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African Journal of Plant Science

Full Length Research Paper

Seasonal variation and modeling of leaf area growth in *Jatropha curcas* L. plants: Implication for understanding the species adaptation in the Sahel of Niger

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***Jatropha curcas* is a tree species introduced in Niger as a trial experiment to offset land degradation and for biofuel production. The objective of this study is to contribute to the understanding of this species' potential for adaptation under the edaphic and climatic conditions of Niger through analysis and modeling of the leaf area dynamics. The nondestructive method is used to evaluate the leaf area growth using four provenances and 120 samples of leaves of *J. curcas* plants. The results show that leaf area is optimal during the wet season of the year with non-significant difference (P > 0.05), while during other periods it is significant (P < 0.05) between provenances. The logarithmic model is the most accurate, and the models developed have a correlation coefficient between 0.95 and 0.99. The error analysis shows a mean absolute percentage of error between 5.92 and 27.43%, depending on the provenances. The accuracies of the developed models were appreciated, with root mean square of error varying from 0.72 to 2.06 cm^2. Contrary to the expectation, for production of *J. curcas* in Niger's Sahelian climate and soil, it is necessary to ensure additional irrigation water to the plants, especially during the dry period of the year.**

Key words: Exotic species, *Jatropha curcas*, adaptation, leaf area models, Niger.

INTRODUCTION

Jatropha curcas is a latex shrub, native to America, from the family of *Euphorbiaceae*. This species is widespread in Africa in the Sudanian and Guinean savannas (Arbonnier, 2009). The tree species is considered for its multifaceted socioeconomic and environmental importance to society (Pandey et al., 2012; Bazongo et al., 2015; Traore et al., 2015). It improves the physicochemical properties of soils and crops' yield. Its action in environmental protection through soil and water erosion control and the potential for carbon sequestration and

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biofuel production is quite remarkable (Openshaw, 2000; Berchmans and Hirata, 2008; Pandey et al., 2012; Bayen et al., 2016). In Niger, this species was introduced in 2000 in the research centers to understand its cycle of growth and development (Zakari, 2013). *J. curcas* can tolerate less than 300 mm of rain per annum and up to 40 °C in temperature (Pandey et al., 2012). The development of this species in the edaphic and climatic conditions of Sahel Niger, marked by less than four moist months and a high maximum temperature in the year, is led necessarily by its adaptation. In semi-arid and arid areas, woody species seek a compromise between survival and safety through leaf and twig shedding during the hard period of the year (Gleason et al., 2016). The transpiring area is an important indicator of the growth and development of the plant. The plant total leaf area provides information on the photosynthetic capacity and on the assessment of water losses by the flow of perspiration (Nemecek-Marshall et al., 1995; Lamade, 1997). Some studies indicate that the leaf area index can be a useful parameter for predicting the effects of vegetation upon microclimate, which could be used to make small-scale climate predictions (Hardwick et al., 2015).

In addition, the leaves are organs oriented for the interception of light, which is necessary for the photosynthesis mechanism. The light is captured by a wide range of chloroplasts straddling the air and the vascular tissues that drain water and export the products of photosynthesis (Lambers et al., 1998). Preliminary observations have shown that *J. curcas* eliminates leaves and twigs or reduces leaf area during periods of limited humidity (Ouédraogo, 2006; Moussa et al., 2017). Ouédraogo (2006) explained this phenomenon as an adaptation of the plant to the conditions of the environment. This kind of physiological mechanism aspect of drought stress could have an impact on the productivity of the species because reducing the leaf area would induce a reduction in CO_2 assimilation rates and photosynthetic activities (Reddy et al., 2004). Despite its wide distribution throughout the world and its tolerance to drought (Ouédraogo, 2006), the development of *J. curcas* is still problematic in the arid conditions of Sahel Niger (Moussa et al., 2017). Thus, it is necessary to refine our knowledge of biology, especially the dynamic and foliar production of *J. curcas* in the edaphic and climatic conditions of Sahel Niger, so as to make better decisions when managing agrosystems. It is specifically necessary to understand the dynamic of the leaf area of this species and to model it according to the time of growth. The modeling of the leaf area of *J. curcas* plants will make it possible to predict and, especially, to determine its rhythm of growth in order to better understand its elasticity. To carry out this study, two hypotheses were posed: (i) the growth of the leaf area in *J. curcas* plants depends on the period of the year (wet, dry, cold) in the Sahel and (ii) the dynamic of the leaf area in *J. curcas* plants follows an appropriate distribution that the analysis makes possible to determine.

