221	211	199
Sifol®	244 a	234	247	242
Average	218	233	234	
C.V. (%)	4.7	14.3	9.4	

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other by the Tukey test at 5% probability.

plant water loss. These characteristics later contribute to a more efficient production of photo assimilates, which are important in seed formation (Taiz and Zeiger, 2015). Silicon supply in the different stages of rice growth and

Table 2. Cultivate Irga 424 and Puitá Inta CL, first germination count test (FGC) and germination test (Germ) on seeds produced as a function of foliar application with silicon sources (Caulim® and Sifol®) at tillering stages, booting and flowering.

Phenological stages of the plants	IRGA 424				Puitá Inta CL			
	FCG (%)		GERM (%)		FCG (%)		GERM (%)	
	Caulim®	Sifol®	Caulim®	Sifol®	Caulim®	Sifol®	Caulim®	Sifol®
Control	77^{a}*	74^{a}	98^{a}	92^{a}	71^{b}	74^{c}	97^{a}	92^{a}
Growth stage	67^{b}	76^{a}	95^{a}	98^{a}	79^{b}	69^{c}	99^{a}	95^{a}
Booting	79^{a}	71^{a}	98^{a}	96^{a}	84^{a}	78^{b}	95^{a}	97^{a}
Flowering	69^{b}	71^{a}	98^{a}	95^{a}	87^{a}	86^{a}	98^{a}	98^{a}
Average	72	72	95	94	80	77	97	95
C.V.(%)	8.7		6.1		7.4		7.5	

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other by the Tukey test at 5% probability.

development can contribute to increased seed yield and to the formation of more vigorous and healthy seeds.

In the evaluations regarding physiological quality, there was a simple effect for silicon sources, with statistical differences for both IRGA 424 and Puitá Inta CL. For the first germination count of cultivar IRGA 424, Sifol® differed statistically from Caulim®, showing the highest average in the harvested lots with application during the vegetative and flowering stages (Table 2). For the first germination count of cultivar Puitá Inta CL, Sifol® showed better results for the lot with application at booting stage. For the lot with Si application at flowering stage there were no statistical differences between Sifol® and Caulim® (Table 2). Regarding the germination percentage, there were no differences between treatments for both cultivars. Germination percentage was above 90% in all cases (Table 2).

Studying seeds treated with different silicon sources and doses, Oliveira et al. (2016) observed an increased vigor in the treatment with Caulim® at 30, 60, 90, and 120 g 100 kg seeds^{-1}. In addition, studying the same cultivars but with aluminum silicate and ground rice husk ash as silicon sources, Tunes et al. (2014) observed an increased seed vigor through the results of root length and field emergence. Study using silicon sources for seed coating have shown that the expression of some important enzymes differs in the germination process (Tunes et al., 2014), which suggests that silicon influences this process. These results corroborate those found in the present study, where silicon treatments promoted increased physiological and sanitary quality of seeds of both cultivars.

Overall averages show that the seeds treated with silicon sources had lower incidence of fungi after harvest compared to the control (Table 3).

Studying the effect of calcium silicate and rice husk ash on fungal spots in irrigated rice seeds, Roma-Almeida et al. (2016) did not observe the reduction of this fungus by applying Si sources. However, Datnoff et al. (2007) provided Si to plants via soil and leaf, observing satisfactory results in the control of various diseases, both in mono- and dicotyledonous plants. According to Figueiredo and Rodrigues (2007), silicon is linked to the induction of a series of metabolic reactions in plants, resulting in the formation of compounds such as phytoalexins and lignins, which confers increased resistance to attack by phytopathogens and pests.

With increasing doses of silicon in rice, Berni and Pradhu (2003) observed a significant decrease in the severity of blast disease caused by the fungus *Pyricularia oryzae*. This fungus was not found in the present study. However, other pathogens found in both treated and untreated seed samples cause spots in grains and seeds (*Phoma* sp., *Curvularia lunata*, *Nigroppora oryzae*, *Alternaria* sp., *Fusarium* sp.). The high incidence of these pathogens has been of concern to producers, as they lead to sterility and decreased seed weight (Malavolta et al., 2007). The use of Sifol® and Caulim®, regardless of application time, proved to be important in this study, minimizing fungal levels in the seeds produced compared to the control average.

Conclusion

Foliar application of silicon through Caulim® and Sifol® proved to be beneficial, contributing to crop seed production. In general, foliar applications of silicon at tillering and boosting stages were efficient to increase the number of seeds produced in cultivar IRGA 424. The use of silicon through Sifol® and Caulim® improved seed quality in both cultivars, which was observed through the first germination count. Moreover, their application minimized fungal levels in harvested seeds. For cultivar IRGA 424, Caulim® can be applied at boosting stage, and Sifol® can be applied at tillering and flowering stages.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Table 3. Cultivate IRGA 424 and Puitá Inta CL - Seed health test for seeds produced as a function of foliar application with silicon sources (Caulim® and Sifol®) at tillering, booting and flowering stages.

Silicon applicatio times	Silicon sources	Health seed test (%) IRGA 424						
		Clad.	**Nig.**	**Alte.**	**Cur.**	**Fusa.**	**Bipo.**	**Pho.**
	Control	83	78	21	5	19	3	0
Tillering	Caulim®	50	50	24	6	25	0	1
	Sifol®	53	32	24	5	25	3	0
Booting	Caulim®	58	60	18	5	12	3	0
	Sifol®	49	50	27	1	14	1	0
Flowering	Caulim®	40	38	22	2	12	1	1
	Sifol®	41	36	29	7	18	1	3
		Health seed test (%) Puitá Inta CL						
	Control	41	36	29	7	18	1	3
Tillering	Caulim®	36	66	22	7	8	2	0
	Sifol®	30	71	23	4	11	1	0
Booting	Caulim®	30	42	24	6	25	3	0
	Sifol®	38	35	21	6	14	0	0
Flowering	Caulim®	34	49	24	3	14	0	0
	Sifol®	31	31	16	5	11	1	1

* Fungi identified in health test: *Cladosporium* sp., *Nigrospora* sp., *Alternaria* sp., *Curvularia* sp., *Fusarium* sp., *Bipolaris oryzae* e *Phoma* sp.

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Full Length Research Paper

Release of fenugreek (*Trigonella foenum-graecum* L.) Variety “Bishoftu”

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Even if there are released Fenugreek varieties in Ethiopia, there is no variety specifically for color quality purpose. This study was conducted based on the objective of evaluation of different colored Fenugreek germplasm for yield and market preference in Ethiopia. Evaluation and preliminary variety trial were conducted at Debrezeit, Chefe donsa and Akaki locations. While national variety trial was conducted at Debrezeit, Chefe donsa, Akaki, Kulumsa, Sinana, Gonder, Sirinka, and Assosa having nine genotypes including check variety. The standard experimental procedures were used in the experiment. The result showed that, there is a significant difference among tested genotypes over locations; however, the objective of the experiment is to evaluate nine fenugreek genotypes including check variety considering both market seed color preference and yield. The study found FG-10(Bishoftu) variety had white seed color. This color is preferred color in international market. This variety therefore, was released for production for all fenugreek growing environments of East Shoa and similar environments.

Key words: Fenugreek, market seed color, quality, Bishoftu.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L) is an annual, self-pollinating, legume crop, believed to be native to the Mediterranean region but now, is widely cultivated in India and other parts of the world (Acharya et al., 2006). It originated from the countries bordering on the eastern shores of the Mediterranean and is widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. (Polhil and Raven, 1981; Petropoulos, 2002; Acharya et al., 2006; Davoud et al., 2010). Ethiopia is also known as the original homeland of fenugreek subspecies Mediterranean, ecotype Abyssinians with its distribution extending to Eritrea and Somalia (Sinskaya, 1950). The word fenugreek in Amharic is “Abish”, and the seed is often used in Ethiopia as a natural herbal

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Table 1. Summary of ecological and climate description of the testing location

Parameter	Kulumsa	Debrezeit	Sinana	Gondor (gorgora)	Sirinka (Jawi)	Assosa
Average annual temperature	15.1	18.7	14.4	20.8	23.2	21.6
Average annual rainfall	1147	892	930	1099	1513	1116
Altitude	2425	1850	2487	1879	2112	1570

medicine in the treatment of diabetes, as a spice in foods, in artificial flavoring of maple syrup, as a condiment and, in the production of steroid and other hormones for the pharmaceutical industry (Jorgensen, 1988). It is used both as an herb (the leaves) and as a spice (the seed).

Fenugreek seed is widely used as a galactagogue (milk producing agent) by nursing mothers to increase inadequate breast milk supply. Since the maple syrup-like flavor is strong and not always liked, the seeds are ground to a powder and administered in capsules. Studies have shown that fenugreek is a potent stimulator of breast milk production and its use was associated with increases in milk production as much as 90%. According to Central Statistics Agency 2013/2014 report (CSA, 2014), the total area under production was 24, 426.24 ha, and the production was estimated to be over 456, 266.15 quintal.

This is the only widely traded leguminous spice, and is useful in improving soil fertility by fixing nitrogen. It is used extensively for medicinal purposes. The fenugreek herb, seed, powders, and extracts are known to possess several pharmacological effects, like hypoglycemic, hypocholesterolemic, antinociceptive, antioxidative, laxative, and fungicidal effects, as well as appetite stimulation (Malhotra, 2011).

So far, the released fenugreek variety in the country is mainly focused on yield aspects and there is no variety released for the purpose of quality. Currently, the world market requires the variety with other quality aspects like seed color. In view of these, this study was conducted with the objective of evaluation of different colored Fenugreek germplasm for yield and market quality (color) for release in Ethiopia.

MATERIALS AND METHODS

Evaluation and preliminary variety trial were conducted at Debrezeit, Chefe donsa and Akaki locations. Eight genotypes which are promoted from preliminary variety trial with control check “Challa” were evaluated as a national variety trial during the season 2015/2016 G.C. This variety trial was conducted as a field trial at Debrezeit, Chefe donsa, Akaki, Kulumsa, Sinana, Gonder, Sirinka, and Assosa. Spacing between plants 10 cm and between raw 30 cm were kept, nine genotypes with recently released control check “Chala” with three replications in RCBD were used. The promoted genotypes are FG-12, FG-45, FG-10, FG-40, FG-48, 52062-01, 52062-02, FG48/01 and CHALA. Plot size of 4 m × 1.2 m (4 row/plot) was used. Spacing of 30 cm b/n row, 3 cm b/n seeds, 50 cm b/n plots and 1 m-1.5 b/n blocks were used for the experiment. Seed rate of 150 seeds/row were used for planting. Yield data will be taken form harvest two central row of each plot (2.4 m^2). Hand weeding was performed during the experiment to make the plot weed free. Data were collected on the following growth and harvest parameters: Days to flowering, days to maturity, thousand seed weight, total biomass, plant height, pod per plant, seed per pod, yield Q/ha and quality were taken. Visual observations were made for the disease reaction of the genotypes. Statistical analysis was carried out using SAS 9.2 version software (Table 1).

RESULTS AND DISCUSSION

Vegetative performance

From the Table 2 there is a signification different P<0.05, among the genotypes over location in all vegetative parameters except seed per pod. The result showed that Genotype FG-48 showed significant and highest thousand seed weight with 2.6 g and FG-10 showed the least with 2.07 g. Number of pods per plant ranges from 3.63 to 4.34. The highest pod per plant was recorded by genotype FG-12 with 4.34 whereas genotype 52062-02 showed the least with 3.63. Table 2 also showed the highest plant height (47.7 cm) by genotype 52062-02 which exceeds the result reported by Issa et al. (2020) which is 34.9 cm.

Yield performance

Table 3 shows that there is a significant difference between genotypes across locations except Kulumsa and rank change observed. There is also significant difference on the genotypes combined location yield performance. Yield variation of different fenugreek varieties was also reported by Anitha et al. (2018) standard check CHALLA showed the highest yield (quintal/hectare) in all individual locations and also combined location. Genotype FG-10 showed the least yield in combined location performance. This result goes in line with results reported by Million (2012). The performance of the genotypes at Gondor location was poor, it is because of the soil type unfavored the crop at the location. From the tested locations, the genotypes performed better at Debrezeit, Chefe donsa and Akaki locations which have soil textural class of vertisol type. There is also a significant genotype by environment interaction. This is due to the genotypes respond in different manner across locations.

The quality of the genotypes in color aspects differ from

Table 2. Combined analysis for vegetative and quality (color) parameters over location.

Genotype	DF	DM	TSW	Biomass	PH	PPP	SPP	Color quality
CHALA	6.43^{bc}	10.80^{ba}	2.17^{b}	1066.7^{ba}	45.20^{ba}	3.79^{ba}	2.83	Green
FG-48/01	6.53^{ba}	10.79^{ba}	2.20^{b}	1066.7^{ba}	46.80^{a}	4.19^{ba}	1.96	Green
FG-48	6.56^{a}	10.39^{b}	2.60^{a}	866.7^{ba}	46.80^{a}	3.89^{ba}	2.86	Green
FG-12	6.53^{ba}	10.88^{a}	2.13^{b}	866.7^{ba}	42.07^{bc}	4.34^{a}	2.46	Grey
52062-01	6.38^{c}	10.51^{ba}	2.20^{b}	966.7^{ba}	47.00^{a}	3.82^{ba}	2.69	Green
FG-10	6.43^{bc}	10.86^{a}	2.07^{b}	866.7^{ba}	39.73^{c}	3.81^{ba}	2.86	White
52062-02	6.45^{bc}	10.72^{ba}	2.37^{ba}	1100.0^{a}	47.07^{a}	3.63^{b}	2.78	Green
FG-40	6.48^{ba}	10.82^{ba}	2.13^{b}	1000.0^{ba}	42.33^{bc}	4.26^{ba}	2.54	Grey
FG-45	6.40^{c}	10.67^{ba}	2.10^{b}	833.3^{b}	43.80^{ba}	4.19^{ba}	2.32	Green
Mean	6.47	10.72	2.22	959.28	44.53	3.99	2.59	-
LSD	0.11	0.45	0.33	247.04	4.19	0.63	ns	-
CV (%)	0.98	2.42	8.69	14.89	5.45	9.17	24.94	-

$P<0.05$. DF: Days to flowering, DM: days to maturity, TSW: thousand seed weight, PH: plant height, PPP: pod per plant, SPP: seed per pod.

Table 3. Fenugreek yield response by location and combined analysis for the year 2015/2016 G.C.

Genotype	Location						Combined analysis yield (quintal/ha)
	Debrezeit	Akaki	Chefe donsa	Kulumsa	Sinana	Gondor	
FG-12	**10.207^{bcd}**	**9.317^{b}**	**17.549^{bcd}**	**8.458**	**10.806^{abc}**	**2.5101^{ab}**	**9.8077^{bcd}**
FG-45	8.828^{cd}	9.496^{ab}	15.608^{cd}	7.542	10.340^{bc}	1.8652^{bc}	8.9465^{cd}
FG-10	**5.811^{d}**	**11.789^{ab}**	**14.299^{d}**	**7.833**	**9.042^{c}**	**1.2892^{c}**	**8.3438^{d}**
FG-40	**9.928^{bcd}**	**11.033^{ab}**	**20.164^{ab}**	**9.792**	**15.104^{a}**	**2.0088^{bc}**	**11.3383^{ab}**
FG-48	7.410^{cd}	12.644^{ab}	17.307^{bcd}	10.014	13.188^{abc}	1.2324^{c}	10.299^{abc}
52062-01	13.735^{ab}	14.583^{ab}	19.437^{abc}	11.111	11.972^{abc}	2.2479^{b}	12.1811^{a}
52062-02	14.900^{a}	12.472^{ab}	19.853^{ab}	10.528	11.132^{abc}	2.7040^{ab}	11.9315^{a}
FG48/01	11.069^{abc}	15.006^{ab}	15.603^{cd}	11.056	14.590^{ab}	1.9135^{bc}	11.5395^{ab}
CHALA	13.564^{ab}	15.969^{a}	23.264^{a}	12.458	12.236^{abc}	3.1958^{a}	13.448^{a}
Mean	10.6057	12.4788	18.1204	9.8657	12.0455	2.1074	10.54843
LSD	4.62	6.602	4.06	ns	4.663	0.84202	1.891
CV	25.17	30.56	12.94	29.25	22.36	23.08	25.78

one another. Genotype FG-10 have ceramic white color while FG-12 and FG-40 have grey color which is different from mostly consumed fenugreek. Since white colored fenugreek has a high market preference, it can be a good option for the producer. The remaining genotypes have normal green color. Genotype FG also have low pungency after drinking which is a good preference for consumer.

Hence, three candidate genotypes which are FG-10, FG-12 and FG-40 applied for registration as a new improved fenugreek variety. Based on the application, the National Variety Release Committee in Ethiopia investigated the performance of FG-10 and visited several locations where the new variety was grown for evaluation, however, the objective of the experiment is to evaluate white and brown seeded fenugreek genotype for quality, and our main focus is to see their response to that of the released "Challa" variety. It is believed that yield can be increased up to some extent by best agronomic management, and due to their seed color quality and have huge potential for export, these three genotypes were selected for verification trial. Among the candidate genotypes FG-10 were selected and released as variety having variety name Bishoftu after it had been evaluated by variety releasing committee.

Recommended ecological zones of adaptation

Fenugreek is a cool season crop and is fairly tolerant to frost and very low (up to freezing) temperature. The plants need relatively cool and low temperature during early stages for better vegetative growth, while a dry and relatively high temperature favors better ripening and high seed production. Thus, it is grown in tropical and temperate, regions owing to its wider adaptability. It is grown from sea level to an altitude of 2000 m. Fenugreek can be grown successfully over all soils (from loam to sandy) but the ideal one is well-drained loam. Soil pH should be between 6.0 and 7.0 for its better growth and development. The crop is fairly tolerant to salinity and can be grown on black vertisols with proper drainage.

Disease reaction

According to visual observation the tested genotype shows different reaction for the fungal major disease powdery mildew and Fusarium wilt at different environment and the diseases severity were not much for the selected candidate variety at high altitude.

Variety maintenance

Breeder and foundation seeds of the variety are maintained by Debre Zeit Agricultural Research Center.

Conclusion

The presence of a variety with good characters than yield is very important. Currently, the world market needs crops with other aspects like quality. The release of these varieties with its distinct color quality brings another choice for the producers as fenugreek is a high source of cash. It is also a variety with important character preferred by importing countries. This variety 'Bishoftu' is characterized by its white color and there is no variety of this kind in Ethiopia. This variety therefore, released for production for all fenugreek growing environments of East Shoa and similar environments. Proper promotion and seed multiplication should be done.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Phenological and grain yield response of hybrid maize varieties, released for differing agro-ecologies, to growing temperatures and planting dates in Ethiopia

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Growing temperatures and planting dates affect phenology and grain yields of maize varieties and farmers have to choose suitable varieties that fit into different planting dates and growing temperatures. A field experiment was conducted to investigate the response of different hybrid maize varieties to different growing temperatures through growing the varieties at different locations varying in altitudes (low land, mid altitude and highlands) under three planting dates. Results revealed that days to seed emergence were influenced by growing temperatures, with days to emergence difference of two weeks observed between Didesa/Uke (high temperature locations) and Holeta (low temperature location). Almost for all varieties except for BH546, days to tasseling and maturity were longer under low temperature at Holeta while they were shorter under high temperature at Didesa and Uke. Early planting resulted in higher grain yields especially at Uke, Bako and Ambo. Grain yield was influenced by the interaction effect of variety and temperature, with BH546 being more yielder than AMH851 under high temperature at Uke. Thus, for most of the tested varieties early planting is recommended, as this will enable the varieties to escape moisture stress that occasionally occurs at grain filling and maturation period, which can seriously affect grain yield.

Key words: Growing temperature, grain yield, maize phenology, maize varieties, planting date.

INTRODUCTION

Maize is one of the major and strategic cereal crops that play an important role in food security and farmers' livelihoods in Ethiopia. Being one of the most important cereals cultivated in Ethiopia both the area and production of maize has shown a sharp increase in the past few decades. Production for instance has increased from 23.9 million quintals in 2004 to 94.9 million quintals in 2018/2019 (CSA, 2004/205; CSA, 2018/2019). Area under improved maize varieties tremendously increased from 14% in 2004 to 59% in 201 (CSA, 2004/2005; CSA, 2018/2019). Maize ranks second after teff (*Eragrositis tef*) in area coverage but ranks first in terms of total

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production and can be grown on a different soil types and temperature regimes in the country. The current national average yield of maize is 4.0 t ha^{-1} (CSA, 2018/2019), which is still low compared to its yield potential. Productivity of maize in Ethiopia ranks second in Sub-Saharan Africa next to South Africa.

In Ethiopia, maize is produced under diverse agro-ecologies ranging from an elevation of 1000 to 2400 masl and from high moisture to moisture stress areas due to the availability of different maize cultivars developed being tailored for each condition (ESA, 2014). Although, maize varieties are released for specific agro-ecology, it was observed that some farmers sometimes grow maize varieties that are not recommended for their area for various reasons including lack of timely supply of the desired suitable varieties (Tesfaye et al., 2019). Temperature and light are the major factors regulating the phenological response of crops including maize (Hatfield and Prueger, 2015). Crop development is usually accelerated under higher temperature (Harrison et al., 2011). This, however, may reduce grain yield through limiting the amount of total solar radiation received by the plant during each developmental stage and in particular at the grain filling stage (White and Reynolds, 2003; Harrison et al., 2011). Thus, temperature regimes seriously affect phenology as well as grain yield (Harrison et al., 2011). However, it may not affect total leaf area as well as total biomass yield (Hatfield, 2016). The influence of temperature on grain yield is related to its effect on number of kernels per ear (Hatfield, 2016).

Planting date also affects maize phenology such as days to tasseling, silking, maturity as well as crop yield (Dahmardeh and Dahmardeh, 2010; Shrestha et al., 2016, 2018; Lizaso et al., 2018; Baum et al., 2019). Delay in planting time and low soil temperatures reduced days to seed emergence (Dos Santos et al., 2019) as well as maize grain yields (Baum et al., 2019). Owing to the difference in maturity and length of growing seasons, the ideal planting dates for hybrid maize vary among locations that highly contrast in growing temperatures and even seasonally with locations due to varying weather (Tsimba et al., 2013). More appropriate planting date was also reported to be dependent on type of maize varieties grown (Beiragi et al., 2011). Thus, it is very important to generate information on the phenological and grain yield responses of maize varieties released in Ethiopia, under different temperatures and planting dates to adjust planting date that best fits each variety at each location. This is because moisture could be limiting if the phenology takes longer period especially in the high altitude areas, where temperatures are lower and each growth phase may take longer time. Therefore, this study was aimed at investigating (i.) the effect of growing temperature on phenology and yields of maize varieties and (ii.) the effect of planting dates on phenology and yields of maize varieties as well as (iii.) to see if there is an interaction effect of varieties, growing temperature and planting date on phenology and yields of maize.