MATERIALS AND METHODS

Site

The trial was conducted at the Faculty of Agronomy in Abdou Moumouni University of Niamey, on a site located at latitude 2° 08'E and 13° 30'N. The meteorological data used were those of Niamey airport station. The data covered a period of ten years. The main climatic factors analyzed were rainfall, temperature, evapotranspiration, and relative humidity. The annual average rainfall was 535 ± 127 mm. The annual average temperature was 29.8 ± 0.98°C. The number of months during which water was used by the roots of plants was three months from July to September (Moussa et al., 2017). Winds were of two types: the harmattan blowing in the hot weather with high intensity from October to March and the monsoon wind of the rainy season. The soil was the leached ferruginous tropical kind. Analysis of soil samples from the site showed a sandy loam texture with a relatively neutral pH equal to 6.46. The total exchange capacity of the soil was 11.33 me/100 g. The available phosphorus and C/N were 36.44 ppm and 11.35% respectively.

Biological and experiment material

The biological *J. curcas* seed materials used in this study came from four provenances, namely Guinea-Bissau, Mali, Mexico, and Senegal. These materials were planted in a randomized complete block design. The block was composed of five lines of seed holes, each measuring 1m x 1m. Along each line, three seeds per seedling from each origin were sown in alphabetical order. The total number of seed holes was twelve on a line and sixty on the block. The block size was 13 m × 6 m.

Trial implementation and monitoring operations

Direct seeding was conducted on August 25, 2008 at four seeds per hole. After seed germination and seedling emergence, thinning to one plant per hole and resowing were carried out on September 25, 2008. In October 2008, corresponding to the inception of the dry season, the setting up of the trial was completed. During the dry period (October 2008-May 2009), regular watering was conducted every three days at twelve liters for three seed holes. In the next rainy season (June-September 2009), weeding was carried out. A phytosanitary treatment was carried out on September 15, 2009 with the Pyrical 480 *EC*. It was applied at a concentration of 70 ml of Pyrical in 15 L of water using a backpack sprayer or about 470 mg/L according to the standard, which was 480 mg/L. The purpose was to limit the damage caused by termites.

Measurement of leaf area

The measured sample was composed of ninety leaves including thirty each from the basal, median, and apical. Leaf area was measured by a nondestructive method. This method determined directly the leaf area without cutting (Lamade, 1997). To do this, the leaf of *J. curcas* was spread carefully on graph paper. The contour of the leaf was drawn using a criterion of 0.5 mm diameter. The area was given by counting the number of mm^2 intercepted by the leaf. These measures were assessed monthly from July to September. The data were used to calculate the average leaf area

Table 1. Correlation coefficient between series of leaf area and time of measurement used for four models.

Provenances	R (Xi, Yi)	R (Xi, lnYi)	R (lnXi, Yi)	R (lnXi, lnYi)
Guinea	0.85	0.77	0.97	0.93
Mali	0.87	0.78	0.95	0.93
Mexico	0.89	0.78	0.96	0.94
Senegal	0.89	0.78	0.95	0.93
All	0.88	0.78	0.96	0.94

for the four provenances based on the measurement period. The total leaf area of a plant gives information on its photosynthetic capacity and the assessment of water loss by transpiration stream (Lamade, 1997; Hardwick et al., 2015). One-way ANOVA was used to compare the location mean of leaf area according to the same period of measurement at 95% of confidence level.

The leaf growth of *J. curcas* from a young age to maturity was followed. The measurements were carried out at the beginning of the rainy season on July 10, 2009 and ended on August, 2009, after leaf area growth remained constant. A sample of thirty young leaves (two days old after identification of the bud) was selected by location. These were labeled using a sewing thread to be distinguished. The area of each sampled leaf was determined every two days at the same hour by the direct method as described earlier. To illustrate the degree of leaf growth, the rate of leaf area multiplication by the time was calculated using the following formula (1):

$$R_i = \frac{LA_i}{T_i} \quad (1)$$

With R_i: rate of leaf area development on time i, LA_i: leaf area on time i and Ti: day of measurement.

Modeling of leaf growth

Leaf area growth monitoring data were used to develop models based on time (day). Thus, initially, four types of models were tested to assess their suitability for the distribution of cloud point. This approach is similar to that of Cai et al. (2017). The linear, exponential, logarithmic, and power models were tested by carrying out the following transformations (2, 3, 4, 5):

$$Y = a + bT \; R^2(T, Y) \quad (2)$$

$$Y = a\exp^{bT} \longrightarrow \ln Y = \ln c + TR^2\,(T, \ln Y) \quad (3)$$

$$Y = a + b\ln T \; R^2\,(\ln T, Y) \quad (4)$$

$$Y = aT^b \longrightarrow \ln Y = \ln a + b\ln T \; R^2\,(\ln T, \; Y) \quad (5)$$

With *Y*: leaf area (*LA*) at a time *T* in day, a, b and c: the model coefficients and R^2: the correlation coefficient between *Y* and *T*.

The best fit of the model was sought on the basis of the strong correlation between leaf area and number of growth days. The logarithmic model of the form Y = a + blnT was used because of the greater correlation between the *Y* (leaf area) and lnT (numeric logarithm of number of days) recorded parameters (Table 1). Thus, the model was developed using the R Commander Software version 2.15.3. The generalized linear model with Gamma link function was used to prevent the back-transformation problem (Ketterings et al., 2001; Packard, 2013). All leaf areas with standardized residues that deviated from the majority of individuals were considered outliers and discarded. This approach has been widely used in many biomass modeling studies (Zuur et al., 2010; Bayen et al., 2016; Moussa and Larwanou, 2018). This resulted in two series of data (leaf area and time), whose variance of residues was relatively homogeneous. Finally, the modeling was based on these last data.

Analysis of error

The model reliability was assessed by examining the mean absolute percentage of error (MAPE) and the root mean square of error (RMSE). The MAPE expresses the average percentage of error in absolute value, which may be misleading when predicting leaf area for a model. The RMSE is a model selection parameter used to aggregate errors into a single predictive power (Fayolle et al., 2013; Yao et al., 2013). The MAPE and RMSE were calculated using the following formulae:

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{LAob - LApred}{LApred}\right| \quad (6)$$

$$RMSE = \sqrt{\frac{1}{n} \times \sum_{i=1}^{n}(LAob - LAped)^2} \quad (7)$$

Where, n is number of measurements, LApred is predicted leaf area and LAob is observed leaf area.

The model is considered to be reliable when MAPE and RMSE are weak. In some cases, according to Sileshi (2014), errors are tolerable in a model with a MAPE less than 10.

RESULTS

Leaf area

Table 2 shows that it was only in August and, to a lesser extent, in July that *J. curcas* plants carried basal, median, and apical leaves. During the other months of the study (September and October), only apical leaves were reported. In July (rainy season), the apical leaf area was 71.31 ± 20.33 cm^2 for Guinea, 69.19 ± 15.16 cm^2 for Mali, 51.02 ± 21.57 cm^2 for Mexico, and 50.14 ± 16.06 cm^2 for Senegal. Statistical analysis showed a highly significant difference between leaf areas in the apical position of these provenances (P = 0.000). The Guinea and Mali

Table 2. Average leaf area (cm^2) by month, position and provenance of *J. curcas.*