METHODOLOGY

Description of the study areas

The experiments were purposively executed at five test locations that represent low altitude, mid-altitude and high altitude agro-ecology to test the phenolgical and yield response of different maize varities under varying temperature regimes. Soils of the four locations (Dedessa,Uke-Kersa, Bako and Holeta) were Nitisols while Ambo site is dominated by pellic vertisols. Figure 1 shows the locations of the study sites in the context of the country map; Table 1 shows coordinates of the study locations and their altitudes; while Table 2 shows characterstics of the tested maize varieties in terms of their altitude requirement, maturity category and disease reaction.

Treatments and design

During 2016 cropping season 5 hybrid maize varieties (all hybrids) namely (BH546, BH661, Limu, Jibat (AMH851) and Kolba (AMH853), each released for differing agro-ecologies were grown at five locations namely Holeta, Ambo, Bako, Didessa and Uke. These locations had different temperature regimes from very low to very high (see min., max. and average temperatures in Table 1). The varieties were planted at three different sowing times and during each sowing time the treatments were replicated three times, with the varieties arranged in randomized complete block design. Rainfall is assumed as not growth limiting in all the study sites since the rainfall recorded at all the sites was more than the rainfall requirement of maize crop (Tables 1 and 2). All the necessary phenological parameters (Days to emergence, tasseling, maturity) as well as grain yield were recorded.

Crop establishment and management

Land preparation was carried out by ploughing three times and leveled using tractor. Row making (0.75 m) and planting were manually done at all location on May 23, May 30 and 6 June, 2016 cropping season. Maize seeds were planted with 0.75 m inter row and 0.30 m intra row spacing (with plant population of 44,444 plants ha^{-1}). A total of 110 kg N ha^{-1} (92 from Urea and 18 N from DAP) and 20 P (46 P_2O_5) from DAP fertilizer were applied uniformly to all plots as a blanket recommendation. Full dose of DAP fertilizer was applied at planting while urea fertilizer was applied in three equal split at planting, 35 day after planting and flag leaf stage.

The experiments were uniformly managed at all locations to control weeds through repeated hand weeding. The first weeding was done with the aid of traditional hoes at 25-30 days after planting/before first urea application. The second and third weeding was done using the same hand-held hoe during the second top dressing of urea application to also incorporate the applied fertilizers. The fourth weeding was done using locally available sickles. Air temperature was recorded by keeping digital thermometer in the field, at the respective locations.

Data collection

Phenology data: Data on days to emergence, days to tasseling and days to maturity were collected, when 90% of the plant

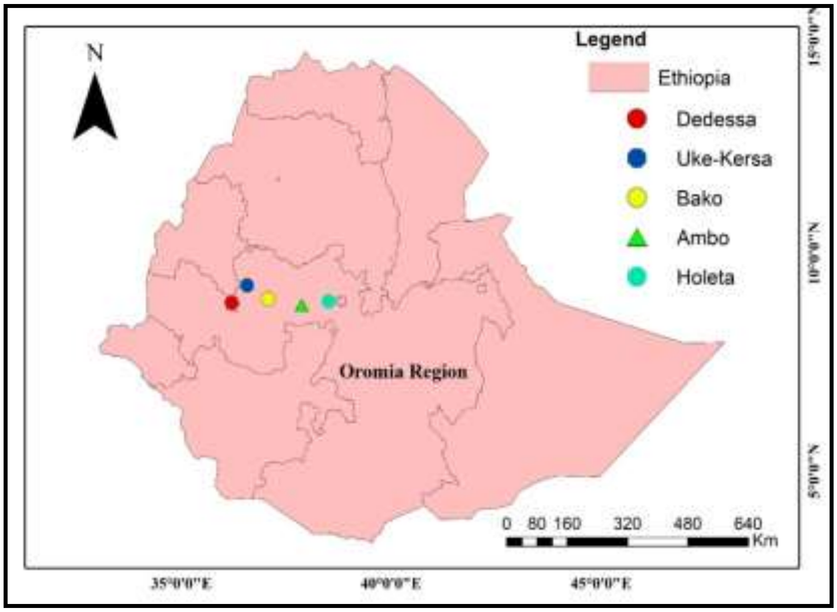

Figure 1. Map showing the five test locations.

Table 1. Temperature, total rainfall, latitude, longitude and altitude of the test locations.

Site	Geographic locations			Air temperature (2016 growing season)			Total rainfall
	Latitude	Longitude	Altitude (masl)	Min (°C)	Max (°C)	Av. (°C)	(mm)
Didessa	9.01008314	36.1704821	1231	20.0	33.0	26.5	2090
Uke-Kersa	9.41832385	36.5398268	1318	18.0	31.0	24.5	2090
Bako	9.10033506	37.0432229	1648	14.0	27.0	20.7	1300
Ambo	8.96768521	37.8597355	2159	10.0	25.0	17.5	1100
Holeta	9.05639602	38.5039351	2352	7.0	21.0	14.0	1040

Table 2. General characteristics of maize varieties tested.

Variety Name	Maturity	Altitude requirement masl	Rainfall requirement (mm)	Reaction to major leaf diseases	Seed colour
BH 661	Late	1600-2200	1000-1500	Tolerant	White
BH 546	Medium	1000-2000	1000-1500	Tolerant	White
AMH 851	Late	1800-2600	1000-1200	Resistant	White
AMH 853	Late	1800-2600	1000-1200	Tolerant	White
Limu	Medium	1200-2000	1000-1500	Tolerant	White

Early 105-120; Medium 120-150 and late 160-180 days.

population has emerged, tasseled and matured respectively.

Grain yield: Harvesting was done at physiological maturity from a net plot area of 4 m × 4.5 m (18 m^2). The total cob weight per plot was determined using hand-held hanging type sensitive balance. The cob weight was converted to field grain weight after determining the shelling percentage of three sample cobs. The field grain weight was then converted to actual grain weight per plot after it was adjusted to the standard moisture content of 12.5% as described in the following formula,

$$Grain\ yield\ (kg\ ha-1) = Cob\ weight * \frac{(100-M)}{(100-12.5)} * 0.81$$

Where, M is the measured moisture content in grain.

Data analysis

Data were analyzed using analysis of variance (ANOVA) procedures using a Statistical Analysis System (SAS), version 9.3 Software, (SAS institute INC., Cary, USA). The ANOVA was computed based on PROC GLM procedure and when ANOVA showed the presence of significant treatment effects, mean separation was carried out using Tukey's test at α=5% level of significance.

RESULTS AND DISCUSSION

Days to emergence

The analysis of variance showed that days to emergence was not influenced by the main effect of variety. However, it was highly influenced by the main effect of growing temperature (P<0.01; Table 3) as well as by the main effect of planting date (P<0.05; Table 3). The interaction effect of variety by temperature and variety by planting date on days to emergence was not significant. However, interaction effects of temperature by planting date and variety by temperature by planting date on days to emergence was significant (P<0.01; Table 3).

Main effects of temperature

Days to emergence was significantly affected by the main effect of temperature. However, termperature and variety did not have interaction effects on days to emergence (Table 3). Sinificantly longer days to emergence was observed at Holeta where the temperature was lower (7/21°C), followed by Ambo (10/25°C). Days to emergence was syonymously shorter for Bako, Uke and Didesa (Figure 2) due to the relatively higher growing temperatures. At these locations, although not measured, the soil temperatures are also higher thus playing significant role in enhancing seed germination and emergence. In line with our observation, Dos Santos et al. (2019) also reported delayed seed emergence of up to two weeks under lower temperature compared to high temperature. They also reported that under extreme low or high soil temperatures percentage seed emergence may even highly decline. Unlike our observation, where we could not see diference in days to emergence between varieties, Kharazmshahi et al. (2015) reported significant effect of maize varieties on days to emergence.

Interaction effect of temperature and planting date:

Days to emergence was significantly influenced both by the main effect of planting date and the interaction effect of growing temperature and planting date. Results indicated that days to emergence did not differ between planting dates for locations such as Uke and Didesa, but differed between planting dates for locations such as Bako, Ambo and Holeta (Table 5). Days to emergence were particularly longer for early planting (May 23) at Ambo and for late planting (June 6) at Holeta under cool temperatures (Table 5). Overall, days to emergence took only seven days at the test locations, which were characterized by warm temperature (Didessa and Uke), while it took between 18 to 22 days at holeta under cool temperature (Table 5). Stone et al. (1998) also reported maize seedling emergence to have occurred after 14 days in cool temperature compared to warm temperature. Likewise, Alessi and Power (1971) reported that seed germination and seedling emergence were delayed if soil temperatures are low and these findings substantiate our observation in the current study.

Days to tasseling

The analysis of variance showed that days to tassling was significantly influenced by the main effects of both variety and growing temperature. However, it was not affected by the main effect of planting date (Table 3). Days to tassling was also influenced by the interaction effects of growing temperature and planting date (P<0.05; Table 3) as well as by the interaction effect of growing temperature and variety (P<0.01; Table 3).

Interaction effects of temperaure, variety and planting date

Days to tasseling was significantly influenced by the interaction effect of growing temperature and variety as well as growing temperature and planting date (Table 3). At Didessa, Uke and Bako under warm growing temperature, days to tasseling was longer for BH661, while at Ambo and Holeta under cool temperatures there was no difference among varieties in terms of days to tasseling (Table 4). For varieties AMH851, AMH853, BH661 and Limu, days to tasseling was significantly the longest at Holeta, where temperature was very low, followed by Ambo and Bako in that order. However, it was synonymously the shortest at Uke and Didesa (Figure 3). Unlike other varieties, for variety BH546, however, days to tassling did not differ between Uke, Didessa and Bako as days to tasseling was synonymously the shortest at all the three locations (Figure 3). Days to tasseling was also significantly influenced by the interaction effect of growing temperature and planting date. Under cool growing temperature at Holeta, days to tasseling was longer for late planting (June 6), whereas at other locations the length of days to tasseling did not significantly differ

Table 3. P values as generated from Analysis of Variance table.

Source	P values			
	Days to emergence	Days to Tasseling	Days to maturity	Grain yield
Variety (V)	0.5 (ns)	<0.01	<0.01	0.24^{ns}
Growing temperature (T)	<0.01	<0.01	<0.01	<0.01
Planting dates (PD)	<0.05	0.08^{ns}	0.07^{ns}	<0.05
V*T	0.58^{ns}	<0.01	<0.05	<0.01
V*PD	0.136^{ns}	0.96^{ns}	0.08^{ns}	0.11^{ns}
T*PD	<0.01	0.05	<0.05	<0.01
V*T*PD	<0.01	0.84^{ns}	0.31^{ns}	0.08^{ns}

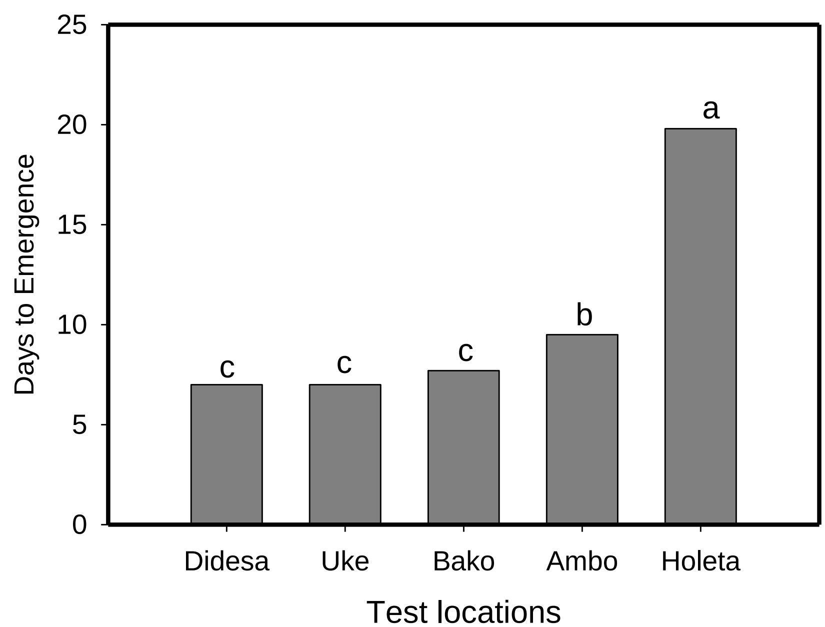

Figure 2. Main effect of location (growing temperature) on days to emergence.

(Table 5). However, contrary to our observation, Shrestha et al. (2016) and Maresma et al. (2019) reported shorter days to silking, for late than for early planting.

Days to maturity

The analysis of variance showed that days to maturity was significantly influenced by the main effects of variety and growing temperature (P<0.01; Table 3) and by the inteaction effect of variety and growing temperature as well as by the interaction effect of growing temperature and planting time (P<0.05 both cases). However, days to maturity was not affected by the main effect of planting time and the interaction of variety and planting time as well as by the interaction effect of variety, growing temperature and planting time (Table 3).

Interaction effect of growing temperaure, variety and planting date

Days to maturity was also significantly influenced by the interaction effect of growing temperature and variety. Under warm temperatures, days to maturity was longer for Bako hybrids (BH661 and BH546); whereas under cool temperatures at Holeta, days to maturity was longer for a pioneer hybrid Limu. Overall, days to maturity steadily increased with the decline in the growing temperature as one moved from Didessa, a low altitude location to Holeta, a high-altitude location. For varieties, AMH851, AMH853 and BH661, days to maturity significantly varied among locations, the longest being at Holeta followed by Ambo and Bako in that order. For the same varieties, days to maturity were the shortest at Didessa. For varieties BH546 and Limu, days to maturity

Table 4. Interaction effects of growing temperature and maize variety on days to tasseling, maturity and grain yields of maize (Effect of varieties at each location).

Growing temperature	Variety	Days to tasseling	Days to maturity	Grain yield (kg ha^{-1})
20/33°C (Didessa)	AMH851	66.0^{b}	125.8^{b}	5383^{a}
	AMH853	66.4^{b}	126.1^{b}	5243^{a}
	BH661	75.4^{a}	139.3^{ab}	6896^{a}
	BH546	75.7^{a}	141.1^{a}	4736^{a}
	Limu	69.2^{b}	129.5^{ab}	5960^{a}
LSD (5%)		6.0	14.0	4155
18/31°C (Uke)	AMH851	67.4^{c}	146.4^{ab}	7258^{b}
	AMH853	66.6^{c}	139.9^{c}	7748^{ab}
	BH661	73.8^{a}	150.2^{a}	8153^{ab}
	BH546	71.1^{b}	146.4^{ab}	9465^{a}
	Limu	70.3^{b}	143.2^{bc}	8515^{ab}
LSD (5%)		2.0	5.3	1844
14/27°C (Bako)	AMH851	74.2^{b}	154.0^{a}	6033^{a}
	AMH853	70.3^{b}	143.1^{b}	7111^{a}
	BH661	82.7^{a}	155.5^{a}	6362^{a}
	BH546	76.2^{ab}	154.6^{a}	8674^{a}
	Limu	76.3^{ab}	154.0^{a}	8271^{a}
LSD (5%)		7.1	5.5	3833
10/25°C (Ambo)	AMH851	91.2^{a}	194.1^{a}	9884^{a}
	AMH853	89.3^{a}	187.3^{a}	9713^{a}
	BH661	99.2^{a}	196.7^{a}	8947^{a}
	BH546	95.8^{a}	192.4^{a}	9007^{a}
	Limu	95.8^{a}	183.4^{a}	8365^{a}
LSD (5%)		2.35	18.4	3493
7/21°C (Holeta)	AMH851	124.1a	185.7ab	ND
	AMH853	122.3a	182.7b	ND
	BH661	123.0a	185.7ab	ND
	BH546	122.7a	185.0ab	ND
	Limu	124.9a	187.3a	ND
LSD (5%)		8.3	3.3	-

ND: Not determined.

did not vary between Holeta and Ambo, where it was significantly the longest and between Didesa and Uke where it was the shortest (Figure 4).

Days to maturity was also significantly influenced by the interaction effect of growing temperature and planting time (Table 3). Under warm temperature, at Uke, late planting (June 6) resulted in longer days to maturity while under cool weather at Holeta, early planting (May 23) resulted in longer days to maturity. Thus, there is no clear trend of planting date effect on days to maturity since the influence of planting date on days to maturity varied with growing temperatures of the locations. At the other three locations, however, days to maturity did not significantly differ between planting dates. According to reports of Shrestha et al. (2016), days to attain different phenological stages decreased with late sowing, which agrees with our observation under lower temperature (7/21°C) at Holeta, where days to maturity decreased with the progress of planting time. Such crop strategy will enable the crop to escape the expected moisture limitation at the end of the season, which usually happens with late planting. However, at a growing temperature of 18/31°C (min/max at Uke) which is warm, days to maturity was longer for late planting (June 6), while under cool temperature at Holeta, early planting (May 23) resulted in longer days to maturity.

Grain yield

The analysis of variance indicated that there was main

Table 5. Interaction effects of growing temperature and planting date on days to emergence, tasseling and maturity (effect of planting dates at each location).

Growing temperature	Planting time	Days to emergence	Days to tasseling	Days to Maturity	Grain yield (kg ha^{-1})
20/33^{O}C (Didessa)	May 23	7^{a}	70.6^{a}	134.2^{a}	4319^{b}
	May 30	7^{a}	71.6^{a}	136.2^{a}	4140^{b}
	June 6	7^{a}	69.5^{a}	126.7^{a}	9035^{a}
LSD (5%)		-	5.4	10.2	1759
18/31^{O}C (Uke)	May 23	7^{a}	69.5^{a}	142.8^{b}	8922^{a}
	May 30	7^{a}	70.4^{a}	145.3^{ab}	7542^{b}
	June 6	7^{a}	69.7^{a}	147.7^{a}	8220^{ab}
LSD (5%)		-	2.7	4.3	1268
14/27^{O}C (Bako)	May 23	8^{a}	73.4^{a}	154.3^{a}	7914^{a}
	May 30	7^{b}	76.7^{a}	154.5^{a}	9251^{a}
	June 6	8^{a}	77.9^{a}	147.9^{a}	4875^{b}
LSD (5%)		0.8	5.6	4.76	1990
10/25^{O}C (Ambo)	May 23	12^{a}	94.3^{a}	188.5^{a}	11439^{a}
	May 30	8^{b}	93.9^{a}	192.7^{a}	9112^{b}
	June 6	8^{b}	93.4^{a}	191.2^{a}	6998^{c}
LSD (5%)		0.72	4.1	12.5	1590
7/21^{O}C (Holeta)	May 23	18^{b}	121.9^{b}	186.8^{a}	ND
	May 30	18^{b}	121.3^{b}	184.0^{b}	ND
	June 6	22^{a}	126.9^{a}	184.8^{ab}	ND
LSD (5%)		2.4	4.9	2.27	

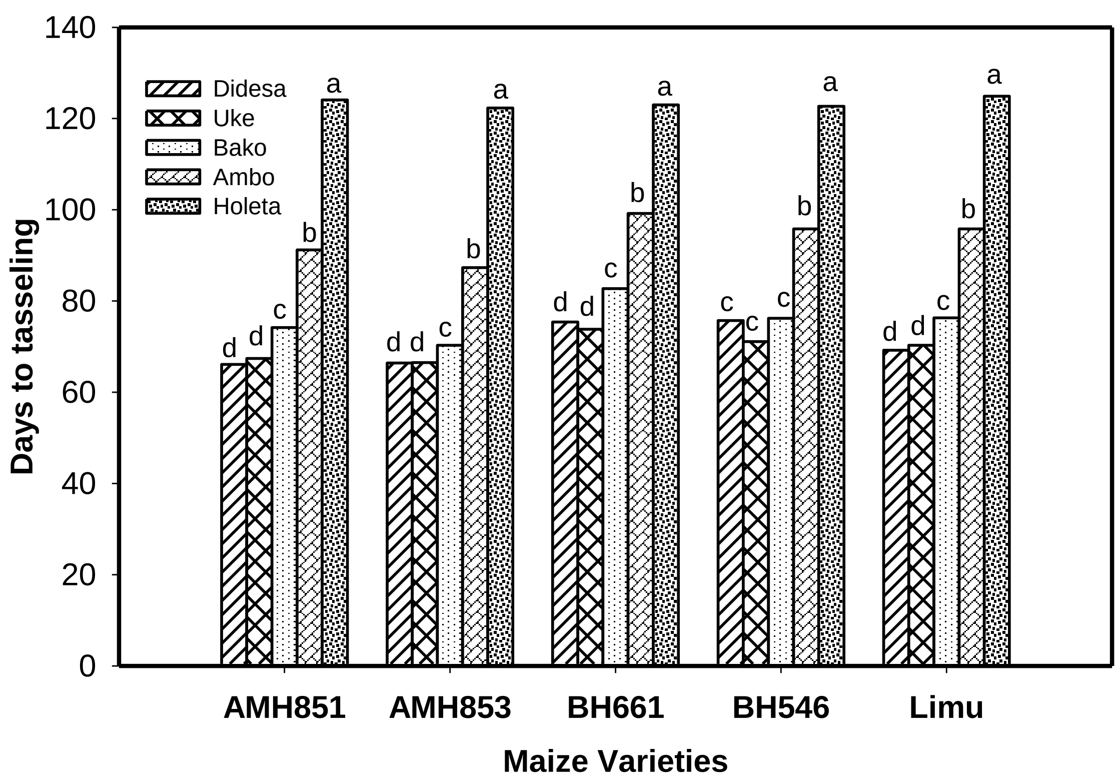

Figure 3. Interaction effect of growing temperatures (locations) and maize varieties on days to tasseling (Effect of growing temperatures/locations on each variety).