Positions	Provenances	July	August	September	October
Apical	Guinea	71.31 ± 20.33[a]	64.10 ± 25.21[a]	50.07 ± 23[c]	43.46 ± 15.01[d]
	Mali	69.19 ± 15.16[d]	67.64 ± 30.05[a]	52.33 ± 23.12[b]	50.45 ± 20.32[b]
	Mexico	51.02 ± 21.57[b]	62.86 ± 18.60[a]	44.85 ± 18.98[d]	44.52 ± 16.15[c]
	Senegal	50.14 ± 16.06[c]	73.74 ± 35.86[a]	61.20 ± 18.60[a]	57.56 ± 25.45[a]
ANOVA		F = 9.7, df= 3/112, P = 0.0000	F = 0.49, df = 3/76, P = 0.68	F = 3.44, df = 3/110, P = 0.019	F = 5.26, df = 3/115, P = 0.002
Median	Guinea	12.47 ± 7.72[d]	14.57 ± 7.02[d]	Fallen	Fallen
	Mali	13.82 ± 6.27[c]	35.96 ± 29.66[a]	Fallen	Fallen
	Mexico	25.02 ± 18.28[a]	19.67 ± 5.47[c]	Fallen	Fallen
	Senegal	20.32 ± 11.64[b]	30.90 ± 13.77[b]	Fallen	Fallen
ANOVA		F = 4.91, df = 3/79, P = 0.003	F = 2.67, df =3/53, P = 0.057	-	-
Basal	Guinea	Fallen	7.83 ± 4.21[d]	Fallen	Fallen
	Mali	3.57 ± 1.93[b]	16.21 ± 5.53[b]	Fallen	Fallen
	Mexico	13.40 ± 9.11[a]	12.44 ± 8.50[c]	Fallen	Fallen
	Senegal	Fallen	32.93 ± 27.65[a]	Fallen	Fallen
ANOVA		F = 11.30, df = 1/13, P = 0.005	F = 1.63, df = 3/16, P = 0.221	-	-

Data in the same column with the same letter are not statistically different at 0.05 probability level.

provenances had the largest apical leaf area compared to Mexico and Senegal in July 2009. The median leaf area was 12.47 ± 7.72 cm^2 in Guinea, 13.82 ± 6.27 cm^2 in Mali, 25.02 ± 18.28 cm^2 in Mexico, and 20.32 ± 11.64 cm^2 in Senegal. Statistical analysis of variance showed a significant difference between leaf areas in the median position of these provenances ($P < 0.05$). In July, the leaf areas of Mexico and Senegal in the median position were wider than those of Guinea and Mali. In the basal position, the leaf area was 3.57 ± 1.93 cm^2 in Mali and 13.40 ± 9.11 cm^2 in Mexico. The Guinea and Senegal provenances did not carry basal leaves in July. The statistical analysis of variance expressed a significant difference between the leaf areas in the basal position of the Mali and Mexico provenances ($P < 0.05$). Mexico had the widest basal leaf area compared to Mali. In August, the leaf area varied slightly compared to the previous month, and no significant differences were found in the apical position ($P > 0.05$). Therefore, significant differences were found in the median and basal positions ($P < 0.05$). In September and October, the apical leaf area of the plants decreased. It was 43.46 ± 15.01 cm^2 for Guinea, 50.45 ± 20.32 cm^2 for Mali, 44.52 ± 16.15 cm^2 for Mexico, and 57.56 ± 25.45 cm^2 for Senegal in October (the period marking the end of the rainy season). The *ANOVA* showed a significant difference between the apical leaf areas of these provenances ($P < 0.05$). The leaf area of Senegal had the highest apical area of the four countries in October.

Growth of leaf area

Figure 1 shows sigmoid curves in three phases: During the first phase, A, young leaves grew slowly. This lasted about six days. Thus, on the sixth day, the leaf area reached 8.53 ± 2.67 cm^2 for Guinea, 7.65 ± 4.86 cm^2 for Mali, 8.50 ± 4.49cm^2 for Mexico, and 6.09 ± 3.08 cm^2 for Senegal. The growth rate of the leaf area was 1.42 cm^2/d for Guinea, 1.27 cm^2/d for Mali, 1.41 cm^2/day for Mexico, and 1.01 cm^2/day for Senegal. The *ANOVA* showed a significant difference among provenances on the sixth day of leaf growth ($F = 3.1$, df = 3/96, $P = 0.031$). The second phase, B, was marked by a strong growth of the leaf area. This phase extended from the seventh to the twentieth day. On the twentieth day, the leaf area reached 57.14 ± 13.37 cm^2 (Guinea), 62.30 ± 9.70 cm^2 (Mali), 73 ± 18 cm^2 (Mexico), and 58.78 ± 22.70 cm^2 (Senegal). The growth rate of the leaf area, which increased strongly and reached its maximum at the twentieth day, was 2.85 cm^2/d (Guinea), 3.11 cm^2/d (Mali), 3.65 cm^2/day (Mexico) and 2.94 cm^2/day (Senegal).

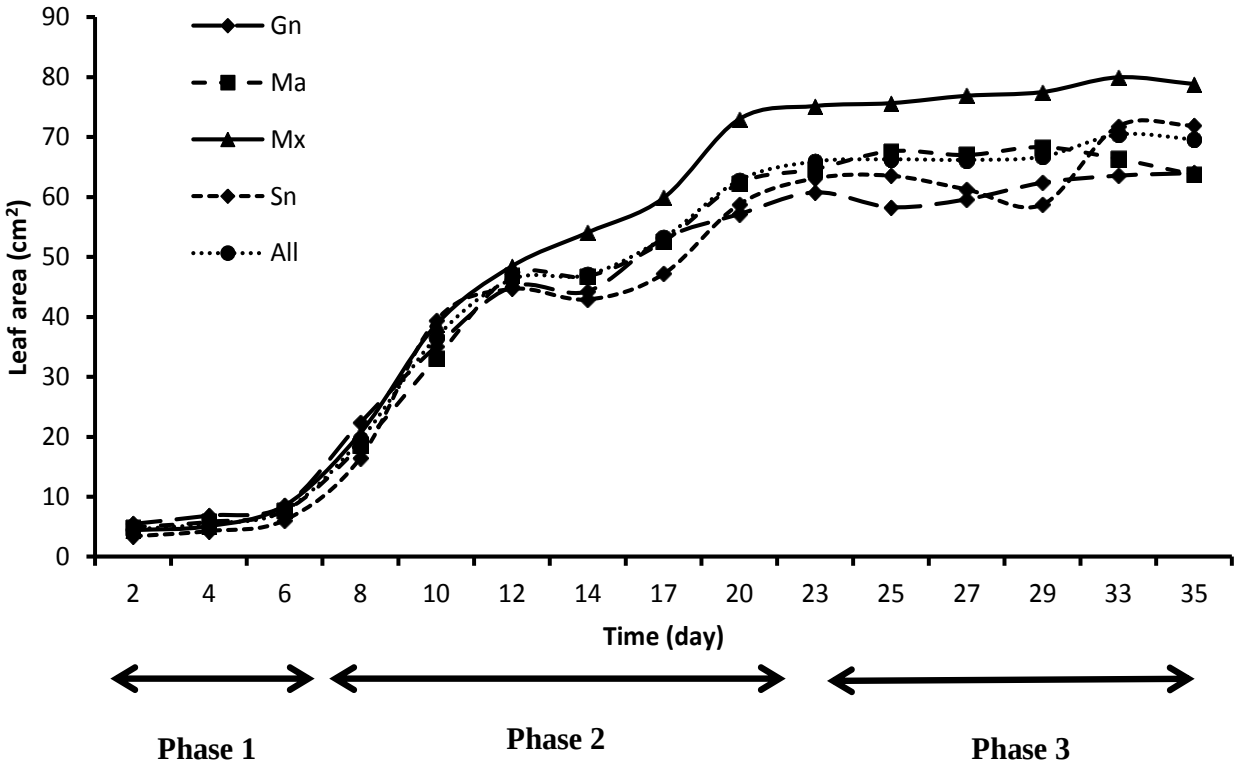

Figure 1. Evolution of leaf area of *J. curcas* by day.

The ANOVA showed a statistically significant difference between provenances (F = 3.68, df = 2/95, P = 0.015). On the twentieth day of growth, the leaves of *J. curcas* plants from Mexico and Mali were wider than those from Guinea and Senegal. Finally, a third phase, C, was marked by the appearance of a plateau with a more or less stationary growth of the leaf area. Thus, until the thirty-fifth day, marking the end of measurement, the average leaf area hardly exceeded 63.98 ± 20.67 cm^2 (Guinea), 63.71 ± 10.23 cm^2 (Mali), 78.83 ± 23.93 cm^2 (Mexico), and 71.90 ± 23.86 cm^2 (Senegal). During this phase, the growth rate of the leaf area decreased and fell on the thirty-fifth day to 1.82 cm^2/d (Guinea), 1.82 cm^2/d (Mali), 2.25 cm^2/d (Mexico), and 2.05 cm^2/d (Senegal). *ANOVA* showed a nonsignificant difference between provenances (F = 0.38, df = 3/55, P = 0.766). On the thirty-fifth day of measurement, the variation in leaf area growth of *J. curcas* plants from Guinea, Mali, Mexico, and Senegal was not significant.