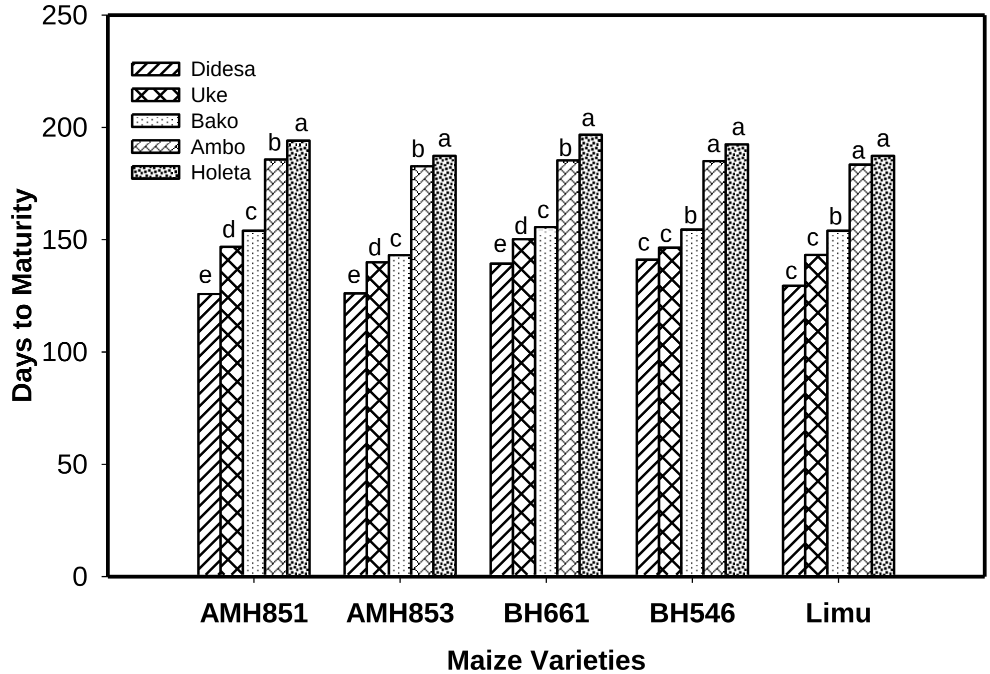

Figure 4. Interaction effect of growing temperatures (locations) and maize varieties on days to maturity (Effect of growing temperatures/locations on each variety)

factor effects of growing temperature (P<0.01) and planting date (P<0.05) on grain yield. There was also an interaction effects of variety and temperaure (P<0.01) as well as planting time and termperature (P<0.01) on grain yield (Table 3).

Interaction effects of temperature, variety and planting date

Although grain yield was significantly affected by the main effect of growing temperature and planting date, this aspect is not discussed in this section since their interaction effect was also significant. Grain yield was influenced by the interaction effect of growing temperature and planting date (P<0.01; Table 3). Under warm temperatures at Didessa, grain yield was significantly higher for late planting (June 6). However, at Uke, where the temperature is still warm, early planting (May 23) gave higher grain yield. At the other two locations (Bako and Ambo) early planting resulted in better grain yield compared to late planting. In line with our findings, Tsimba et al. (2013) also reported high grain yield for early planting of maize compared to late planting, as delayed planting may expose the plant to terminal drought at grain filling stage. Similarly, Varma et al. (2014) also recommended early or at least mid planting for better maize seed yields and quality, which confirms our finding. Many other literatures also witnessed that grain yield is highly influenced by planting date, although the effect is context specific as early planting increases grain yield in some regions (Shrestha et al., 2018; Lizaso et al., 2018; Baum et al., 2019) or reduces grain yield in other regions (Dahmardeh, 2012). There could be yield penalty or complete crop failure if optimum planting window could not be met, especially under the current unpredictable weather changes such as early rain stops as observed in some regions of the country during some years. Lizaso et al. (2018) ascribed the grain yield difference between planting dates to difference in kernel weight since he observed higher kernel weight for the first sowing date which resulted in higher grain yields. Early sowing which resulted in higher yields, however, might be associated to sufficient moisture during grain filling, in our context, not with temperature unlike his speculation.

Grain yield was also affected by the interaction effect of variety and temperature. At Uke, under warm temperature, BH546 gave significantly higher yield compared to AMH851 (Table 4) whereas grain yield did not vary among varieties at the other locations. For varieties, AMH851 and AMH853, grain yield was significantly higher at Ambo than the other three locations (Table 4) and this is acceptable as these varieties were released for similar highland agro-ecologies. For all varieties, the lowest grain yield was recorded under higher temperature at Didesa (Figure 5). Grain yield at Uke and Bako did not show much difference. The reason for the yield reduction at Didesa, where temperature was

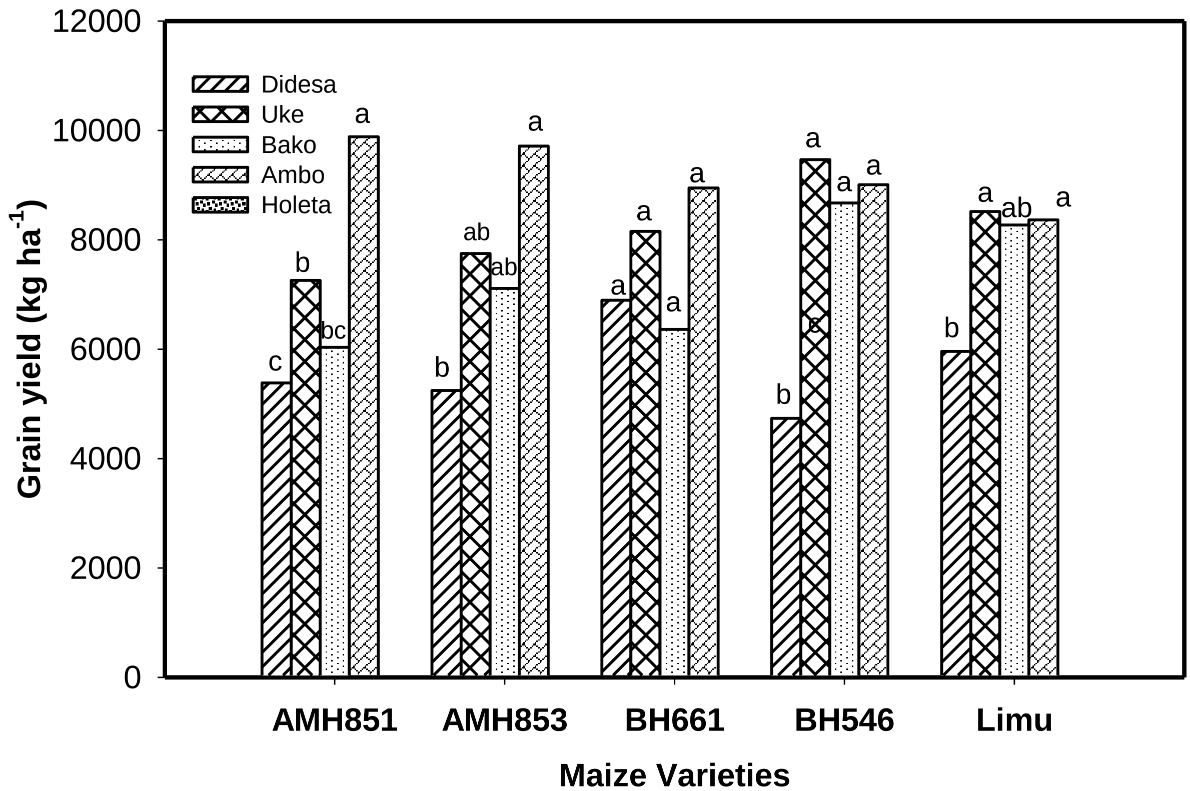

Figure 5. Interaction effect of growing temperatures/locations and varieties on grain yields (Effect of growing temperatures/locations on each variety)

very warm compared to the other locations could be related to higher dark respiration, which negatively affects the crops ability of conserving the carbon fixed through photosynthesis (poor carbon budgeting) as suggested by Hatfield (2016). The reduction in grain yield thus could be related to poor assimilate portioning to the grain resulting in less kenel weight and kenel number per cob as reported by Lizaso et al. (2018). In our study we did not see the interaction effect of maize variety and planting dates, although this was possible in other studies (Beiragi et al., 2011).

Conclusion

Days to seed emergence was influenced by the growing temperature, with days to emergence difference of two weeks observed between Didesa/Uke (high temperature locations) and Holeta (low temperature location). Almost for all varieties except for BH546, days to tasseling and maturity were longer under low temperature at Holeta while they were shorter under high temperature at Didesa and Uke. Early planting resulted in higher grain yields especially at Uke, Bako and Ambo. Yield performance was influenced by the interaction effect of variety and temperature, with BH546 being more yielder than AMH851 under high temperature at Uke. Based on the result of the current study, early planting is recommended, as this will enable the varieties to escape moisture stress that occasionally occurs at grain filling and maturation period, which can seriously affect grain yield. However, such experiments should be repeated to confirm consistence of the results across years to reach reliable conclusion.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of methyl jasmonate on *Acacia senegal* (Hashab trees) production and characteristics of gum

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The study aimed to investigate the effect of the application of different concentrations of Methyl Jasmonate (JA-Me) on yield and quality of gum in Hashab (*Acacia senegal*) trees. Moreover, also studied the anatomy of the treated trees, and characteristics of gums produced. The study was carried out in two locations, Gedarif State (high rainfall area-clay soil) and North Kordofan State (low rainfall area- sand soil) in eastern and western Sudan respectively. JA-Me was applied at three different concentrations (50, 100 and 150 mg/L) as foliar spray and covered with plastic bags for 4-16hours to allow the JA-Me to enter into the tissues. The results showed that a suitable concentration of JA-Me for maximum gum yield per tree was 100 mg\L. The gum ducts in *A. senegal* trees treated by JA-Mewas wider compared to those of untreated (control) trees and finally one large duct was formed, where gum appeared as drops on the bark of the tree. There was no effect of JA-Me (100 mg/L) application on gum shape and colour when compared to the untreated control. The gum produced was red coloured spherical nodules with high solubility in cold water. The significance of the study and its outcomes is very important to treat the trees in areas of gum Arabic trees which have less capability to produce gums due to climatic changes and drought occurred in the areas of Acacia belt in Sudan. The study focused on areas of high production affected by environmental changes.

Key words: Sudan, gum Arabic, gum yield, natural hormones, gum duct, gummosis, Jasmonate.

INTRODUCTION

Jasmonates are a new group of additives plant used to enhance the biological activities and to regulate the growth of some fruit crops like, peach, cherry and apricot, and also promote the gum production. Jasmonates are involved in plant wound responses and defence against insects and fungal elicitors (Mabood et al., 2005). The gummosis in trees in response to the stresses regulated by jasmonates is similar to the mode of action of ethylene. Also, it stimulates anthocyanin accumulation in peach shoots (Saniewski et al., 1998). The effect of JA-Me on the induction of gum was studied in relation to peach (*Prunuspersica* Bactsch), and the studies have shown the induction of various potential defense-relatedproteins in white spruce by jasmonate treatment (Richard et al., 1999,

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Figure 1. Sudan Map , Site I in Gadaref State and Um Rawaba Area Site II in North of Kordofan State.

2000; Lapointe et al., 2001).

This study was carried out based on the areas of less production, and the effect of climate change and other unknown reasons. It is important to find a way to increase the production due to the dependence of local communities on this tree and the use of the product for income generation and is considered as an economic tree in Sudan. The main objectives of this study; were to assess the effect of JA-Me on gum production of Hashab:; (*Acacia senegal*) trees, characteristics of gums produces, and to study the anatomy of the trees.

MATERIALS AND METHODS

Study area

Site I (Gadaref State)

This study was carried out in El Rawashda and Wad Kabo Reserved Forest which is situated at approximately latitude 14° 15´N and longitude 35° 45´E. These forests occur in southern central clay plains at 650 m altitude to the Atbara River. The elevation of El Rawashda Forest is about 540-550 m. The site is characterized by low rain fall savannah woodland on claysoil where *A. senegal*, *Acacia mellifera* and *Acacia seyal* are the dominant tree species.

Site II (Kordofan State)

Kordofan State located at Western of Sudan which is known as drought areas, situated at approximately latitude 14° 22´N and longitude 29° 32´E.The state is surrounded by White Nile State and Darfur Sates. The site occurred in semi desert zone and characterized by sandy soil, locally called *qoz*, and the communities depend on rainfed agriculture and gum production from the trees of *A. senegal*. The study area located within the gum belt which is crossed Sudan from East to West (Figure 1).

Treatment of trees by JA-Me

The forest was planted and divided by compartments, each compartments were arranged in a randomized complete block design. Ten trees with more or less the same age, height, diameter and morphological shape in each block were selected randomly and tapped with a tool called “sonky” and labeled. The JA-Me solution was applied as a foliar spray, and the solution was prepared by dissolving JA-Me in the wetting agent water + alcohol. Two experiments were conducted.

Figure 2. Black Polythene bags used for the protection of the inoculated branches of A. senegal trees.

Experiment I

The method described by Vincent et al. (2001) was followed for the application of JA-Me solution. The solution was applied on both sides of the trunk surface of the tree. Two squares (10×12 cm) were made by cutting 0.5 m of the bark with sonky (local tapping device) in two parts. The two parts were coated with prepared100 mg/L JA-Me in water with 0.1% Tween 20, using a cotton pad for wetting the bark for 5 min.

Experiment II

Trees were originally planted at a spacing of 4 m × 4 m. Three 1 ha blocks of a uniform tree density and stand structure (compartments) at both experimental sites were selected randomly, enumerated, measured and classified according to size. The trees was selected randomly, and two branches per tree were tapped. Forty healthy trees of the same size and age were selected randomly in the two different sites in the same forest for treatment of JA-Me as a foliar spray. The concentration prepared by using amount of JA-Me in mg in one liter of distilled water using the 50, 100 and 150 mg (Molarity calculation). The first ten trees were treated with a concentration of 50 mg/L of JA-Me, and the second was treated with a concentration of100 mg/L, third was treated with 150 mg/L and the last ten trees were left untreated as a control. After spraying branches with JA-Me, the leaves were covered with black polythene bags immediately, and left for 4-16 h (Figures 1 and 2). The gum was collected after two weeks.

Anatomy of treated branches

Preparation of stem samples for microscopy

Method 1

Samples of Hashab branches were collected and cut in strips of 20 mm length and 4 mm width and dropped in fixative 2% paraformaldehyde and 1.25% glutaraldehyde buffered in 50 mmol/L L-piperazine-N-N'-bisacid, pH 7.2). The above strips were left for one night at room temperature, then rinsed with the same buffer, the strips were sectioned (1 µm thick), were cut with a diamond knife, dried onto gelatin-coated slides, and stained with Stevenel's blue. A drop of Canada balsam was placed on the sections followed by a cover slip that was sealed to the slide with nail polish. These sections branches were examined and photographed with photomicroscope.

Method 2

Three to six pieces of Hashab logs from branches with size 5-8 cm in length were cut from stems and branches of treated trees. The pieces were transported directly from the field to the laboratory and preserved in the formaldehyde (10 ml of 40%), ethanol (50 ml of 95%) and glacial acetic acid (5 ml) (FAA) (Figure 3). The preservation period was 7-10 days before sectioning. Staining was done by using the safranin-Fast green type. Clove oil mixed with ethanol (1:1. v/v), series of dilutions (50, 70, 90 and 95%) were prepared. After preparation of sections, by using the slide – microtome and staining the permanent section slides of different treatments were prepared, and examined microscopically to study the structure of gum ducts in all treatment (Sass, 1958).

Collection of gum

After two weeks the gum samples from each treatment were collected per tree and weighed.

Gum characteristics

Nitrogen determination

The nitrogen content of the gum from treated and untreated trees was determined according to a microKjeldahl method AOAC (1984).

Figure 3. Samples of A. senegal branches used for anatomical study.

Viscosity determination

The viscosity of gum from all treated and untreated trees (control) was determined by using a Brook-field model DV – 1+1 viscometer. One gram of gum was dissolved in 100 ml of distilled water in a conical flask to make a 1% solution, about 4% of NaOH solution. The solutions were filtered through 3 µm Millipore filter into clean containers and the viscosity determined using a Cannon Ubbelohde (M130) semi micro dilution viscometer size 75. The viscometer was cleaned by washing with distilled water and dried in acetone. Exactly 2ml samples were pipetted into the reservoir and the viscometer was placed into the holder and inserted into a constant temperature water bath set at 25ºC. The initial relative viscosity was determined. Three subsequent readings for the flow time were taken. Further dilutions of the samples were made *in-situ* by adding appropriate amounts of the solvent and the flow time for each concentration was determined as described previously. This was replicated three times for all the gum samples using a viscometer and the viscosity was expressed in centipoises (cps). The intrinsic viscosity was determined according to the following equations:

Relative viscosity

Reduced Viscosity = $\{V-V_0\}/\ V_0 x C$

Intrinsic Viscosity = $\{V-V_0\} C_0/ V_0 x C$

Where: V = viscosity of the solvent.
V_0 = viscosity of the gum solution.
C = concentration of the gum solution.
C_0 =concentration of solvent.

Specific optical rotation

The specific optical rotation of gum collected from all treatments and control trees was determined by ADP 220 Polarimeter according to AOAC (1984).

Ash value

The ash value of gum from both control and treated trees was determined according to FAO (1990).

Elemental analysis of the produced gum

The cationic composition of gum produced from both treated and untreated trees was determined according to AOAC (1984). One milliliter extract of each sample of gum was placed in 50 ml of distilled water in a conical flask. Three drops of NaOH, with a small amount of peroxide indicator were added with 0.01N EDTA to the violet end point, the contents of the flask were titrated. Calcium, magnesium, manganese, iron, and phosphorus, in the diluted extracts were determined volumetrically by titration against EDTA. The percentage of Fe^{++}, Ca^{++}, P^{-3}, Mg^{++}, and Mn^{++} were calculated as follows:

Fe^{++}, Na^{+} ,Ca^{++} , P^{-3} and Mg^{++}:

Atomic Absorption = V x N. EDTA x 1000/Volume of extract= mg/L

Where:
N = normality of EDTA = 0.01
V = volume of EDTA used
Mg/L × equivalent wt = mg/L (ppm)

m.wt × 100/10^7× weight of the sample =Ca^{++} or Mg^{++}……….

Where: m.wt = molecular weight of element

Statistical analysis

The data checked for homogeneity prior to statistical analysis, and then the statistical analysis was performed with the JMP (3.2.2) statistical software by SAS (JMP 1970s) for gum yield comparison among all the treatment. One way ANOVA and two-way ANOVA were used for all replication in a randomized complete block design. When significant differences were detected, a comparison of all means values was done by Tukey-Kramer HSD all Duncan at Alpha =0.05 combined regression modeling for comparison between the effect of JA-Me and season was also used.

RESULTS AND DISCUSSION

The results presented in Figures 4 and 5 show that the highest amount of gum yield per tree was obtained from trees treated with JA-Me 100 show significant result when compared with JA-Me 50, JA-Me 150 and untreated control. These results are important for determining the appropriate concentration to increase the production of Hashab trees. The effect of 100 mg/L JA-Me on *A. senegal* trees enhanced the flow of gum from the surface bark. Figure 5 shows that when *A. senegal* trees were treated with 100 mg/L JA – Me, gum was exuded from all the surface bark. The produced lumps are pale yellow and relatively large size.

As shown in Figure 6 only JA-Me at 100 mg/L affected significantly the gum production per tree (P<0.0001). There is no significant effect of seasons on gum yield per tree (P=0.13). The survival and growth of the *A. senegal* trees are negatively affected by the frequent drought accentuated with intensive tapping causing a drastic reduction in gum production per area. There is strong belief among local farmers that *A. senegal* trees infested by the insect *Agrilus nubeculosus* (Garaha) produce more gum. The ecology of the insect is yet unknown, but preliminary investigations have shown that there are two microorganisms associated with this insect and might induce gum exudation as a defensive mechanism (Khalil, 2003, Khalil et al., 2011).

This study showed that the JA-Me can induce gum in *A. senegal* trees tapped stems and branches when applied at the concentration of 100 mg/L as a foliar spray, when compared with the concentrations 50, 150 mg/L and control. It induced significant gum yield per tree. This is in agreement with the findings obtained by Saniewski et al. (1998, 2003, 2004, 2006) who stated that JA-Me induced gum formation in plum shoot and fruits, peach, cherry shoots and apricot, in tulip bulbs and stone-fruit trees and their fruits.

The present study indicated that JA-Me induced gums in *A. Senegal* trees, the gum accumulated into the surface of the bark in tapped and untapped branches and bark. This is in conformity with other researchers such as Abeles (1973) and Boothby (1983), who confirmed that the gummosis promoted by Jasmonate (JA-Me). When JA-Me was applied as a spray onto the surfaces of the leaves and small branches of *A. senegal*, the tree gum yield has increased significantly. This study is in line with different researches; reported that; gummosis in trees occurred in response to stresses was regulated by JA-Me.

The gum ducts in *A. senegal* trees treated by JA-Me compared to those of untreated (control) trees (Figure 7), treatments with JA-Me has widened the gum ducts and finally one large duct was formed, where gum appeared as drops on the bark of the tree (Figure 5). Some results showed that traumatic resin ducts might be induced by JA-Me signaling in a dose-dependent manner (Hudgins et al., 2004; Franceschi et al., 2005) and ethylene may cause the resin ducts (found in the bark) to make more resin (Hudgins et al., 2004; Hudgins et al., 2004; Franceschi et al., 2005). The application of JA-Me to *A. senegal* trees has affected the anatomy of the trees (Figure 7). This is similar to the results obtained by Vincent et al. (2001) who stated that when JA-Me was applied to the surface of the trunk of 30+year-old trees has stimulated the production of a compound which induced anatomical changes similar to those caused by wounding or fungal inoculation. To that end, the application of JA-Me to *A. senegal* trees enhanced the flow of gum from the outer bark in large amount. This result is similar to that of Vincent et al. (2001) who proved that the application of JA-Me treatment led to enhanced resin flow from Norway spruce bark wounds. The present study showed that the gum duct in *A. senegal* tree originated directly from the pith through the wood tissues towards the outer bark in tapped area, through the cross section and longitudinal section in all treated trees with JA-Me and micro-organisms. These results are similar to the findings of Morrison and Polito (1985) who stated that, gum ducts have been found in both woody tissues and fruits in all cultivated *Prunus* species

The gum ducts were found to differ in width in treatments with JA-Me, in case of untreated tree the gum was found to exude from the tapped area in the bark while trees treated with 100 mg/L of JA-Megum exuded from both tapped and untapped surface of the bark. These results are similar to those obtained by Christiansen et al. (1999a) Franceschi et al. (2000) Nagy et al. (2000) whose findings showed that the exogenous application of JA-Meto mature or young spruce tree bark led to enhanced resin flow from Norway spruce bark wounds.

All treatments: untreated trees (control), JA-Me (100 mg/L) produced red coloured gum, spherical nodules with high solubility in cold water. Table 1 show the physical and

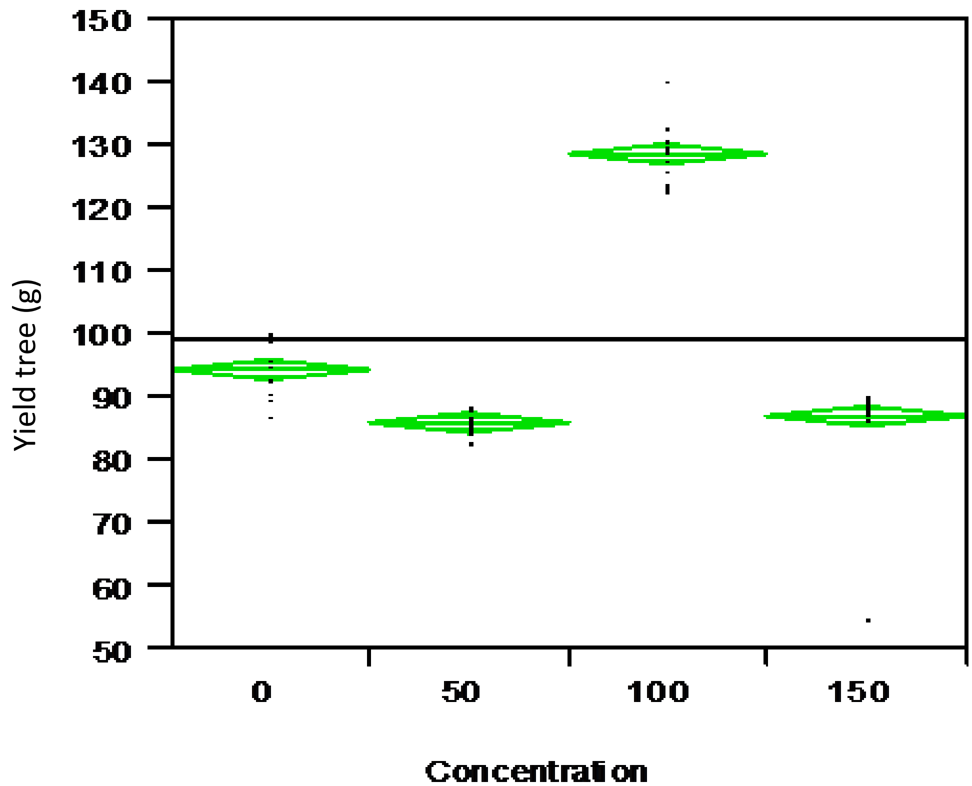
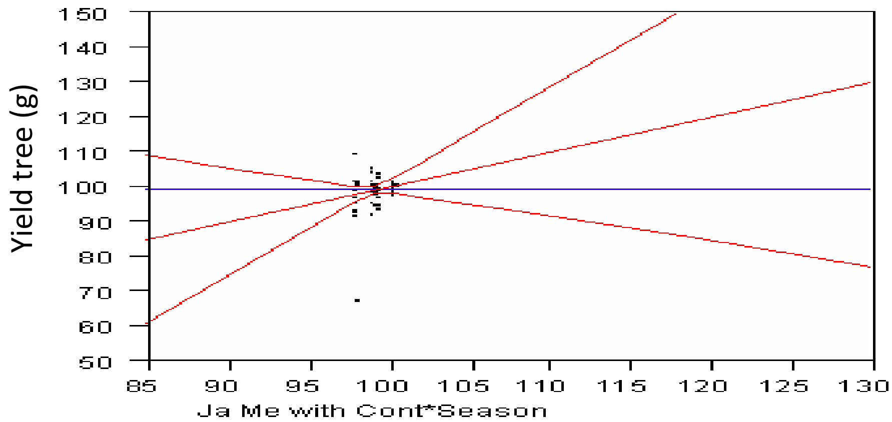


Figure 4. Effect of various concentrations of JA-Me in mg/L on gum yield per tree. 0= Control. Diamonds are means of 10 replicates ± S.E =1.0517.

Figure 5. Effect of JA-Me 100 mg/L on *A. senegal* tree gumming.



Season	2.3494	0.1297
Ja Me with Cont	373.4917	<0.0001
Ja Me with Cont*Season	0.9387	0.4266

Figure 6. Effect of JA-Me concentrations, control and seasons and their interaction on gum yield per tree.

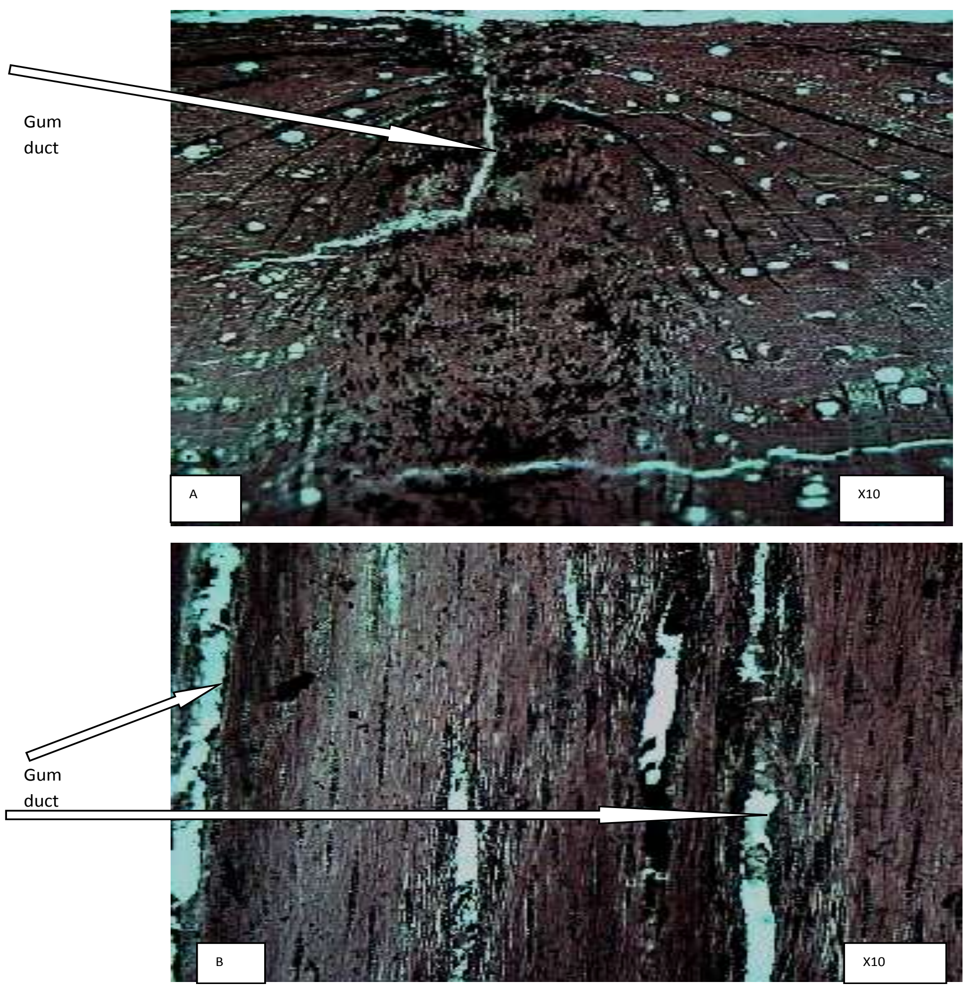


Figure 7. T.S (A) and L.S (B) wood sections of *A. senegal* tree untreated trees showing few and relatively small gum duct.

Table 1. Physical and chemical characteristics of gum.

Specification	Value
Optical rotation*	- 28°*
Viscosity cm^{-3}/g^{-3} ***	3.274*
Ash value **	2.629*
Protein%****	2.263*
Nitrogen%*****	0.362*

* Mean values of 3 determinations.

chemical characteristics of gum.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Correlation between Arbuscular Mycorrhiza (AM) fungi and plant growth of two cassava (*Manihot esculenta* Crantz) clones under Bentex 'T'(Benomyl+Thiram) soil treatments

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Growth response of two clones of *Manihot esculenta* Crantz (Cassava), TMS 30555 and TMS 30572 to Bentex T soil treatment was studied. Mycorrhizal root colonization in relation to growth parameters such as stomata size, plant water content, plant foliation, as well as height and stem circumference was examined. Bentex T, a fungicide which could be used to limit the growth of Arbuscular Mycorrhizal (AM) fungi was added to soil at the concentrations of zero 0 (control), 50, 100, 500 and 1000 µg a.i. /g soil. Growth parameters had minimal variations (p> 0.05) between treatments in both clones of the plant. However, clonal differences at (p< 0.01) occurred in some of the growth parameters. The level of root colonization by the AM fungi affected the growth response of the plant. The untreated soil (control) with the highest AM fungi root colonization (84%) had the least plant foliation (15 and 16) and height (34.1 and 28.5 cm) for TMS 30572 and TMS 30555, respectively. The highest values obtained for stomata size (width and length) were at 50 µg/g bentex concentration; 0.040 and 0.019mm for TMS 30572 and 0.017 and 0.007 for TMS 30555, respectively. The least value obtained for the stomata size was at the zero (0) µg/g bentex concentration. Plants from soil treated with 100 µg/g bentex T concentration had the highest amount of water; 75% for TMS 30572 and 76% for TMS 30555. The untreated soil had plants with the least amount of water. Implications of Bentex T soil treatment of cassava plants was discussed in relation to mycorrhizal colonization rating and some growth parameters of the test plant.

Key words: AM fungal colonization rating, growth response, cassava, Bentex T Soil Treatment

INTRODUCTION

Manihot esculenta Crantz (Cassava) is a dicotyledonous plant belonging to the Euphorbiaceae. The plant originated in North East Brazil and has spread to various parts of the world. In Nigeria, it exhibits great potential in alleviating food shortage problems due to its high yielding ability, wide ecological adaptability and low input

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requirements (Onwueme, 1978). Cassava is one of the most exploited crops in world agriculture, occupying approximately 20 million hectares with a production of about 276 million tons of roots (FAO, 2014). To achieve the higher productivity needed to meet current and future demand, agriculture must literally, return to its roots by rediscovering the importance of healthy soil, drawing on natural sources of crop nutrition since the overuse of mineral fertiliser in agricultural production has carried significant costs to the environment. Crop nutrition can enhance by such biological associations between plant roots and soil mycorrhizae (FAO, 2012). Cassava can grow and produce reasonable yields on soils where other crops would fail. It is highly tolerant of soils with low levels of phosphorus and can generally grow even with no application of phosphorus fertilizer. That is because cassava has formed a mutually beneficial association with a group of soil fungi called arbuscular mycorrhizae (AM) fungi (Howler, 2017). Present in practically all natural soils, mycorrhizae penetrate the cassava roots and feed on the sugars it produces, in exchange for enhanced phosphorus uptake by roots of the plant. Commercial inoculants of AM fungi have also been used. The combined inoculation of *Glomus clarum*, fungi in a study was significant in cassava and fostered better performance in plant growth over time (Lopes et al., 2019). However, one major drawback in the use of commercial inoculants is that the species used might not survive the competition with local AM fungi communities (Rodriguez and Sanders, 2015).

Arbuscular Mycorrhizal (AM) fungi symbiotic associations form when host roots and compatible fungi are both active in close proximity and soil conditions are favorable. The ability of cassava to yield reasonably well in soils low in phosphorus is reported to be due mainly to the crops responsiveness to AM fungi (Kang et al., 1980). It is well known that cassava obligately depends on AM fungi for phosphorus uptake and that this is increased with mycorrhizal associations (Howeler, 2017). In addition to the nutritional function they provide, AM fungi can enhance plant tolerance to both biotic and abiotic stresses (Augé et al., 2015). An ideal experimental system for the study of AM fungi and their effect on cassava growth rate would involve a fungicide that could be used specifically to eliminate or reduce AM fungi, with little or no effect on the remaining biota (Schreiner and Bethlenfalvay, 1996). One of the most widely used of such fungicide is Bentex T (20% benomyl (methyl 1-(butyl carbamoyl) benzimidazol -2-yl carbamate plus 20% Thiram). The measurement of mycorrhizal contribution to crop growth and phosphorus uptake by comparing with a control with inhibited or decreased AM fungi formation has its inherent problem. The main problems in this approach concerns soil sampling, creation of a non-mycorrhizal control and choice of test plant. However, creating the non-mycorrhizal control by benomyl turned out to decrease mycorrhization in a satisfactory degree.

The effects of fungicide targeted on AM fungi are of interest to agriculture since the inhibition of these beneficial microorganisms may counteract benefit derived from them (Schreiner and Bethlenfalvay (1996). It is on the basis of this that this work sought to establish the relationship between arbuscular mycorrhizal colonization rating and cassava growth rate in two clones (TMS 30555 and 30572) of the plant.

MATERIALS AND METHODS

Screened house experiment

Ten-day old seedlings from 2-bud node stem cuttings of two clones of *M. esculenta* Crantz, TMS 30555 and TMS 30572, obtained from a farm stead in Benin City were transplanted from moist sawdust into fungicide-treated soil in polyethene bags at the Department of Crop Science, University of Benin, Benin City. A complete randomized experimental design (CRD) in which five different treatments (bentex T dilutions) were replicated four times and duplicated for each clone of cassava giving a total of 40 samples per clone. Each polybag contained 5 kg of sandy soil taken from a field plot 100 meters from the screened house were the experiment was carried out. The fungicide used for the study Bentex 'T' contained 20% benomyl (methy 1-(butylcarbamoyl) benzimidazole-2-yl carbamate) and 20% Thiram (Tetramethylthiuram disulfide) a seed protectant fungicide as active ingredients (a.i). Fungicide was added to soil at the rate of zero (0), 50, 100, 500 and 1000 µg/g a.i. These rates constituted the five treatments in the study with the zero titre as control.

Measurement of stomatal size of plant

Fresh leaf sample detached from the parent plant was immediately painted with a quick-drying substance, cosmetic nail vanish (Theons UK), to make a leaf impression on the ad axial surface. The leaf impression was carefully removed and placed on a clean slide. A drop of glycerine was added and a cover slip placed on the impression (Hsiao and Fischer, 1975). The size of the stomata aperture was measured under a microscope (x40). The width and the length of the pores were obtained using a calibrated eye piece. Stomata pores appeared as holes.

Determination of plant water status

Shoots of plant were harvested as 08.00 h in the morning and the fresh weight determined. Plants were placed in appropriately labeled sealable paper and over dried at 80°C for 3-5 days. The weights of the dried plants were determined as follows:

$$\%\, water\ content = \frac{fresh\ weight - dry\ weight}{fresh\ weight} \times 100$$

Differences in the plant water status between treatment levels were assessed using the analysis of variance test (Slavik, 1974).

Evaluation of the effects of Bentex T soil treatment on plant foliation, height and stem girth

At the 14th week after planting measurements were taken of the foliation, height and stem circumference of 4 test plants from each treatment levels. Stem circumference data were taken at 2 cm from

Table 1. Stomata size (width and length) and number per unit surface area of cassava grown in soil treated with different concentrations of Bentex T for TMS 30572.

Mean values	Soil treatment (bentex titre – µg/g soil)		
	Zero (control)	50	100
Width of stomata (mm)	0.026	0.040	0.029
Length of stomata(mm)	0.008	0.019	0.013
Number of stomata(per mm^2 leaf area)	11.25	12.0	8.0
Statistical significance		0.040	

p > 0.05 = Significant.

Table 2. Stomata size (width and length) and number per unit surface area of Cassava grown in soil treated with different concentrations of Bentex T for TMS30555.

Mean values	Soil treatment (bentex concentration – µg/g soil)		
	Zero (control)	50	100
Width of stomata(mm)	0.015	0.017	0.014
Length of stomata(mm)	0.006	0.007	0.006
Number of stomata(per mm^2 leaf area)	10.75	12.75	9.75
Statistical significance		0.514	

p > 0.05 = Not Significant.

ground level, while height measurements were from the base of the plant to the tip of the school using a long meter rule. Foliation was done by a manual counting of leaves. Differences in height, foliation and girth data were assessed for significance, using the analysis of variance (ANOVA) test.

Estimation of Mycorrhizal colonization rating in cassava rootlets

The method of Philip and Hayman (1970) was used Root segments of cassava cut 1cm each and fixed in F.A.A. (13 ml formalin, 5 ml glacial acetic acid, 200 ml 50% ethanol) were heated at 90°C for about 1 hour in 10% KOH. This removed the host cytoplasm and most of the nucleus and the roots became clear with the vascular cylinder distinctly visible. The roots were then rinsed in water and acidified with dilute HCl. They were stained by simmering for 5 min in 0.05% trypan blue in lactophenol. Root segments were then mounted on slides temporarily in lactophenol. Slight pressure on the cover slip flattened KOH – treated roots for observation within a limited range of focus (x10). Quantitative estimates of root infection were made on 1cm segments by recording the number of segments with any infection and the amount of infection per unit length of root (Philips and Hayman, 1970).

RESULTS

Plant stomatal size

Data obtained on the size of stomata opening and number of stomata per leaf area for cassava clone TMS 30522 is given in Tables 1 and 2. The highest values obtained for stomatal size (width and length) were at the 50 µg/g bentex concentration; 0.040 and 0.019 mm for TMS 30522 and 0.017 and 0.007 for TMS 30555. The least value obtained for stomata size (width and length) were at the zero (0) bentex concentration (control); 0.026 and 0.008 for TMS 30572 and 0.015 and 0.006 for TMS 30555. Mean number of stomata per lower leaf area obtained ranged between 8.0 -12.0 per mm^2 leaf area for TMS 30572 and 9.75- 11.75 per mm^2 leaf area for TMS 30555.

Plant water content

Data obtained on the water content of two clones of cassava plant (TMS 30572 and 30555) are shown in Table 3. Plant water content varied significantly at $p < 0.05$ between treatment levels in both clones of the plant. Plants from soil amended with 100 µg/g bentex concentration had the highest amount of water; 75% for TMS 30572 and 76% for TMS 30555. Analysis of variance carried out on the data showed a significance difference at ($p < 0.05$) between treatments in water content of the cassava clones.

Plant foliation, height and stem circumference

Plant treated with 500 and 1000 µg/g bentex concentration had evident phytotoxic symptoms; chlorotic dry leaves, stunted growth and heavy defoliation characterized these plants. These plants eventually withered and died after about 8 weeks of planting. Data

Table 3. Water content (%) of cassava plant (TMS 30572 and 30555) grown in soil treated with different concentrations of Bentex T.

Cassava	**Soil treatment (bentex titre-µg/g soil)**		
Clones	Zero (control)	50	100
TMS 30572	72	72	75
TMS 30555	73	72	76
Statistical significance		0.001	

$p < 0.05$ = Significant.

Table 4a. Cassava seedlings foliation, stem circumference and height of plant for TMS 30572.

Mean values	**Soil treatment (bentex concentration- µg/g soil)**			
	Zero (control)	**50**	**100**	**Statistical sig.**
Plant foliation	15.3	18.3	16.5	0.086
Stem circ. (cm)	1.00	1.12	0.95	0.091
Plant height (cm)	35.1	34.1	37.3	0.499

$p > 0.05$ = Not significant.

Table 4b. Cassava seedlings foliation, stem circumference and height of plant for TMS 30555.

Mean values	**Soil treatment (bentex titre -µg/g soil)**			
	Zero (control)	**50**	**100**	**Statistical sig.**
Plant foliation	16.0	17.0	16.5	0.045
Stem circ. (cm)	1.01	0.05	0.78	0.005
Plant height (cm)	28.5	38.5	35.5	0.052

$p > 0.05$ = Not significant.

obtained on the number of leaves per plant of two clones of cassava plant (TMS 30572 and 30555) at 14 weeks of planting shows that plants from soil treated with 50 µg/g bentex had the highest number of leaves; 18 for TMS 30572 and 17 for TMS 30555. The untreated soil (control) had plant with the least number of leaves, 15 for TMS 30572 and 16 for TMS 30555 (Tables 4a and b). On the mean height of two clones of cassava plant (TMS 30572 and 30555) at the 14th week of planting. Plants from soil amended with 50 µg/g bentex concentration had the highest plant height; 37.3 cm for TMS 30572 and 38.5 cm. The untreated soil (control) however have the least plant height; 34.1 and 28.5cm for TMS 30572 and 30555 respectively (Tables 4a and b). Differences in measurements between treatments at $p > 0.05$ were not significant in both clones of the plant. Measurement of stem girth of the two clones of cassava plant (TMS 30572 and 30555) at 14 weeks planting ranged between 0.95-1.12 in TMS 30572 and 0.73-1.01 cm in TMS 30555 (Table 4a and b). Differences in measurements between treatments was not significant ($p > 0.05$) in TMS 30572.

Mycorrhizal colonization ratings

The result of mycorrhizal colonization ratings in cassava rootlets after the 14th week of planting in two clones of cassava plant (TMS 30555 and 30572) revealed that the degree of infection by AM fungi infection varied proportionally with the amount of Bentex T applied (Table 5). The infection rates in both clones of the plant were highest among roots of the untreated soil (control); 70 and 84% for TMS 30555 and 30572 respectively and lowest in roots of plants with soil amended with 100 µg/g Bentex T; 48 and 58% for TMS 30555 and 30572 respectively. Endophytes infection ratings for plants grown in soil treated with 500 and 1000 µg/g Bentex T could not be determined because of the phytotoxic effect Bentex T had on the test plant at those titre. Analysis of variance (ANOVA) carried out on the infection data

Table 5. Mycorrhizal colonization ratings (%) of roots segments from 14 weeks old cassava (TMS 30572 and 30555) grown in soil treated with different concentrations of Bentex T.

Sample	Soil treatment (Bentex titre µg/g soil)			
	Zero (control)	50	100	Statistical significance
TMS 30572	84	72	58	0.243
TMS 30555	70	52	48	0.093

C infected number out of 100 root segments examined (%).
p > 0.05 = Not significant.

Table 6. ANOVA for Mycorrhizal colonization rates in both TMS 30572 and 30555.