Model of growth of leaf area

The logarithmic models expressing the leaf area as a function of the measurement time were statistically representative for each provenance. The correlation was between 0.95 and 0.99. For the five models, the MAPE was 5.92, 27.43, 9.13, 13.11, and 7.09% for Guinea, Mali, Mexico, Senegal, and all provenances, respectively. It was highest for Mali and Senegal. The RMSE was equal to 0.81 cm^2 (Guinea), 2.06 cm^2 (Mali), 1.14 cm^2 (Mexico), 1.82 cm^2 (Senegal), and 0.72 cm^2 (all provenances). This error was highest in Mali and Senegal (Table 3).

DISCUSSION

The introduction of new plant material in an environment presupposes knowledge of its growth and development cycle. *J. curcas* is a plant known for its ability to produce biofuel and contribute to improving the living conditions of rural communities. This plant has behaved well in the western part of West Africa (Ouedraégo, 2006). In Niger, in the Sahelian region, the first experiments showed an additional need for irrigation water for this species to complete its development cycle (Moussa et al., 2017; Zakari, 2013). Analysis of seasonal variation of leaf area is an important indicator in understanding the degree of adaptation of the plant to the environment (Cai et al., 2017).

A good compromise between water losses and gas exchange, particularly carbon dioxide, is a good indicator for improving biomass production and climate adaptation (Tardieu, 2005; Kim et al., 2017). Very often these exchanges take place through the leaf area. The

Table 3. Statistical parameters of model according to provenances.

	N	a	b	R^2	MAPE (%)	RMSE (cm^2)	Models	P-value
Guinea	20	-20.45	24.33	0.98	5.92	0.81	-20.45+ 24.33lnT	***
Mali	31	-32.7	30.10	0.95	27.43	2.06	- 32.7 + 30,10lnT	***
Mexico	21	-29.4	31.50	0.99	9.13	1.14	- 29.4 + 31.50lnT	***
Senegal	31	-41.4	31.50	0.98	13.11	1.82	- 41.4 + 31.50lnT	***
All	104	-27.7	28.20	0.95	7.09	0.72	- 27.7 + 28.20lnT	***

N: number of leaf, a and b: coefficient of model, R^2: coefficient of correlation, MAPE: mean absolute percentage of error, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, NS $P > 0.05$.

development of the leaf area of *J. curcas* was most important during the wetter period (August) of the year.

From a general point of view, the water deficit leads to an architectural adjustment in the plant, which begins with the reduction of the transpiring area. In the worst case, leaves are eliminated, and small branches are taken to ensure survival (Gleason et al., 2016). Under the conditions of this study, where the water balance shows that the number of months during which the water is usable by the roots does not exceed three, namely July, August, and September (Moussa et al., 2017), the development of *J. curcas* under the dependence of rain water is of great concern. Despite a significant correlation being established between growth and adaptation in *Euphorbiaceae*, (Gleason et al., 2016), it nonetheless remains the case that *J. curcas* production requires water supplementation. Such observations of other growth parameters were made by Moussa et al. (2017), in particular on the growth in height, diameter, and multiplication of leaves and branches of *J. curcas* in Niger. In general, when the moisture decreases as in October, the plants lose water by perspiration through the leaves, and the leaf area decreases. Then, when water deficit is observed, the rate of cell division of the plant decreases; the cell walls, which have to deform to allow cell growth, become more rigid; and turgor decreases (Smith et al., 1997; François et al., 2006). This variability of leaf area by the season of the year for the same species has been observed elsewhere in *Ulmus japonica* (Cai et al., 2017). Even if the two species are different, this reflects the natural behavior of certain plant species in relation to the climatic conditions of the environment. The same observations were made between the dry periods of the year and the reduction in leaf area in many plant species by Kim et al. (2017). For the growth of young leaves of *J. curcas*, there are three phases: a slow phase, a phase of strong growth, and a stationary phase. The slow phase of early growth is due to the introduction of the compounds or structures necessary for growth. The active phase can be explained by accelerated cell divisions and growths. Finally, the stationary phase is the result of growth arrest and senescence of the cells (Heller, 1985). The peak of leaf growth is reached on average on the thirtieth day and usually there are fallen leaves after two months of growth. Ouédraogo (2006) observed a stunting of the leaves of *J. curcas* in a nursery on the forty-third day. The growth of leaves could also depend on environmental conditions and the intrinsic nature of the species. This has been demonstrated by the fact that leaf life and physiological function increase from deciduous to evergreen species and can ranged from 165 to 509 days, respectively (Brodribb et al., 2002; Athokpam et al., 2013).