Source of variation	SS	df	MS	F	P-value^{α}	F crit.
TMS	1231.7	1	1232.7	75.983	*0.0070	4.4139
Treatment	2360.3	2	1180.2	72.750	*0.0024	3.5546
Interaction	112.33	2	56.167	3.462	^{NS}0.0534	3.5546
Within	292.00	18	16.222			
Total	3397.3	23				

α* significant at p < 0.01, F = frequency p = calculated value.
NS= Not Significant, F crit. = table value, SS = sum of squares, df = degree of freedom, MS = mean square.

showed no significant difference at (p> 0.05) in the mean mycorrhizal infection between the treatments in both clones of the plant; AM fungi root infections from the unamended soil was not significantly different from those of 50 and 100 µg/g Bentex T treated soils. However, clonal differences were significant at p<0.01 in the mycorrhizal colonization rating (Table 6).

DISCUSSION

The level of root colonization of arbuscular mycorrhiza (AM) fungi affected the growth responses (stomatal size, plant water content, plant height, foliation and stem circumference) of the test cassava plant in both clones of the plant. This is in line with report in a study carried out by Séry et al. (2016) who noted that there was a difference in the way the two native arbuscular mycorrhiza fungi species impacted cassava plant growth in green house conditions stating that AM fungi contributed to the growth and development of the test cassava plant. This study however reports the contrary, as AM colonization of test cassava plant led to growth depression. Habte (1994) reports that the growth response of *M. esculenta* Crantz (cassava) to fungicide treatment ranged from growth promotion to growth depression. Plant growth responses to colonization by mycorrhizal fungi have been observed to have a similar trend (Allen, 1992). This is consistent with this study. Under low Phosphorus (P) conditions, inoculation with in-vitro produced AM fungi inoculants has been effectively employed to reduce P fertiliser requirement for cassava and improve yield (Aliyu et al., 2019). The same response was observed for cotton plants grown in upland Asian soils (Gao et al., 2020). However, as earlier stated, the presence of AM fungi lead to growth depression in the test cassava plant as was observed in the study. The 50µg/g bentex treated soil at the 14th week of cultivation thrived more than the untreated test plant. This can be attributed to competition for carbon and nitrogen between plant and fungus (Hodge and Storer 2015). Plant stomata size was highest at the 50 µg/g bentex concentration and the least value obtained for stomata width and length were at the zero (0) µg/g bentex concentration (Tables 1 and 2). Plant water content varied significantly at p < 0.05 between treatment levels in both clones of the plant. Plants from soil treated with 100 µg/g bentex concentration had the highest amount of water; 75% for TMS 30572 and 76% for TMS 30555 (Table 3a). Augé et al. (2015) reported similarly that arbuscular mycorrhiza symbiosis alters stomatal conductance of host plant more under drought than under amply watered conditions. Mycorrhizal colonization has been reported to improve plant growth and yield by enhancing nutrient absorption in cassava, increase water stress tolerance and nematode resistance (Séry et al., 2016); however, exudes from the hyphae of fungi can stimulate growth of other soil organisms such as bacteria, and this can change the overall structure of the communities (Varela-cervero et al., 2016). This may also be responsible for the depression in growth in the untreated soil (control) reported in this study. Analysis of variance carried out on the data showed significance difference at (p< 0.05) between treatments in water content of the cassava clones (Table 3). Studies have indicated that when water is available, cassava maintains a high stomatal conductance and can keep internal CO_2 concentration high, but when water is limiting, it closes stomata in response even small decreases in soil water potential (Cock, 1985). This observation is consistent with the study.

The level of root colonization by AM fungi affected the growth responses of the cassava plant. Plants with higher mycorrization had reduced stomatal size, plant foliation, height and circumference as compared to the plant with less AM fungi root infection. The untreated soil (control) had plant with the least number of leaves, 15 for TMS 30572 and 16 for TMS 30555 and the least plant height; 34.1 and 28.5cm for TMS 30572 and 30555 respectively (Tables 4a and b). However, studies have shown that AM fungi have benefits that facilitate and improve soil fertility (Azcón-Aguilar and Barea 2015). Hence, as earlier stated, reduced growth responses in the untreated soils may be due to competition between symbionts. Data obtained on the number of leaves per plant of two clones of cassava plant (TMS 30572 and 30555) at 14 weeks of planting shows that plants from soil treated with 50 µg/g bentex had the highest number of leaves; 18 for TMS 30572 and 17 for TMS 30555. On the mean height of two clones of cassava plant (TMS 30572 and 30555) at 14 weeks of planting. Plants from soil treated with 50 µg/g bentex concentration had the highest plant height; 37.3 cm for TMS 30572 and 38.5 cm (Tables 4a and b). Differences in height measurements between treatments at $p>0.05$ were not significant in both clones of the plant (Tables 5a and b). Measurement of stem circumference of the two clones of cassava plant (TMS 30572 and 30555) at the 14th week planting ranged between 0.95 – 1.12 in TMS 30572 and 0.73 -1.01 cm in TMS 30555 (Tables 4a and b). Differences in measurements between treatments was not significant ($p >0.05$) in TMS 30572.

The result of mycorrhizal colonization ratings in cassava rootlets at the 14th week of planting in two clones of cassava plant (TMS 30555 and 30572) revealed that the degree of infection by AM fungi infection varied proportionally with the amount of Bentex T applied (Table 5). The infection rates in both clones of the plant were highest among roots of the untreated soil (control); 70 and 84% for TMS 30555 and 30572 respectively and lowest in roots of plants with soil treated with 100 µg/g Bentex T; 48 and 58% for TMS 30555 and 30572 respectively. The mycorrhizal infection ratings in both clones of the plant were highest among roots with no bentex application (control) and lowest in roots of plants with the highest amount of bentex application. This is consistent with previous findings (Boatman et al., 1978). Mycorrhizal infection ratings for plants grown in soil treated with 500 and 1000 µg/g Bentex T could not be determined because of the phytotoxic effect Bentex T had on the test plant at those titre. Analysis of variance (ANOVA) carried out on the infection data showed no significant difference at ($p>0.05$) in the mean mycorrhizal infection between the treatments in both clones of the plant. AM fungi root infections from the untreated soil was not significantly different from those of 50 and 100 µg/g Bentex T treated soils (Table 5). However, clonal differences were significant at $p<0.01$ in the mycorrhizal infection rating (Table 6). This is in line with studies done by Walder and Vander Heijden (2015) who reported that factors such as environmental conditions and functional diversity, can affect nutrient exchange between the fungi and plant partners. However, some studies carried out with different varieties of cassava suggest that the level of root colonization of AM fungi was probably more dependent on the environmental conditions than on the plant variety (Begoude et al., 2016).

Conclusion

The use of arbuscular mycorrhiza (AM) fungi inoculation in sustainable agriculture is now widespread. The potential of these AM fungi species to promote growth in two clones of cassava species TMS 30555 and 30572 was evaluated. Fungicide was added to soil at the rate of zero (0), 50, 100, 500 and 1000 µg active ingredient per gram in a complete randomized experimental design (CRD). Growth responses observed varied from treatment to treatment and from parameter to parameter. The highest root colonization by AM fungi was observed in the untreated soil. Again, the least value obtained for stomata size, water content, plant foliation, height and circumference was on the untreated soil (control). This clearly shows a level of growth depression in the untreated soil. This growth depression exhibited in the untreated soil due to root colonization by AM fungi may be as a result of competition for carbon and nitrogen between plant and fungus. Clonal variations were observed for most of the parameters studied in the two clones of the cassava plant. The degree of dependency on AM fungi by a host plant has been found to be related to the host species, especially the structure of the host root system, the fungus and soil conditions. Different plant species vary in the response to AM fungi. Even within cultivars and hybrids of the same plant, response to AM fungi may differ.

The measurement of mycorrhizal contribution to crop growth and phosphorus uptake by comparing with a control with inhibited or decreased AM fungi formation has its inherent problem. The main problems in this approach concerns soil sampling, creation of a non-mycorrhizal control and choice of test plant. Mycorrhiza technology should therefore be employed in such a way that it will benefit the plant so its use is not counterproductive.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of DEM resolution on the RUSLE-LS factor and its implications on soil and water management policies through the land cover seasonality

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This study aimed to demonstrate the effects of spatial resolution in modeling water erosion by the Revised Universal Soil Loss Equation (RUSLE). In this study, three specific objectives were defined: Evaluation of the effect of the geographic information source on water erosion, seasonal effects on the potential production of sediments, as well as public policies concerning the different scenarios. The topographic factor (LS) of this equation was determined using four digital terrain models, with different spatial resolutions (10 and 30 m). The results of this factor prove to be influenced by the resolution of the DEM used. The spatial modeling of water erosion was carried out by combining the various input variables of the RUSLE model. The analysis of the obtained erosion maps revealed that its production is influenced by the spatial resolution and by the seasonality, demonstrating that the DEM obtained via DRONE presented the lowest values of soil loss potential in any scenario. Thus, it was verified a need to implement practices for the management of soil cover and conservation to reduce vulnerability to water erosion in the watershed.

Key words: Soil erosion, geographic information system (GIS), watershed, modeling, hydrology.

INTRODUCTION

Soil erosion is characterized as a natural and continuous phenomenon, which may occur to a greater or lesser extent, depending on the degree of association between various factors, such as relief, climatic conditions represented by the intensity of rain, dynamics of water movement in the soil (infiltration and redistribution processes), soil type (texture, hydraulic permeability, porous continuity), and land use and occupation (expansion of agricultural frontiers, waterproofing of urban areas and demographic growth). According to Lepsch (2010), the combinations of these factors lead to ecological and economic losses.

The accelerated erosive process causes changes in the surface runoff and, consequently, in the hydrological dynamics of the watershed, with effects on the decrease of water availability in periods of drought, increased peak flow in the rainy period with a propensity to flooding generation, silting up watercourses, besides the impacts

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on water quality (Nunes and Roig, 2015; Botelho et al., 2018), and also increasing public spending in an attempt to reverse or mitigate this environmental imbalance.

According to Souza Júnior et al. (2017), the current Brazilian model of water management contributes to the increase of problems associated with water scarcity, which becomes necessary to improve the articulation mechanisms between water resources plans with the use of integrated assessment tools, and among these tools, the mapping of the areas most susceptible to erosion.

These tools, therefore, allow the construction of much more effective risk management (Vörösmarty et al., 2010; Kirchhoff et al., 2013), based on the use of hydrological modeling, contributing to improving the efficiency of public spending and management activities of the watershed, since management practices are directly dependent on the estimated soil loss (Ganasri and Ramesh, 2016).

The use of mathematical models for this purpose is essential due to the operating costs for direct measures in large areas, which become impractical from a financial point of view; methodological restrictions and, mainly, the time to obtain the information (Panagos et al., 2015; Rodrigues et al., 2017).

According to Karydas et al. (2014) and Hrabalíková and Janeček (2017), among the more than 80 models currently in existence to estimate potential soil erosion, varying in time and space scales, the models of the USLE family are still the most used. The revised universal soil loss equation - RUSLE (Renard et al., 1997) aims to estimate water erosion from climatic, pedological, and topographic variables, besides the conditions of use, management, and soil conservation practices.

According to Minella et al. (2010), the topographic factor (LS) represents the dynamics of surface runoff in the erosion process in the studied area. Its determination has limitations due to the complexity of the relief, resulting in erroneous estimates of erosion rates, leading to the need to incorporate the concepts of unit stream power (Yang, 1972), associated with the accumulated flow (Desmet and Govers, 1996) and geoprocessing techniques.

Thus, according to Zanin et al. (2017), the accuracy of erosion modeling is based on the ability to be able to explain the physical factors that determined the output result and on the accuracy of each physical factor of input. In this way, the results obtained will have a significant impact on the need to prevent natural disasters due to erosion processes.

In this sense, spatial resolution can have significant effects on soil loss assessment models (Datta and Schack-Kirchner, 2010), and their choice must be guided to reduce errors in topographic attributes (Wu et al., 2005), which allows a better analysis of the assessed area and, consequently, in the development and improvement of watershed management and conservation policies by management committees.

Considering that there is a growing demand for information on environmental impacts, whether resulting from agricultural, industrial activities or urbanization processes on water resources, this work aims to evaluate the effects of spatial resolution in determining the LS factor of RUSLE in a watershed located between the second and third plateau of Paraná. And, also its effect on the potential estimate of erosion based on the seasonal variation of land use and occupation, considering the central months of the seasons and how this information can impact soil management and conservation from a perspective development of public policies.

MATERIALS AND METHODS

Study area

The study area is in an affluent micro watershed of the Pitangui River with a total area of 604.9 ha. This watershed belongs to the watershed of the Tibagi River, in Castro - PR (Figure 1), inserted in the region called Campos Gerais, with a predominance of Latossolo Bruno Ácrico (LBw2) according to survey and soil recognition in the State of Paraná. This region stands out for grown of soybeans, corn, and beans, and it is considered one of the most important milk-producing regions in the country.

Maps database

The mapping of land use and cover considered the central months of the seasons, to be able to assess the effects of seasonality in the estimation of sediment production by the watershed, as well as the effect of anthropogenic dynamics in it. For this, four images obtained from the ESA base of the Sentinel-2 satellite were selected, with a spatial resolution of 10 m and, subsequently, their supervised classification was performed, obtaining the distribution of use as shown in Table 1, generating maps in the scale of 1: 25,000 (Figure 2).

Four databases were used to generate different digital elevation models with their respective spatial resolutions. The first DEM was obtained from an SRTM image prepared by the USGS with a resolution of 30 m (LS1), made available by the Instituto Nacional de Pesquisas Espacias - INPE (National Space Research Institute) through the TOPODATA project. The second DEM was based on contour lines provided by the Laboratório de Pesquisas Aplicadas em Geomorfologia e Geotecnologia - LAGEO-UFPR (Laboratory for Applied Research in Geomorphology and Geotechnology) with a resolution of 10 m (LS2). The third DEM from the database was made available by the Instituto de Terras, Cartografia e Geociências do Paraná - ITCG (Institute of Lands, Cartography, and Geosciences of Paraná), also with 10 m resolution (LS3). And, the fourth DEM was produced from an unmanned aerial vehicle image (DRONE), where the contour lines were extracted through aerophotagrametry, with a resolution of 10 m (LS4).

Potential soil loss estimation

The RUSLE was structured in a GIS environment, allowing the generation of individual and spatial maps of each component of the model aiming to develop the identification map of the area’s most vulnerable to water erosion, varying the spatial resolution and consequently the topographic factor and, the use of the soil from

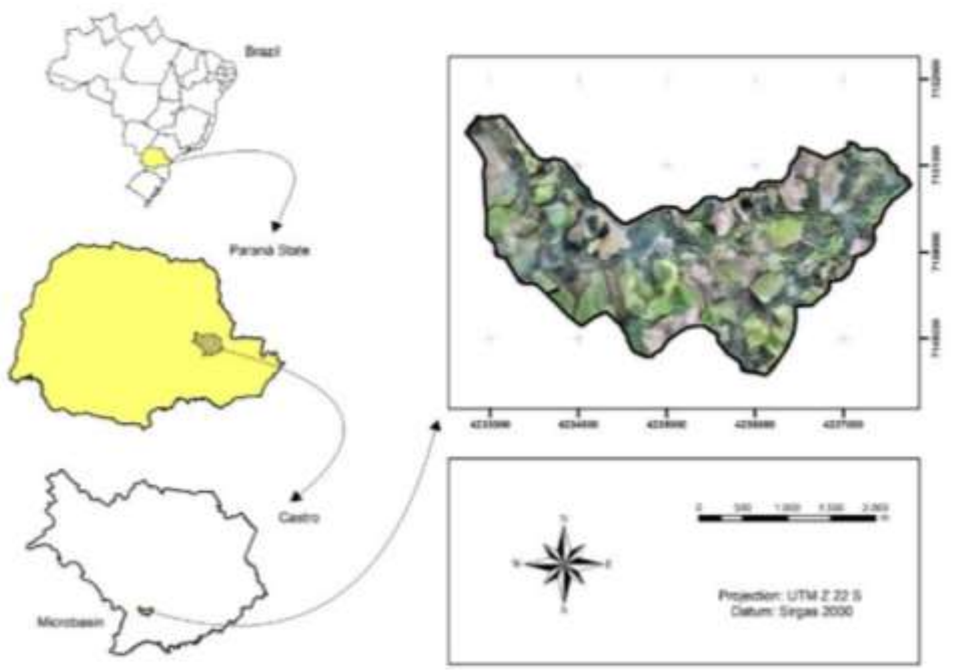

Figure 1. Location map of the study area.

Table 1. Percentage distribution of classes of use relative to central months.

Data	Land cover	Area (ha)	% Distribution	Total (ha)
January 5th, 2018 (Summer)	Annual cropping	260.35	43.04	604.9
	Native forest	216.92	35.86	
	Fallow agriculture	65.57	10.84	
	Pasture land	61.94	10.24	
	Dirt road	0.12	0.02	
April 13th, 2018 (Autumn)	Annual cropping	93.28	15.42	604.9
	Native forest	203.13	33.58	
	Fallow agriculture	164.17	27.14	
	Pasture land	144.21	23.84	
	Dirt road	0.12	0.02	
July 18th, 2018 (Winter)	Annual cropping	117.77	19.47	604.9
	Native forest	203.13	33.58	
	Fallow agriculture	102.05	16.87	
	Pasture land	181.83	30.06	
	Dirt road	0.12	0.02	
October 30th, 2018 (Spring)	Annual cropping	38.47	6.36	604.9
	Native forest	203.00	33.56	
	Fallow agriculture	278.56	46.05	
	Pasture land	84.75	14.01	
	Dirt road	0.12	0.02	

the representative images of the central months of the climatic seasons (January, April, July, and October). Then a linear combination of the factors that characterize erosion was performed, according to Equation 1:

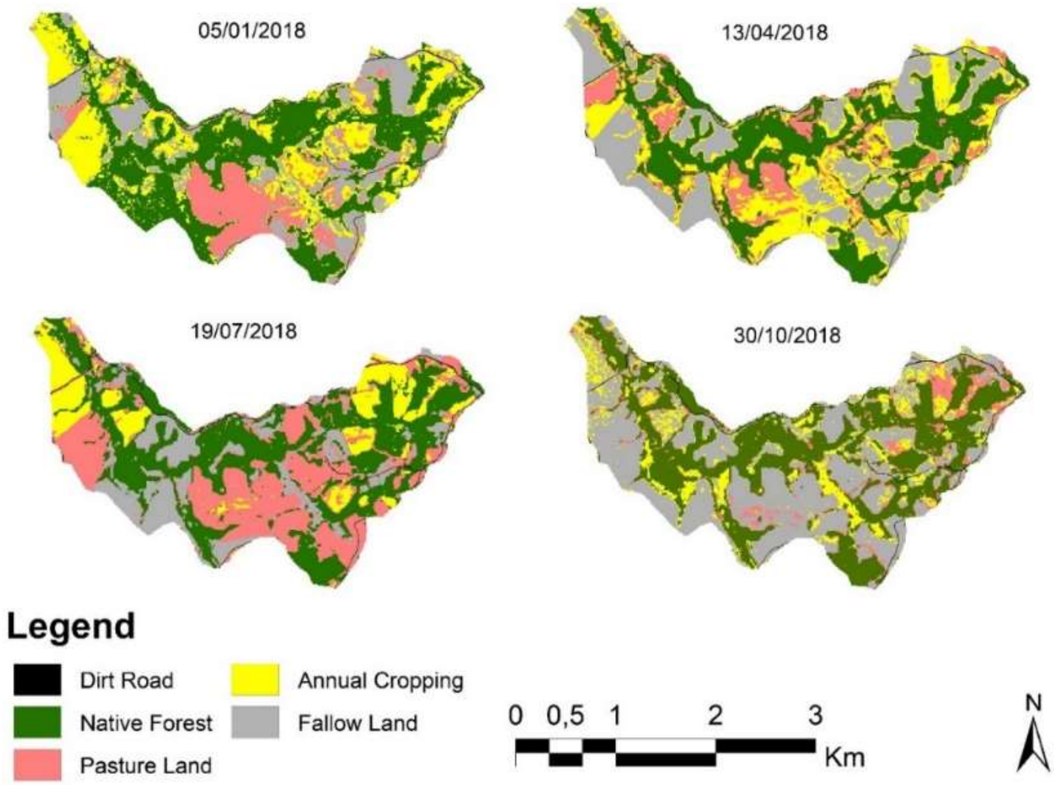

Figure 2. Land use classification map for the central months of the climatic seasons.

$$A = R \cdot K \cdot LS \cdot C \cdot P \quad (1)$$

where A represents the average annual rate of soil erosion per unit area (t ha^{-1} year^{-1}); R is the average annual rainfall erosivity factor (MJ mm ha^{-1} h^{-1} year^{-1}); K is the factor corresponding to soil erodibility (t ha MJ^{-1} mm^{-1}); LS is the topographic factor represented by the length and slope (dimensionless); C corresponds to the soil cover factor (dimensionless), and P is the factor associated with conservationist erosion control practices (dimensionless).

Rain erosivity

Erosivity represents the potential of rain to cause erosion due to the detachment of solid particles due to the kinetic energy of the rain. For the state of Paraná, research aimed at estimating this parameter began in the 1980s, as shown by Netto et al. (2018), with emphasis on the works of Castro Filho et al. (1982), Rufino (1986), Rufino et al. (1993), and Waltrick et al. (2015).

However, even in the condition highlighted earlier, the annual erosivity map for the watershed may not represent the necessary spatial distribution, and unique R values end up being used to characterize an entire watershed. In this sense, it was decided to use a multivariate statistical model, developed by Mello et al. (2013), in which it is proposed to estimate the average annual erosivity as a function of the latitude, longitude, and altitude of each cell in the watershed, allowing to characterize in a distributed way the rain erosivity. It should be noted that this model has been widely used, according to the studies by Oliveira et al. (2014a, b), Rodrigues et al. (2017), Steinmetz et al. (2018), among others. The model developed by Mello et al. (2013) for the southern region of Brazil is as follows:

$$R = 2610770 - 60.44 \cdot A + 98839 \cdot LO - 1114.68 \cdot LA^2 + 938.47 \cdot LO^2 - 1.182 \cdot A \cdot LO + 1.1885 \cdot LA^2 \cdot LO^2 + 0.01494 \cdot LA^2 \cdot LO^3 \quad (2)$$

where R is average annual erosivity (MJ mm ha^{-1} year^{-1}), A is altitude (m), LA corresponds to latitude, and LO refers to longitude, both in negative decimal degrees.

Soil erodibility

Erodibility represents the soil's intrinsic vulnerability to erosion, due to the ease of detachment of solid particles by the impact of the raindrop. This factor can be estimated by different methodologies. According to Marques et al. (2019), one of the alternatives to measure the K factor is from direct measurements in experimental fields under natural or simulated rain, however, under these conditions the estimate becomes costly and time-consuming, even considering the standard method (Lin et al., 2019). Besides this methodology, erodibility can be estimated using pedotransfer function, which uses multiple regression models (Young and Mutchler, 1977; Bertoni and Lombardi Neto, 2005; Marques et al., 2019).

On the other hand, Marques et al. (2019) report that for countries like Brazil, the determination of this parameter is hampered by costs and, therefore, the use of predefined values for some soil classes are commonly used (Beskow et al., 2009). Thus, as the watershed has a ruling class of soils (Latossolo Bruno Ácrico), the value adopted was 0,018 t h MJ^{-1}mm^{-1}, according to Albuquerque et al. (2000).

Table 2. Classes of topographic factor LS.

LS	Class
0 - 1.2	Very low
1.2 - 1.7	Slightly low
1.7 - 3.3	Low
3.3 - 5.5	Moderate
5.5 - 7.5	Moderately high
7.5 - 20	High
> 20	Very high

Table 3. C values for coverage and land use conditions.

Land use	C-factor	Source
Annual cropping	0.253	Bertoni and Lombardi Neto (2008)
Native forest	0.012	Farinasso et al. (2006)
Fallow land	0.5	Panagos et al. (2015)
Pasture land	0.015	Tomazoni et al. (2005)
Dirt road	1	-

Topographic factor

Ahamed et al. (2000) showed that the effect of slope length and its gradient on the erosion process intensity could be determined with the aid of a GIS and in watershed scales, by combining a digital elevation model of the terrain (DEM) with processing algorithms to obtain the cell length and slope in a distributed way.

In the particular case of RUSLE, the calculation of the LS factor incorporates a vital concept associated with the contribution of surface runoff from upstream to downstream, giving a more appropriate physical interpretation to the erosive process than that adopted in the calculation by USLE.

The procedure presented by Moore and Burch (1986) via GIS and equation proposed by Zhang et al. (2013) and Abdo and Salloum (2017) was adopted and used to estimate the value of the LS factor, according to Equation 3.

$$LS = \left(\frac{FA \cdot CS}{22.13}\right)^{0.6} \cdot \left(\frac{\sin(S) \cdot 0.01745}{0.09}\right)^{0.6} \quad (3)$$

where FA is the flow accumulation expressed as the number of grid cells, CS is the raster spatial resolution (m), and S is the slope in degrees.

The LS factor explains the effect of topography on erosion by RUSLE and is calculated as the product slope length sub-factor (L) and slope sub-factor (S). These two subfactors combined represent the ratio of soil loss at a given length and slope of any point from a slope of the unit that has a length of 22.13 m and a slope of 9%, where all other conditions are the same. Thus, the values associated with the LS are not absolute but reference to the value of 1. If <1.0, it represents areas less erosive than the standard reference condition. If > 1.0, it represents more erosive conditions than the aforementioned reference (Yang, 2015).

The proposal made by Ruthes et al. (2012) was adopted to classify the LS factor, which adapted the classification by Fornelos and Neves (2007), and it is presented in Table 2.

Erosion control practice factor (P) and cover management factor (C)

Factor P represents management practices that contribute to erosion control. However, due to the difficulty in identifying such practices through satellite images, it was decided to adopt their value equal to 1, as seen in similar works, especially those of Vemu and Pinnamaneni (2011), Pradhan et al. (2012), Silva et al. (2012), Oliveira et al. (2014), Bera (2017), and Steinmetz et al. (2018).

Factor C represents the conditions that can be easily changed to contain soil erosion, ranging from 0 to 1, where values close to 1 indicate areas with almost null vegetation cover and, therefore, more susceptible to water erosion. The classes of use were defined, as well as their percentage of occupation (Table 3) using satellite images of the central months of the climatic seasons. Then C values were adopted according to studies published for the same uses in Brazil, which are shown in Table 3.

RESULTS AND DISCUSSION

About the DEMs produced by the different databases, variations were observed concerning the altitudes between the models, based on the layout of the topographic dividers initially obtained from the LAGEO-UFPR base.

The DEM generated from DRONE showed values that ranged from 930.49 to 997.86 m, with an image of 60,490 pixels and a resolution of 10 m. The image obtained via SRTM, in turn, had an elevation ranging from 939.22 to 1022.66 m, with approximately 6,910 pixels and a resolution of 30 m. Considering the LAGEO-UFPR and ITCG bases, both with a spatial resolution of 10 m and 60.490 pixels, a small difference was observed, the first

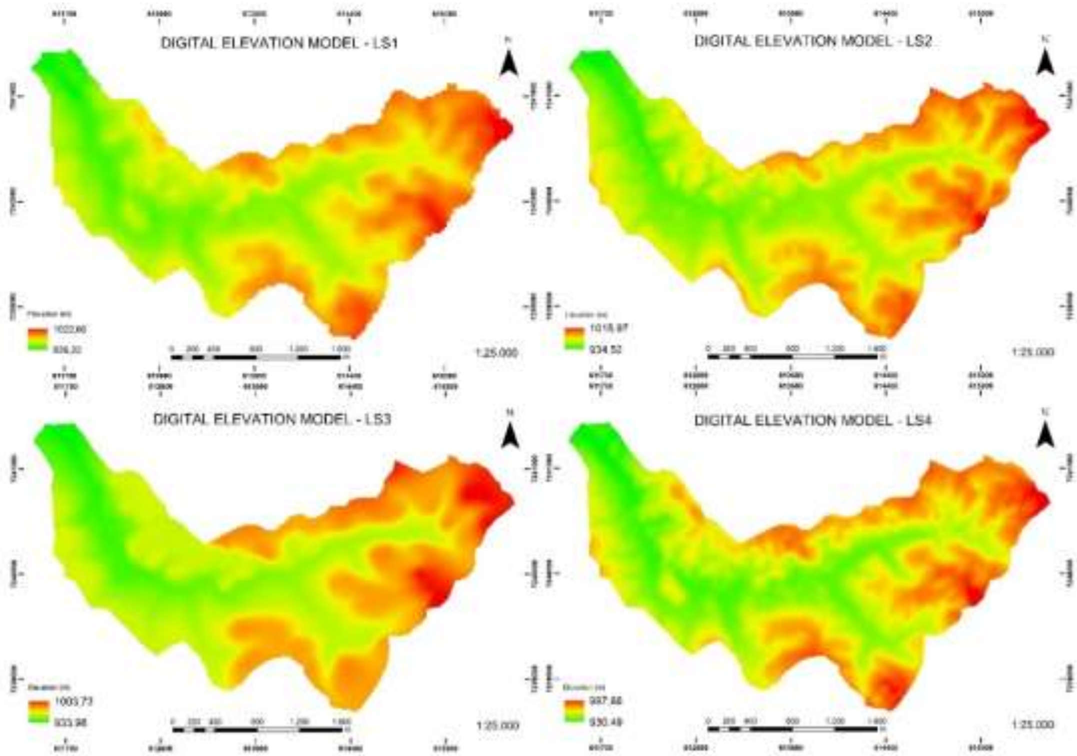

Figure 3. Digital elevation model from the bases SRTM, LAGEO, ITCG, and from the DRONE.

Table 4. Classes for the LS factor.

Classification	LS1	LS2	LS3	LS4
	(ha)			
Very low (0 - 1.2)	234.99	455.05	413.42	556.52
Slightly low (1.2 - 1.7)	84.33	67.74	96.53	19.35
Low (1.7 - 3.3)	174.87	59.9	79.86	16.78
Moderate (3.3 - 5.5)	75.15	16.56	10.16	7.28
Moderately high (5.5 - 7.5)	19.44	4.46	2.95	2.67
High (7.5 - 20)	29.88	1.22	1.98	1.44
Very high (>20)	3.24	-	-	0.04

had altitudes between 934.52 and 1015.97 m, and the second had altitudes between 933.98 and 1003.73 m, as can be seen from the analysis of Figure 3.

It verifies that in both maps, it is in the northeastern portion that the highest topographic elevations are concentrated and, such differences between altimetric values, can lead to an increase in the values of the LS factor and, consequently, in the production of sediments from the watershed (Figure 3).

Once the DEMs were obtained, flow accumulation maps were generated, thus allowing the application of Equation 3 to obtain the LS factor. Once the LS maps were generated, the classification proposed by Ruthes et al. (2012) was followed, which was adapted from the classification initially proposed by Fornelos and Neves (2007) (Table 4).

The results presented in Table 4 demonstrate the effect of spatial resolution in the formation of DEM and, consequently, in the spatial pattern of the LS factor, with reflections in the erosion prediction, for example, in the DEM with a resolution of 30 m, there was the lowest percentage of area in the "very low" class.

The occupation of the first three LS classes in more than 90% of the area was similar between the models LS2, LS3, and LS4, with similarity higher than 95% in the models from cartographic basis. In contrast, the LS1 model, which showed a difference of more than 10% in the first three classes, also being the one with the highest

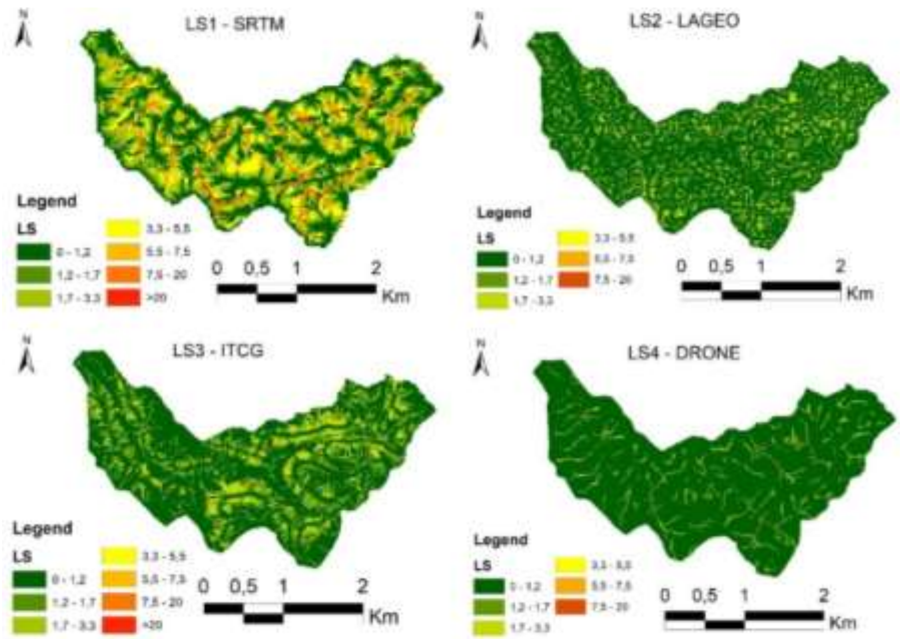

Figure 4. Spatial distribution of the LS factor.

percentage of area in the last three classes, even if not concentrated in the watershed.

About the topographic factor from the SRTM image, this map presented a maximum value of 38.10, with an average of 2.29, and among all generated maps, the one that presented the highest LS values. LS2 had a maximum value of 13.55 and an average of 0.86. LS3 map had 16.98 and 0.93 for the maximum and average value, respectively. About the map obtained via DRONE, the maximum value obtained was 21.02, and the average was 0.52. Figure 4 shows the spatial distribution of the LS factor in the watershed for each image studied.

This behavior can also be observed in Zhang et al. (2008), who when evaluating the effects of the resolution and the data source in the erosion modelling in two American forest watersheds, observed that both the resolution and the source of the models generated shapes and varied structures. This led to different lengths and slopes of reliefs and channels, producing different predictions of water erosion.

For Yang (2015), LS values calculated from different sources reveal that higher DEM resolutions produce more detailed LS maps; and lower quality resolutions tend to overestimate the value of this factor. These differences are more noticeable for a higher range of LS (LS>10), as shown in Figure 5.

About the LS4, this image obtained a better representation of the landscape, since a high resolution allows us to absorb and capture with greater precision the geomorphological aspects of the surface. In contrast, the LS2 and LS3 images showed a distribution between similar classes.

As shown by Aziz et al. (2012), depending on the input source, estimation methods, and procedures used to generate the DEM, the DEM may contain errors, which can affect the estimate not only of the LS factor but also of other parameters derived from the DEM. Thus, the choice of which resolution to use should consider which images are available and which one can represent all the characteristics of the watershed.

Zhao et al. (2010) show that the prediction of soil loss by RUSLE through high-resolution DEM is more appropriate since other resolutions may not represent the impact of deviation terraces in reducing soil loss.

These results reinforce the importance of adapting the mapping objectives according to the DEM, since changes in cell size cause differences between the slope maps, which can generate results that are either more conservative or more alarming from environmental management, affecting the adoption of conservationist policies in a given area.

According to Beskow et al. (2009), areas with LS greater than 10 are considered to be highly vulnerable to erosion, and therefore, erosion control mechanisms should be encouraged (Steinmetz et al., 2018), such as maintaining and improving vegetation cover and conservation practices of soil.

The results regarding the behavior of the LS factor as a function of spatial resolution, demonstrate that there is, effectively, a difference in the direction of flow and, consequently, in the topographic effect in the formation of water erosion, as was observed in the work of Panagos et al. (2015) who assessed water erosion in Europe.

Contrarily, only the topographic condition is not enough

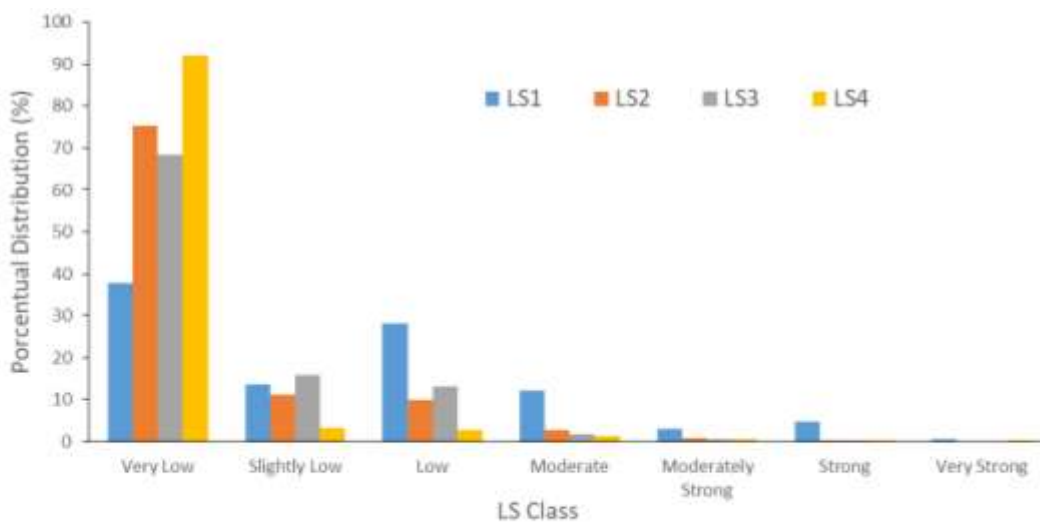

Figure 5. Percentage distribution of LS classes from spatial resolution.

to analyze the production of sediments in a watershed and, therefore, one must evaluate together with the other factors of RUSLE, mainly with the maps of land use and cover.

Given the spatial distribution of the LS factor, it is expected that maps obtained with the best resolutions, tend to produce more accurate DEM. Which results in less soil loss, as well as higher chances of success in monitoring areas degraded by water erosion, since most studies in this line in Brazil use SRTM 30 m data.

Once the other factors of RUSLE were consolidated, it was applied to estimate soil loss in different scenarios, since the process is dynamic and can range according to the use and occupation of the land. Thus, the distribution of erosion by climatic season is shown in Figure 6, for each of the resolutions, which allows identifying which periods require watershed management activities.

For the conditions presented, it is noted that with the decrease of the natural areas from autumn and with another small reduction in spring, areas susceptible to erosion processes increase, represented by the increase of fallow agriculture areas in autumn and spring. There was an increase in the area occupied with pasture between autumn and winter, with a reduction in the fallow area, which is traditionally characterized by the presence of exposed soil.

About the summer, the average potential erosion was higher on the map produced for the LS1 condition, with an average production of 45.08 t ha^{-1} $year^{-1}$ of sediments. In contrast, the best condition obtained was from the DRONE image, with an average production of 10.64 t ha^{-1} $year^{-1}$. For the LS2 and LS3 images, the average potential sediment production was 18.75 and 19.78 t ha^{-1} $year^{-1}$, respectively.

About the periods, the highest estimated values in any scenario were in the areas close to the watershed outlet, where there is intense agricultural activity, and in the areas identified as a dirt road. According to Minella et al. (2007), these areas are the main sources of sediment production in a hydrographic watershed, and once the source of erosion has been identified, the implications for soil and water conservation can be assessed.

Less conservationist soil coverings can be replaced by coverings with less erosive potential, especially in places with higher LS values, as suggested by Caten et al. (2012). It is also possible to readjust the layout of rural roads, in addition to the build of rainwater catchment watersheds, reducing the kinetic energy of surface runoff and, consequently, the transport of suspended solids and the dragging of material to other areas of the watershed, as proposed by Casarin and Oliveria (2009).

About the period corresponding to autumn, there was a reduction in the area destined to native forest and conventional agriculture. This period is characterized by the soil preparation for the next harvest and the insertion of cattle in the watershed, justifying the increase in the fallow and pasture areas, respectively. Table 5 presents a summary of the main results found in the scenarios evaluated for water erosion.

The increase in the fallow area tends to increase sediment production. However, the observed increase occurred in flatter areas, decreasing the effect of the LS factor. In contrast, in the areas with higher LS, the pasture was inserted, potentially reducing the effect of erosion. In this period, an average value of 43.07 t ha^{-1} $year^{-1}$ was observed for LS1, 19.41 t ha^{-1} $year^{-1}$ for LS2, 21.20 t ha^{-1} $year^{-1}$ for LS3, and 9.92 t ha^{-1} $year^{-1}$ for LS4. For winter, the average sediment potential decreased in all scenarios, with LS1 presenting 32.45 t ha^{-1} $year^{-1}$; LS2 with 13.46 t ha^{-1} $year^{-1}$; LS3 with an average value of 13.37 t ha^{-1} $year^{-1}$ and LS4 presented 7.24 t ha^{-1} $year^{-1}$.

One of the reasons that may explain this reduction

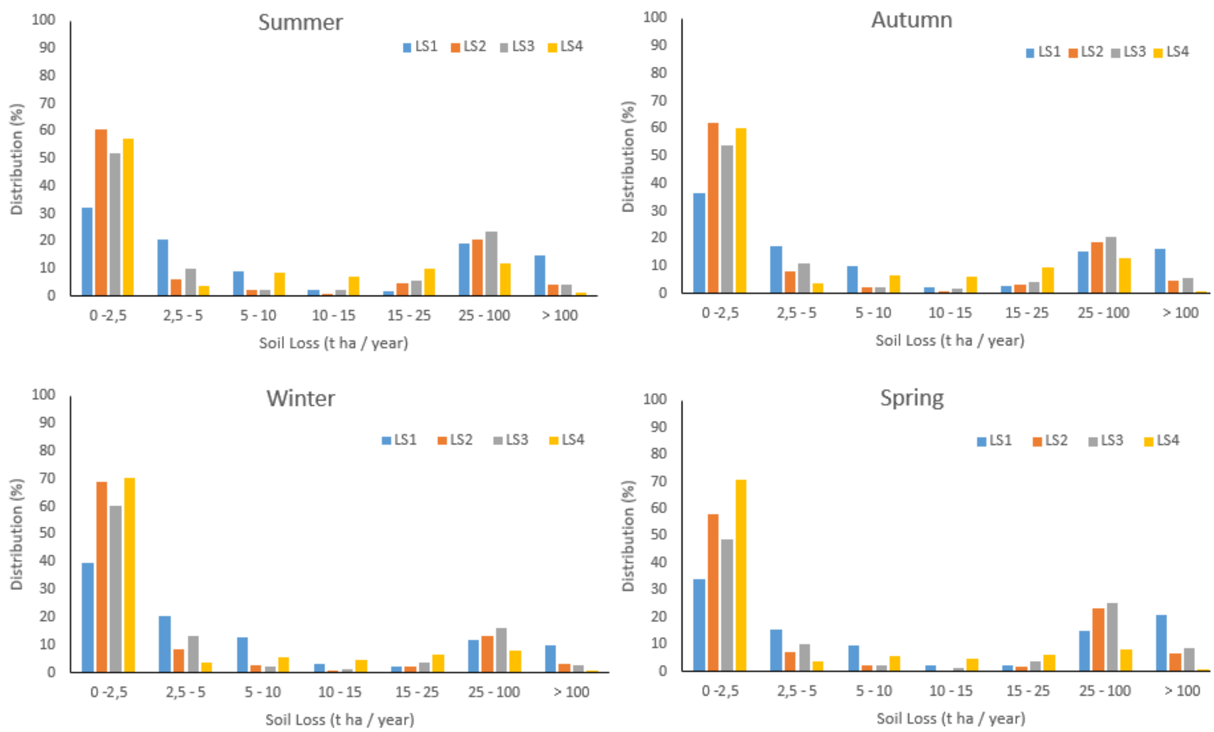

Figure 6. Distribution of the potential erosion in the evaluated scenarios.

Table 5. Estimated soil loss values, according to seasonality.