Moreover, Humphries (1966) observed three relationships between area and cell number of successive leaves with the photoperiod: in phase (1), cell number increased at a greater rate than leaf area; in phase (2), leaf area decreased while cell number increased; in phase (3), cell number and leaf area decreased proportionally. These results corroborate those found in this study. For the data collected during this experimentation period, the logarithmic model responded the most compared to the other models. For the same species, leaf growth variation was confirmed by Cai et al. (2017). Depending on the time of year, the leaf area growth models developed by these authors can move from a linear model to a power model. The models developed in this study gave high correlation coefficients of close to 1. The validation of these models is not limited to the simple estimation of the correlation coefficient. A model can have a high coefficient of correlation and hide important errors. The analysis of MAPE revealed high errors between 5.92 and 27.43% in each of these models (Sileshi, 2014). These errors were higher in the Mali and Senegal provenances. This reflects the variability of leaf growth of these two provenances under the conditions of the experiment, which seemed more homogeneous in Guinea and Mexico. The low MAPE values of less than 10% indicate that these models were performing well. Even if these errors were high in Mali and Senegal, it is still possible to use the generic model of which they are weak. The accuracy of these models also remains significant with the low values of the RMSE, especially for the generic model and the provenances of Guinea and Mexico. Indeed, it is highly rare in the literature to find leaf area growth patterns. However, in some cases leaf area growth models have been developed with precisions of 93.96% and 96%, depending on the season of the year

(Cai et al., 2017).

Conclusion

This study illustrated the dynamics of the leaf area of *J. curcas* plants in sandy soil and the Sahelian climate of Niger. The growth of *J. curcas* leaves was more effective during the wet period of the year, including July and August, with a nonsignificant difference between provenances. At the lowest water deficit (September and October), *J. curcas* plants lost their medial and basal leaves and also reduced their transpiring area. To ensure the production of *J. curcas* under these conditions, it is necessarily important to provide the plant with a supplement of irrigation water at the end of the rainy season. The present study also saw the development of models of leaf area growth of *J. curcas* plants. To reach its full growth, the leaf took thirty days, following a sigmoid curve. The logarithmic model was chosen as the most efficient among the four types of tested models. These results are useful in understanding the cycle of growth and development of this plant in Niger. Better still, they will guide its management in agrosystems by the knowledge of its critical period of water need. The model thus developed can improve the production process of the species by predicting its leaf area to ensure good water efficiency.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The major factors influencing coffee quality in Ethiopia: The case of wild Arabica coffee (*Coffea arabica* L.) from its natural habitat of southwest and southeast Afromontane rainforests

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Coffee quality is a complex trait involving sensory and bean characteristics as well as biochemical contents. The objective of this study was to assess the major factors influencing the quality of wild Arabica coffee (*Coffea arabica* L.) in the natural coffee forests of southwest and southeast Ethiopia. Results revealed that both natural (soil, aspect, elevation, climate, geographic location) and human factors (cherry harvesting/ handing, theft, forest management) considerably influenced the quality of wild Arabica coffee. The soil factor affected every component of coffee quality (cup quality, bean characteristics and biochemical contents). The cup quality of coffee varied with soil properties, especially with available P and soil texture. The bean size distribution was also affected by soil properties; there was significant positive relationship between soil pH, sand or Mn and the proportion of bold beans (retained on screen 17). Soil organic matter, total N and sand content were inversely correlated with caffeine content, but available P and clay content were positively correlated with caffeine. Increase in elevation led to increase in bean size up to the elevation of about 1600 m above sea level, but thereafter no more increase in bean size (hump-shaped relationship, not monotonic). Bean size increased with increase in longitude, but it decreased with increase in latitude. Cup quality was also significantly influenced by coffee harvesting and handling, but its influence was not noticed on bean size and biochemical contents. Coffee quality is therefore the resultant of an interaction of different natural and human factors prevailing in the respective area.