Season		LS1	LS2	LS3	LS4
	Maximum soil loss (t ha^{-1} year^{-1})	2,411.57	612.28	1073.52	838.50
Summer	Average soil loss (t ha^{-1} year^{-1})	45.08	18.75	19.78	10.64
	Standard deviation (t ha^{-1} year^{-1})	112.67	41.64	40.12	28.32
	Maximum soil loss (t ha^{-1} year^{-1})	1,134.10	733.58	751.23	463.29
Autumn	Average soil loss (t ha^{-1} year^{-1})	43.07	19.41	22.10	9.92
	Standard deviation (t ha^{-1} year^{-1})	88.62	46.53	45.95	22.17
	Maximum soil loss (t ha^{-1} year^{-1})	2,411.57	681.08	652.24	936.81
Winter	Average soil loss (t ha^{-1} year^{-1})	32.45	13.46	13.37	7.24
	Standard deviation (t ha^{-1} year^{-1})	112.21	38.37	32.53	26.24
	Maximum soil loss (t ha^{-1} year^{-1})	1,911.74	733.58	1073.52	597.07
Spring	Average soil loss (t ha^{-1} year^{-1})	53.77	25.31	27.66	13.04
	Standard deviation (t ha^{-1} year^{-1})	106.95	52.44	51.96	27.12

concerning the previous period is the decrease of the fallow area (C = 0.5) and an increase of the pasture area.

For the spring, there was an increase in the average values of potential water erosion, with LS1 presenting 53.77 t ha^{-1} year^{-1}; LS2 had an estimated average production of 25.31 t ha^{-1} year^{-1}; LS3 with approximately 27.66 t ha^{-1} year^{-1}, and LS4 producing 13.04 t ha^{-1} year^{-1}. In the winter, most of the areas were occupied with fallow, with a decrease in pasture areas and native forests.

Traditionally, both autumn and winter period is characterized by the low amount of rainfall events in Brazil, and therefore less sediment transport when compared to periods of convective rain (spring/summer). This behavior can explain the low values of avarege soil loss found in those periods associated to changes in land use and land management that may increase soil erosion, but without significant precipitation, soil losses tend to be less.

The importance of plant cover in controlling water erosion is widely accepted. In the short term, vegetation influences erosion mainly by intercepting rainfall and protecting the soil surface against the impact of rainfall drops, and by intercepting runoff. In the long term, vegetation influences the fluxes of water and sediments by increasing the soil-aggregate stability and cohesion as well as by improving water infiltration (Zuazo and Pleguezuelo, 2008).

According to Beutler et al. (2003), in the spring/summer period, on average, soil losses are twice as high as in the autumn/winter period. Although the values found in this study are not of this magnitude, the estimated losses follow this pattern of behavior in the analyzed periods.

In the case of modeling soil erosion, a higher resolution provides better and more accurate results and will reduce uncertainty, while lower resolution provides generalized results (Mondal et al., 2017).

The analysis of temporal changes occurred serves as a subsidy for the implementation of more efficient erosion control practices, especially about soil management. Mainly because most studies of the potential for water erosion using RUSLE in Brazil, do not consider seasonality and, many times, have overestimated values.

It is observed that natural resources are under increasing pressure due to climate change, population growth, and competing demands for the use of the resource. Which demands not only more effective governance but also a significant improvement in accessibility, especially in the use of information about these possible impacts at the watershed scale, as shown by Vörösmarty et al. (2010), Pahl-Wostl (2007), Kirchhoff et al. (2013), among others.

According to Rodrigues et al. (2017), this type of analysis presents itself as an effective tool to estimate the vulnerability to erosion, since it allows the identification of the most susceptible areas and subsidizes ecological services aimed at sustainability, a key aspect in the development and formalization of public policies for watershed management

Therefore, the use of high-resolution images is an alternative that goes in the direction of this process of development and improvement of environmental policies. Which allows access to more accurate information and, consequently, in reducing costs, be it in the dimensioning of rural roads, in construction of terracing systems or, in the reforestation of the most critical areas for water erosion within the watershed.

Conclusion

Soil loss was estimated to the entire study area, ranging from 0 to more than 2000 t ha^{-1} $year^{-1}$. The most susceptible areas were found in areas with the highest LS values and those classified as dirty roads, therefore reinforcing the need for conservation practices to promote sustainable agricultural practices on more steep terrain. The results found in this study stand out as one of the pioneer studies of this nature for Paraná state, thus playing an essential role in the soil and water resources management of the region. Future studies should focus on direct field measurements of soil loss in this watershed to validate the results estimated according to the RUSLE.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic diversity of elite wheat mutant lines using morphological characters and molecular markers

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Genetic diversity is the material basis for crop improvement. The genetic diversity of 17 wheat genotypes was evaluated using 25 agro-morphological characters and 10 simple sequence repeat (SSR) markers. The objective of this study was to determine the genetic diversity of elite stem rust resistant mutant lines in comparison with their adaptable but susceptible parent varieties using morphological traits and molecular markers. The results obtained showed significant variation in morphological traits and molecular makers existed. Morphological diversity between mutant lines and their parent varieties was mainly separated by grain yield per spike, 1000 grain weight and maturity time period. The dendrogram based on 10 SSR markers grouped the 17 genotypes into three major clusters and six sub-clusters with mutants clustering with their respective parents. 10 SSR primer pairs yielded 13 polymorphic loci with a percentage of 92.86%. The mean number of alleles per locus in each group was 2.0 and the mean number of polymorphic alleles per locus was 1.9286. The gene diversity ranged from 0 to 0.4893 for each sample. Results showed it is possible to classify genetic diversity of elite wheat genotypes and select them for the highest genetic diversity. The results can be used in selecting diverse parents in breeding programs and also in maintaining genetic variation in the germplasm.

Key words: Genetic diversity, molecular markers, morphological traits, wheat.

INTRODUCTION

Wheat (*Triticum aestivum.*L) contributes to food security in Kenya and his ranked second important cereal crop after maize (KALRO, 2016). However, its productivity is low due to abiotic and biotic stresses (Njau et al., 2010). Wheat is a self-pollinating crop that has been bred and developed for specific end-use quality traits and to grow within a specific production environment. Genetic variability holds the potential to deal with multiple biotic and abiotic stresses. Knowledge of genetic diversity of a crop is important in the development and improvement of a particular crop species. Evaluation of genetic diversity among adapted germplasm provides predictive estimates of genetic variations among segregating progeny for new varieties development. It is desirable therefore to have a large genetic diversity for the creation of new genotypes.

Morphological traits and molecular markers play an important role in the analysis of variance in genetic diversity studies. Use of morphological traits alone is unreliable because they are greatly influenced by the

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environment (Takumi et al., 2009). A genotype may exhibit different morphological traits for two different locations (Stepien et al., 2007). Molecular markers increase the breeding progress for traits that are difficult to select under field conditions and those that are controlled by multiple genes. Simple Sequence Repeats (SSRs) were considered the best molecular markers because they are able to identify and differentiate genotypes within a particular species. Their co-dominant inheritance and the high level of polymorphism in a large sample of elite germplasm make them useful (Prasad et al., 2000). Genetic diversity is important for adaptability and survival of genotypes against the threats of biotic and abiotic stresses. Wheat breeding in Kenya has attempted to develop resistant wheat varieties through utilization of resistant genes, but virulence is still being reported in most these varieties (Njau et al., 2009). The objective of this study was to assess genetic diversity of eight pre-selected elite mutant lines in comparison with their adaptive but stem rust susceptible parents and seven other commercial checks using morphological traits and molecular markers.

MATERIALS AND METHODS

Experimental local sites

Three major wheat growing sites were used in Kenya; the first site was at University of Eldoret, on 0°34'N; 35° 18 'E, at 2,153 m above sea level. The average temperature is 18°C with average annual rainfall of 1,100 mm. Second site was at KALRO-Kitale, on 0°33'S; 35° 55'E, at 2,900 m above sea level with average temperatures of 15°C and average rainfall of 1,800 mm. Third site was KALRO-Njoro, on 0°20'S; 35° 56'E, at 2,185 m above sea level with average temperatures of 20°C and average annual rainfall of 900 mm. The experiments were carried out in 2012/2013.

Plant materials

Seventeen wheat genotypes were used in this study comprising of eight pre-selected mutant lines as illustrated in Table 1, from University of Eldoret: SP-9, SP-16, SP-20, SP-21, SP-26, SP-29, SP-31 and SP-34. Two parental varieties: Njoro II (SP-N) and Kwale (SP-K) and seven other commercial checks: Duma (SP-D), Pasa (SP-P), Simba (SP-S), Farasi (SP-F), Robin (SP-R), KS Mwamba (SP-M) and Chozi (SP-C) all sourced from KALRO Njoro Seed Unit. The two parents and the seven commercial check varieties used in this study are popular and moderately susceptible commercial wheat varieties grown in Kenya.

Field experimental procedures

The 17 genotypes were established based on Complete Randomized Block Design with three replications per location. Field experimental plots were 6 rows by 2 m in length with 20 cm inter-row by 5 cm intra-row spacing. Seeds were hand planted with Di-ammonium Phosphate (DAP 18:46:0) at a rate of 125 kg/ha, followed by an application of Urea at 75 kg/ha at tillering and booting stages. Irrigation was carried out when the soils were dried to maintain soil moisture. Wheat agronomic practices were carried out as recommended by Kinyua and Ochieng (2005).

Green house experimental procedure

Seeds (10) of each genotype were planted in the greenhouse in pots using completely randomized design (CRD).

Data collected for morphological analysis

Plants were selected at random for 25 morphological characters and were evaluated to determine morphological diversity. They were divided into qualitative and quantitative traits and were measured or observed as follows: germination (%), plant height (cm), spike length (cm) and flag leaf area (cm^2). Growth habit, seed shape, re-curved flag leaf, spike shape, flag leaf attitude, straw pith, spike density, grain colour, lower glume, sprouting, awns, and shriveling were observed. Number of tillers (no.), lodging (no.), number of grains per spikelet (no.), number of spikelet's per spike (no.), awn length (cm), seed diameter (cm), 50% heading (Days), 50% maturity period (Days), grain yield per spike (g), and 1000 seed weight (g).

Statistical analyses

Analyses of variance (ANOVA) were performed on the quantitative traits using Genstat computer software (Gensat 15th Edition, 2012). Data were subjected to general linear model and analyzed as a RCBD:

$$Y_{ijkl} = \mu + \lambda_i + \pi_{(j)i} + t_k + \lambda t_{jk} + \varepsilon_{ijkl}$$

where Y_{ijkl} = plot observations, μ = overall mean of experiment, λ_i = Season effect, $\pi_{(j)i}$ = Replication within season effect, t_k = genotype effect, λt_{jk} = Interaction of genotype effect, ε_{ijkl} = Residual effect.

Qualitative data were subjected to frequency distribution analyses and assigned numerical values and computed in excel using Shannon-Weavers diversity index (H') = ($\log_e$Pi) **/** $\log_e$n; where H' = Shannons-weaver diversity index, Pi = Frequency proportion of each qualitative trait, n = number of classes per qualitative trait; H' value ranges from 0-1 (0 = absences of diversity and 1 maximum diversity).

Molecular analysis

SSR markers as seen in Table 2 were used to investigate the relationship among the 17 genotypes. 10 seeds of each genotype were planted in pots in the greenhouse and after 4 weeks, leaf tissues were selected randomly from each genotype, cut and crushed together in the laboratory using a mortar and pestle. DNA extraction was performed following modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle, 1990). 45 µl of SDS was added and mixed thoroughly. Samples were then incubated at 65°C in a water bath for 1 h and cooled down for 5 min before adding 220 µl of 5M Potassium Acetate. Samples were then put in ice for 15 min and later on centrifuged for 10 min at 13000 rpm. 700 µl of the supernatant was transferred into a microfuge tube and 600 µl of Chloroform and Isoamyl Alcohol in the ratio (24:1) added and centrifuged again for 10 min at 13000 rpm. 600 µl of the supernatant was transferred into a new tube and ice cold isopropanol added to the samples. They were centrifuged again for 10 min at 13000 rpm to pellet the DNA. The supernatant was poured leaving the pellet. 500 µl of 70% ethanol was added to the DNA pellets and centrifuged at 6500 rpm for 5 min and then gently poured off. The pellets were air dried for 1 h and re-suspended in50 µl distilled water then stored at 4°C.

Table 1. Samples identities and their descriptions.

Sample No.	Sample abbreviation	Sample description
1	SP-D	Duma
2	SP-P	Pasa
3	SP-S	Simba
4	SP-F	Farasi
5	SP-R	Robin
6	SP-N	Njoro II (Parent)
7	SP-M	KS Mwamba
8	SP-C	Chozi
9	SP-K	Kwale (Parent)
10	SP-9	Njoro II Mutant
11	SP-16	Njoro II Mutant
12	SP-20	Njoro II Mutant
13	SP-21	Njoro II Mutant
14	SP-26	Kwale Mutant
15	SP-29	Kwale Mutant
16	SP-31	Kwale Mutant
17	SP-34	Kwale Mutant

Concentration and purity of the extracted DNA was determined using Nanodrop200 spectrophotometer (Thermo Fisher Scientific Inc.) and Gel electrophoresis. All samples exhibited good quality and quantity of DNA for PCR amplification. 1 g of agarose was added to 100 ml of TBE buffer (Tris Boric Edta) and casted to make the gel that was used to quantify the DNA samples (1% gel). Nanodrop spectrophotometer 200 (Applied Biosystems) was used to quantify the extracts. The extracted total nucleic acid was suspended in distilled water and 1 ul of each sample loaded on the spectrophotometer pedant and its absorbance measured. Extracts were run in a 1% agarose gel containing Ethidium bromide staining dye at voltage of 100 V and a current of 400 mA for 30 min and visualized on a UV Trans illuminator.

The study used a scoring method where (1) represented presence of expected band, while (0) was absence of band. Genetic variation at each locus was characterized in terms of observed number of alleles (na), observed heterozygosity (HO), expected heterozygosity (HE), gene diversity and Shannon's diversity index (I) using the genetic analysis packages POPGENE Version 1.32 (Yeh et al., 2000).

In addition, Hardy-Weinberg equilibrium (HWE) was tested by the Chi-squared test. Gene diversity (GD) and polymorphic information content (PIC) were measured by calculating the shared allele frequencies (Weir, 1996) using PowerMarker 3.25 (Liu and Muse, 2005). UPGMA algorithm was used to construct an unrooted phylogram from a distance matrix based on (Nei, 1973) genetic distances, using MEGA4 software implemented in PowerMarker 3.25 (Liu and Muse, 2005).

RESULTS

Morphological diversity of the wheat genotypes

Qualitative traits

Results of qualitative traits showed 94% of the genotypes had erect growth habit, 34% had shriveled grains, 24% had hard red grains, while 36% were soft white grains. Computed diversity ranged from 0.27 to 0.85. Low diversity values for straw pith (0.27) indicated a low variation while shriveled grains showed high variation (0.85) among the 17 genotypes (Table 3).

Quantitative traits

The combined analysis of variance (ANOVA) showed the 17 genotypes were significantly different ($P < 0.05$) for seed weight, seed diameter, spike length, grain yield per spike and number of grains per spike. The seasonal effects were significant ($P < 0.05$) for seed weight, seed diameter, days to maturity, spike length, plant height and grain yield per spike. But no significant difference ($P < 0.05$) was observed from awn length and days to 50% ear emergence. Significant ($P < 0.001$) genotype × season (G × S) interaction were observed for seed weight, seed diameter, spike length, grain yield per spike and number of grains per spike (Table 4).

Pearson's moment correlation

Pearson's correlation (r) showed significant positive correlation between seed weight and seed diameter, seed weight and grain yield per spike. However, significant negative correlation was between seed weight and number of tillers, seed weight and maturity period (Table 6).

Genetic diversity of 17 wheat genotypes based on SSR markers

Total of 10 polymorphic SSR primers were detected after

Table 2. 10 sets of specific stem rust resistance gene marker primers were used.

Resistance gene	Linked marker	Nucleotide sequence	Expected band size	Reference
Sr2	gmw533	F 5'-AAGGCGAATCAAACGGAATA-3' R 5'-GTTGCTTTAGGGGAAAAGCC-3'	120	Börner et al. (2000)
RSr22	WMC633	F 5'- ACA CCA GCG GGG ATA TTT GTT AC -3' R 5'- GTG CAC AAG ACA TGA GGT GGA TT -3'	117	Bhavani et al. (2008)
Sr28	wmc332	F 5'- CAT TTA CAA AGC GCA TGA AGC C -3' R 5'- GAA AAC TTT GGG AAC AAG AGC A -3'	214,217 and 220	Rouse and Jin (2011)
Sr25	BF145935	F 5'- CTT CAC CTC CAA GGA GTT CCA C -3' R 5'- GCG TAC CTG ATC ACC ACC TTG AAG G -3'	180,198 and 202	Liu et al. (2010)
Sr25	Gb	F 5'- CAT CCT TGG GGA CCT C -3' R 5'- CCA GCT CGC ATA CAT CCA -3'	130	Liu et al. (2010)
Sr26	Sr26#43	F 5'- AAT CGT CCA CAT TGG CTT CT -3' R 5'- CGC AAC AAA ATC ATG CAC TA -3'	207 +ve, 303 – ve	Mago et al. (2005)
Lr34	CsLV34	F 5'-GTTGGTTAAGACTGGTGATGG-3' R 5'-TGCTTCCTATTGCTGAATAGT-3'	150	Lagudah et al. (2006)
Lr67	CFD71	F 5'- CAA TAA GTA GGC CGG GAC AA -3' R 5'- TGT GCC AGT TGA GTT TGC TC -3'	214	Bhavani et al. (2008)
Sr 33	Xcfd15	F 5'- CTC CCG TAT TGA GCA GGA AG -3' R 5'- GGC AGG TGT GGT GAT GAT CT -3'	150-220	Lagudah et al. (2006)
Sr31	csSr32# 1	F 5'- CTC CCG TAT TGA GCA GGA-3' R 5'- CCA GCT CGC ATA CAT CCA -3'	210	Bhavani et al. (2008)

screening 20 markers on 17 genotypes. Most primers had 2 alleles and the alleles sizes were within the expected range. The 10 SSR primer pairs yielded a total 13 polymorphic loci with a percentage of 92.86%. The mean number of different alleles per locus in each group was 2.0 and the mean number of polymorphic alleles per locus was 1.9286 (Figure 1).

The expected and observed moments of heterozygosity was calculated to estimate the number of heterozygous loci. The expected heterozygosity (HE) and observed heterozygosity (HO) ranged from 121.53 to 1.49 and from 22.75 to 0.642, respectively (Figure 2).

The number of alleles obtained was low compared to other studies (Blair et al., 2010). This finding can be attributed to high genetic similarity between the accessions or crossbreeding between the accessions. The gene frequency varied from 0.8824 for *Sr*2 allele 2 to as low as 0.05882 for *Sr*21 allele 3 (Figure 3).

Phylogenetic analysis of the markers was done using DARwin 6.0. 8. Single data dissimilarity was calculated and factorial coordinates calculated from the resulting dissimilarity data to determine segregation of individual samples (Figure 4).

Genotypes were segregated into 4 groups with each group having discrete individuals a. 1, 2, 7, 8, 17, b. 3, c. 6, 9, 10, 13, 14, 15, 16, d. 12, 15. The keys of 17 genotypes based on analysis of 10 SSR markers is as follow: 1-SP-D, 2-SP-P, 3-SP-S, 4-SP-F, 5-SP-R, 6-SP-N, 7-SP-M, 8-SP-C, 9-SP-K, 10-SP-9, 11-SP-16, 12-SP-20, 13-SP-21, 14-SP-26, 15-SP-29, 16-SP-31 and 17-SP-34.

Cluster analysis

Unrooted phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) agglomerative hierarchical clustering (Figure

Table 3. Frequency distribution for qualitative traits evaluated in 17 wheat genotypes.

Trait	Frequency %	H'[a]
Flag leaf (recurved)		
Medium to high	6	0.65
Low to medium	94	
Shriveled grains		
Plump	24	
Intermediate	42	0.85
Shriveled	34	
Grain shape		
Oval	48	
Oblong	18	0.75
Elliptical	34	
Flag leaf attitude		
Erect	6	
Semi-erect	74	0.53
Drooping	20	
Sprouting tendency		
None	6	
Low	42	0.79
Medium	52	
Spike density		
Sparse	6	
Medium	18	0.67
Dense	76	
Awns length		
Medium long	97	0.34
Short awns	3	
Grain colour		
white	36	
Intermediate	40	0.71
Hard Red	24	
Spike shape		
Tapering	12	
Parallel sided	82	0.65
Semi clavate	6	
Straw pith at maturity		
Thin	94	0.27
Medium	6	
Brush hair		
Short	64	0.61
Medium	30	

Table 3. Contd.

Long	6	
Growth habit		
Erect	94	
Semi-erect	6	0.57
Intermediate	0	

H'[a] : Shannon-Weaver index.

Table 4. Mean squares from combined ANOVA for different quantitative traits.

Source	D.F	SW	SD	AL	M	SL	H	GY	EE	GS
Rep.	2	12.23	0.02	1.36	15.45	1.81	68.23	5341.90	1.56	490.11
Genotype	16	113.12**	0.85**	1.77	0.98	14.55**	80.32	2072.96**	2.29	547.17**
Season	1	265.86**	11.9**	1.80	63.48**	217.10**	8114.26**	885.53**	0.54	1.84
G × S	16	11.14**	0.29**	0.73	2.96	1.91**	53.18	2858.11**	1.36	416.12**
Error	32	3.22	0.18	0.88	1.94	0.849	53.70	827.44	1.99	86.99

Locus	Sample Size	na*	ne*	h*	I*
A	17	2.0000	1.7101	0.4152	0.6058
B	17	2.0000	1.9931	0.4983	0.6914
C	17	2.0000	1.8408	0.4567	0.6492
D	17	1.0000	1.0000	0.0000	0.0000
E	17	2.0000	1.8408	0.4567	0.6492
F	17	2.0000	1.5622	0.3599	0.5456
G	17	2.0000	1.2620	0.2076	0.3622
H	17	2.0000	1.2620	0.2076	0.3622
I	17	2.0000	1.1245	0.1107	0.2237
J	17	2.0000	1.9396	0.4844	0.6775
K	17	2.0000	1.9931	0.4983	0.6914
L	17	2.0000	1.5622	0.3599	0.5456
M	17	2.0000	1.4098	0.2907	0.4660
N	17	2.0000	1.5622	0.3599	0.5456
Mean	17	1.9286	1.5759	0.3361	0.5011
St. Dev		0.2673	0.3281	0.1545	0.2023

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* h = Nei's (1973) gene diversity
* I = Shannon's Information index [Lewontin (1972)]

Figure 1. Summary statistics of 10 SSR markers analyzed on 17 wheat genotypes.

5). The dendrogram generated from the results showed the evaluated wheat genotypes segregate into three major clusters and six sub-clusters. The mutants clustered with their respective parental varieties as their resistance profiles were similar or related significantly all the mutants segregated with their parental varieties and hence resistance profiles of parents can be used as references to characterize resistance of the mutant lines to stem rust.

DISCUSSION

Morphological diversity existed between mutants, their parents and other commercial checks used in this study. Both qualitative and quantitative traits showed diversity. This was supported by the average Shannons-Weavers index for qualitative traits (Table 3). The genotype × season (G × S) interaction observed on number of grains per spike, maturity time period, number of tillers

Observed and expected moments of K, the number of heterozygous loci between two randomly chosen gametes in a population : where M1 to M4 are the first four observed moments and EM1 to EM4 are the respective expected values; Xi=Mi/EMi −1.

pop ID	M1	M2	M3	M4
1	4.7059	7.5017	1.4924	121.5397

pop ID	EM1	EM2	EM3	EM4
1	4.7059	2.8136	0.6427	22.7521

pop ID	X2	X3	X4
1	1.6663	1.3221	4.3419

Figure 2. Values of HO and HE for the population.

Gene Frequency :

Allele \ Locus	sr22A	sr22B	sr22C	sr26D	sr26E	sr26F	sr2G	sr2H
Allele 1								
Allele 2	0.7059	0.4706	0.6471	1.0000	0.6471	0.7647	0.8824	0.8824
Allele 3	0.2941	0.5294	0.3529		0.3529	0.2353	0.1176	0.1176

Allele \ Locus	sr2I	sr28J	sr28K	sr25bfL	sr25bfM	sr25gbN
Allele 1						
Allele 2	0.9412	0.4118	0.5294	0.7647	0.8235	0.7647
Allele 3	0.0588	0.5882	0.4706	0.2353	0.1765	0.2353

Figure 3. The gene frequency data.

and 1000 seed weight showed the influence of seasonal differences (Table 4). With the necessity for early maturing varieties, there exists a correlation between growth habits, heading time and maturity time period with most genotypes having erect growth habit. Two types of grain textures the soft white grains and the hard red grains were exhibited by the genotypes. The hard red grains are most preferred by bakers and farmers and weighed from 35 to 45 g while the soft white grains weighed 30 to 35 g/1000 grains weight with a moisture content of 13.5%.

Spike traits and number of tillers per plant were major traits that separated the mutants from their parent varieties (Table 5). The number of tillers and number of grains per spike was greatly influenced by the nutrients supplied and environmental conditions. Differences in grain texture and spike traits contributed significantly to variability between the mutants and their parent. The mutants and their parents clustered into three major clusters with mutants clustering with their respective parents an indication of closer relationships. Positive correlations were observed between seed weight and grain yields per spike. Seed weight affected grain yield per spike which influence the final yield. Correlation between number of grains per spike, seed weight and grain yield per spike have been recorded by Leilah and Al-Khateeb (2005), who observed a negative correlation between number of grains per spike and seed weight as

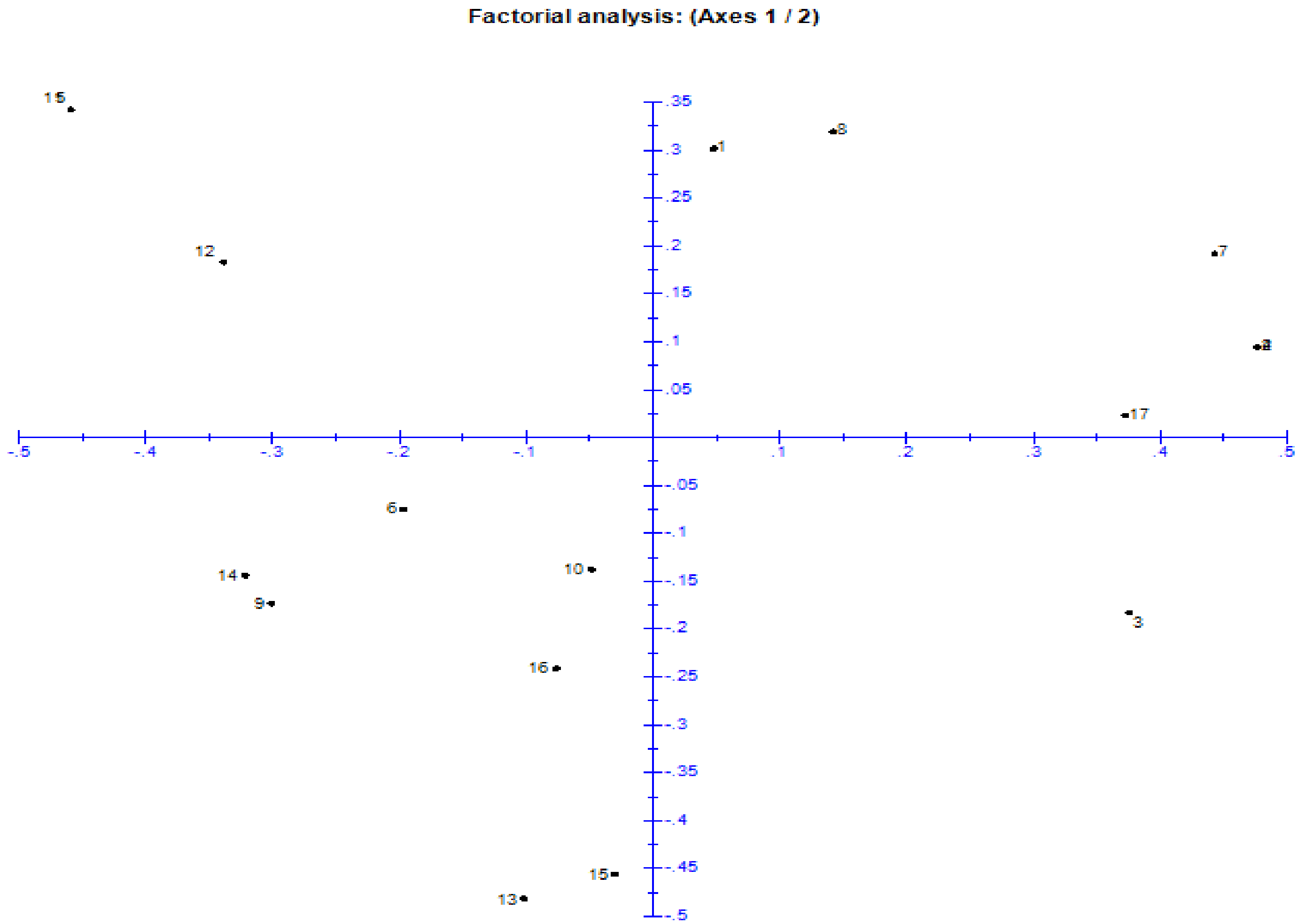

Figure 4. Factorial analysis.

more grains per spike would tend to reduce the size of grains. Negative correlation was observed between spike length and number of tillers per plant and this was attributed to reduction in food to cater for grains per spike. Negative correlation was also observed between maturity period and grain yield per spike. Genotypes with longer maturity periods had reduced seed weight due to unreliable weather conditions.

The polymorphic SSRs markers used were usefully in producing informative bands (Plate 1). Most of the SSR used were polymorphic across the 17 genotypes and a total of 13 alleles were detected with an average number of 2 alleles per locus. According to Salem et al. (2008), the number of alleles per locus ranged from 2 alleles to 7 alleles with an average of 3.2 alleles per locus while Jain et al. (2004) also reported that the number allele per locus ranged from 3 to as high as 22 with an average of 7.8 alleles per locus. Gene diversity ranged from 0 to 0.4893 for each sample, with an average of 0.3361 (Figure 3). The genetic distance analysis separated the 17 genotypes into 3 major clusters and 6 sub-clusters. The genotypes belonging to the same sub-cluster were genetically similar while those belonging to the different sub-clusters were different from each other. The SSRs used in this study demonstrate the ability of SSRs to produce unique DNA profiles and establish discrete identity. Wide range of genetic diversity was observed and it is possible to classify the genetic diversity of the elite mutant lines and select mutant lines for the highest genetic diversity. These findings demonstrated the usefulness and efficiency of SSRs markers in analyzing genomic diversity. According to Hayden et al. (2006), genotypes with the most distinct DNA profile contain the greatest number of novel genes and are likely to carry unique and potentially agronomical useful genes. The genetic diversity levels observed is potentially valuable in predicting sources for selection of genetic diversity with an objective of broadening the wheat genetic base and also have increased progeny performance for complex traits such as yield and disease resistance in

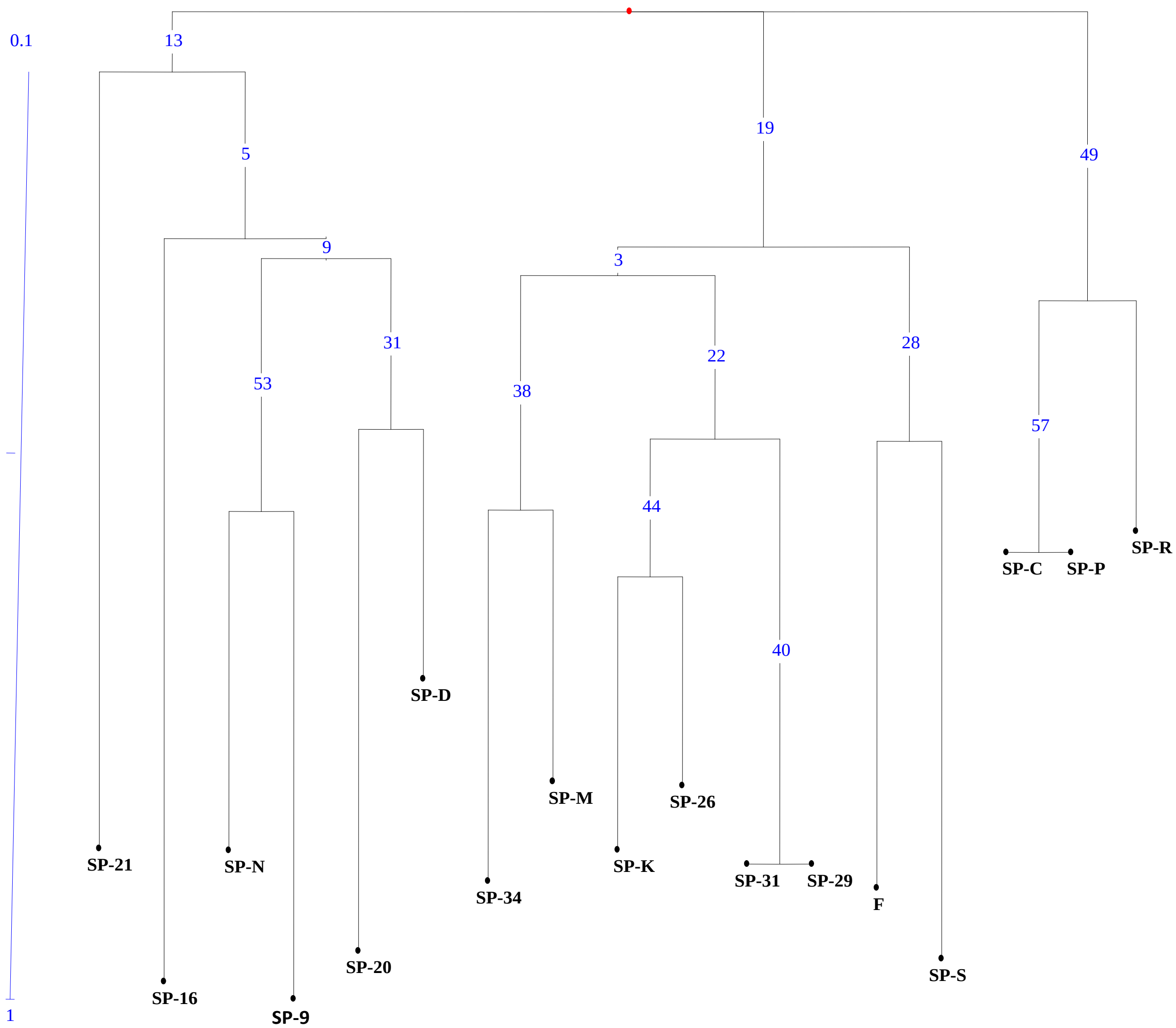

Figure 5. Dendrogram based on genetic similarities discriminated all the wheat genotypes.

wheat production.

Conclusion

Considerable amounts of genetic diversity were observed between the mutants, parents and commercial checks varieties. There was low genetic distance between the genotypes in each sub-cluster attributed to the high genetic similarity between the mutants, their parents and the commercial checks. Observed heterozygosity was higher than expected heterozygosity due to the high genetic variations between the genotypes and within the groupings there were high similarities due to the close relationships and the effects of intense selection in search of the good quality attributes. *Sr*2 was the most polymorphic marker of the ten SSRs as it exhibited greater ability to distinguish between the different genotypes. These results confirmed the relationships between the parents and their respective mutants being placed into the same groupings on the basis of their genetic similarities. Genetic diversity studies is important in developing strategies in wheat breeding as it can be used in selecting genotypes with certain desired traits for breeding programs. The SSRs confirmed morphological traits information about wheat genetic similarities and variations mostly being separated by their grain characteristics. These results can be used in selecting diverse parents in breeding in order to utilize their genetic potential for progeny improvements. This study contributed to stable wheat production by discovering traits relationship that can be used in breeding purposes for adaptation to various desired conditions while the informative SSR markers can be used to map out traits

Table 5. Mean of different quantitative traits of 17 wheat genotypes.

ID	Quantitative traits												
	1000 SW (g)	**SD (cm)**	**GT (no.)**	**M (days)**	**SL (cm)**	**L (1-9)**	**H (cm)**	**EE (day)**	**GY (g)**	**FLA (cm^2)**	**AL (cm)**	**S (no.)**	**GS (no.)**
SP-D	29.7^{f}	0.22^{c}	3^{c}	115^{d}	9.1^{d}	4^{e}	83^{d}	65^{a}	1.15^{h}	29.5^{g}	6.9^{a}	13^{d}	29^{d}
SP-P	25.3^{g}	0.21^{d}	1^{d}	135^{b}	8.9^{e}	3^{f}	88^{a}	70^{a}	0.84^{i}	28.6^{h}	7.0^{a}	11^{e}	24^{e}
SP-S	38.5^{d}	0.25^{b}	3^{c}	115^{d}	10.7^{b}	6^{c}	82^{e}	65^{a}	1.55^{e}	36.4^{d}	6.8^{a}	15^{c}	36^{b}
SP-F	37.2^{d}	0.25^{c}	3^{c}	120^{d}	9.6^{c}	5^{d}	82^{e}	65^{a}	1.24^{g}	32.1^{f}	7.0^{a}	13^{d}	33^{c}
SP-R	33.8^{e}	0.24^{b}	3^{c}	120^{d}	9.7^{c}	6^{c}	83^{d}	65^{a}	1.32^{f}	35.3^{d}	7.2^{a}	13^{b}	34^{c}
SP-N	41.5^{c}	0.26^{a}	3^{c}	125^{c}	11.1^{b}	5^{d}	82^{e}	70^{a}	1.78^{c}	38.6^{c}	6.9^{a}	16^{c}	40^{a}
SP-M	24.6^{h}	0.20^{d}	2^{d}	125^{c}	7.1^{g}	3^{f}	76^{h}	65^{a}	0.82^{j}	27.7^{i}	6.8^{a}	9^{f}	20^{f}
SP-C	25.8^{g}	0.21^{d}	1^{e}	135^{b}	8.7^{e}	3^{f}	87^{b}	70^{a}	0.91^{i}	28.4^{h}	7.1^{a}	12^{d}	25^{d}
SP-K	35.4^{e}	0.23^{c}	2^{d}	145^{a}	9.5^{c}	5^{d}	89^{a}	70^{a}	1.37^{f}	32.8^{e}	7.3^{a}	13^{d}	32^{c}
SP-9	37.3^{d}	0.24^{b}	2^{d}	115^{d}	9.9^{c}	5^{d}	86^{c}	65^{a}	1.45^{e}	35.1^{d}	6.8^{a}	14^{d}	34^{c}
SP-16	39.7^{c}	0.25^{b}	4^{b}	115^{d}	10.8^{b}	7^{b}	86^{c}	70^{a}	1.67^{d}	38.5^{c}	7.0^{a}	16^{c}	38^{b}
SP-20	30.5^{f}	0.23^{c}	2^{d}	115^{d}	9.3^{d}	3^{f}	86^{c}	65^{a}	1.15^{h}	34.5^{e}	6.8^{a}	12^{d}	28^{d}
SP-21	47.8^{a}	0.28^{a}	4^{b}	115^{d}	12.5^{a}	7^{b}	86^{c}	70^{a}	2.12^{a}	42.8^{a}	6.8^{a}	18^{b}	44^{a}
SP-26	44.6^{b}	0.26^{a}	5^{a}	130^{c}	10.9^{b}	8^{a}	80^{f}	70^{a}	2.04^{b}	40.3^{b}	6.8^{a}	20^{a}	45^{a}
SP-29	28.9^{f}	0.21^{d}	3^{c}	130^{c}	9.5^{c}	4^{e}	79^{g}	70^{a}	1.13^{h}	32.2^{f}	6.7^{a}	13^{d}	30^{c}
SP-31	35.1^{e}	0.23^{c}	4^{b}	130^{c}	9.6^{c}	7^{b}	79^{g}	70^{a}	1.32^{f}	35.8^{d}	6.8^{a}	15^{c}	35^{b}
SP-34	27.5^{g}	0.21^{d}	4^{b}	130^{c}	9.3^{d}	4^{e}	79^{g}	70^{a}	0.97^{i}	33.1^{e}	6.8^{a}	12^{e}	28^{d}
Mean	34.3	0.234	2.9	124.5	9.8	5.0	83.0	67	1.34	34.2	6.8	13.8	32.5

, * Significant relationship between the variables at $P \leq 0.05$, 0.001, respectively. 1,000 seed weight= SW, Number of tillers=GT, Maturity periods (days) =M, Spike length=SL, lodging=L, plant height=H, Grain yields per spike=GY, Flag leaf area=FLA, Awn length=AL, Spikelets per spike=S, Seed diameter=SD, Ear emergence=EE, Grains per spike= gs.

Table 6. Pearson's correlation coefficient for the different quantitative traits of 17 genotypes

Correlation	SW	SD	GT	M	S	GS	PH	SL	GY
SW	1								
SD	0.772**	1							
GT	- 0.678**	-0.554*	1						
M	-0.638*	-0.510*	0.257	1					
S	0.296	0.427	0.007	0.256	1				
GS	0.767*	0.412	0.731*	0.364	0.490	1			
PH	0.273	0.381**	0.074	0.098	0.351	-0.124	1		
SL	-0.150	0.321	-0.539*	0.429	0.608	0.280	-0.116	1	
GY	0.938**	0.740**	-0.561**	-0.533*	0.880*	0.724**	0.202	0.718**	

*Significant ($P \leq 0.05$), **Significant ($P \leq 0.01$).

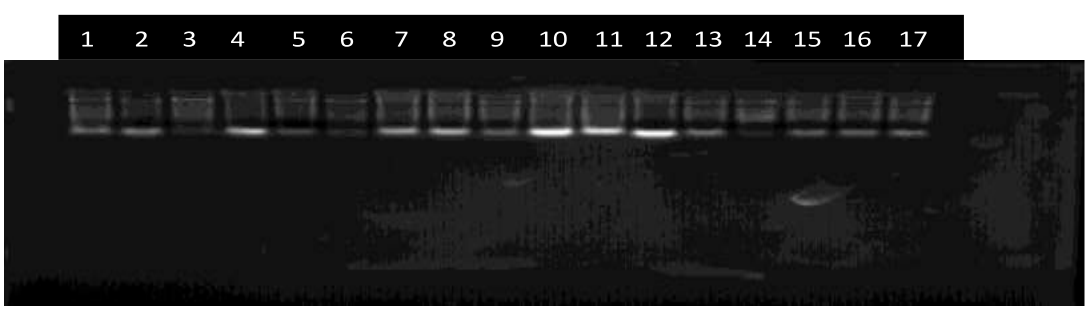

Plate 1. Quantification gel image.

and aid marker assisted selections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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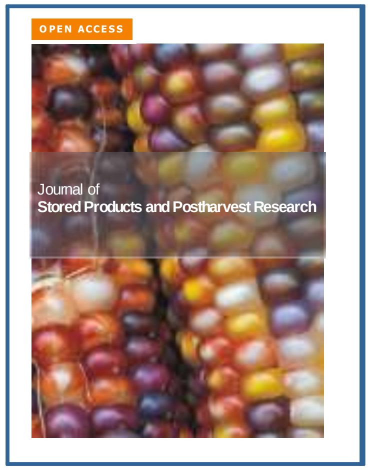

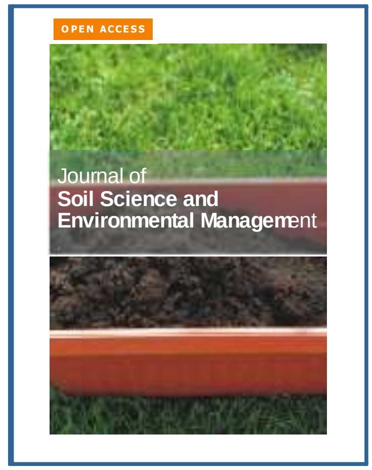

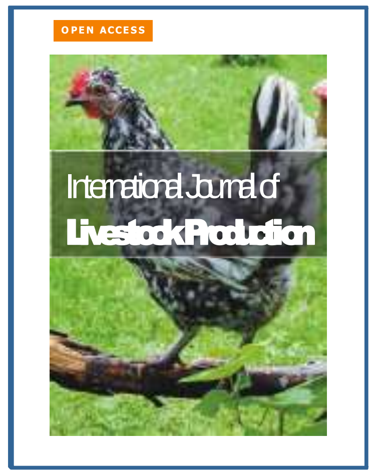

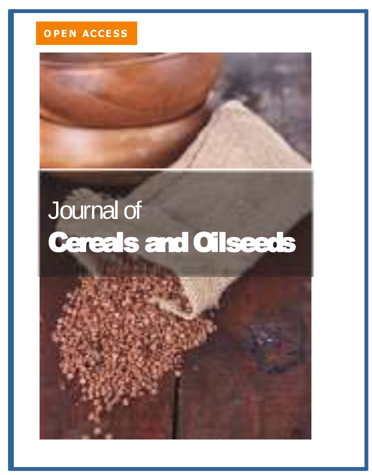

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