Key words: Arabica coffee, bean size, biochemical content, cup quality, environment, management/handling.

INTRODUCTION

Food quality is an important feature because the food people choose depends largely on its quality (Vaclavik and Christian, 2008). According to Caplan (1978), quality is fitness for a purpose. Quality refers to the degree of excellence of a food and includes all the characteristics of a food that are significant and that make the food

acceptable (Vaclavik and Christian, 2008). The International Organization for Standardization (ISO) defines quality as 'the ability of a set of inherent characteristics of a product, system or process to fulfill requirement of customers and other interested parties' (ISO, 2000). According to Lochner and Mater (1990), quality is a measure of the extent to which customer requirements and expectations are satisfied. Fisken (1990) defined it as 'quality is a fuzzy and relative term and it is in a constant motion'. The quality of a product is not absolute; it always depends upon the requirements of the consumer (Hay and Porter, 2006). Thus, method or group of methods designed to control the quality of a defined product may be applicable in a particular situation, but they are subject to a constant evolution (Costell, 2002). This means quality is a subjective term, and it can have different meanings depending upon the context in which the term is used.

According to Feria-Morales (2002), the quality of green coffee mostly depends on the way in which the coffee is grown, harvested and processed. Therefore, coffee quality is hard to define and agree on as the definition of quality varies for different stakeholders across the commodity (production-to-consumer) chain. This means what one stakeholder perceives as quality may not be so thought of by another. At the farmer level, coffee quality is a combination of production level, price and ease of culture; at the exporter or importer level, coffee quality is linked to bean size, lack of defects, regularity of provisioning, tonnage available, physical characteristics and price; at the roaster level, coffee quality depends on moisture content, stability of the characteristics, origin, price, biochemical compounds and organoleptic quality; at the consumer level, coffee quality deals with price, taste and flavour, effects on health and alertness, geographical origin, environmental and sociological aspects such as organic coffee, fair trade, shade coffee, etc. (Feria-Morales, 2002; Leroy et al., 2006; Perriot et al., 2006). It is a joint effort by all the key players of the coffee production-to-consumer chain (Prodolliet, 2004). According to Neilson (2007), quality is embodied not only in taste and/or physical attributes, but also through a plethora of social, environmental, ethical, safety and other concerns. Thus, quality is a key link between different stakeholders in the coffee sector, and hence coffee quality assessment is an important step in coffee trade (Gichimu et al., 2012).

It is generally accepted that coffee quality depends on different factors, such as the species/varieties, the environmental conditions (soil, rainfall, elevation, slope aspect, etc.), geographic locations (latitude, longitude), methods of processing, etc. (Decazy et al., 2003; Wintgens, 2004a, b; Avelino et al., 2005; da Silva et al., 2005; Knopp et al., 2006; Läderach et al., 2006; Leroy et al., 2006; Läderach, 2007; Yadessa et al., 2008a; Barbosa et al., 2012). And these factors vary from country to country and from place to place, and hence contributing to the quality variations in coffees around the world. Coffee quality is therefore the result of an interaction of these natural and human factors. Although Ethiopia is the birthplace of Arabica coffee, factors influencing coffee quality are less studied in the country as compared to other Arabica coffee producing countries. Since the country is the source of gene pool for Arabica coffee, it would have been the source of Arabica coffee research information in general and its quality in particular. Thus, to grow and produce good quality coffee, species/variety, important environmental factors, management factors, socio-economic factors, etc. that affect coffee quality should be taken into account. It is hypothesized that the major factors that affect the quality of wild Arabica coffee (*Coffea arabica* L.) from the natural coffee forest ecosystems are distinct since these natural coffee forests are the origin of Arabica coffee and found only in Ethiopia (not in other countries like Brazil, Vietnam, Colombia, etc.). Ethiopia has a unique position regarding Arabica coffee world as it is the birthplace or origin of *C. arabica*, and the natural conditions for coffee growing are almost ideal in Ethiopia (Krug and de Poerck, 1968). The objective of the present study was thus to assess the major factors (both natural and human) influencing the quality of wild Arabica coffee (*C. arabica* L.) in the natural coffee forests of southwest and southeast Ethiopia, and then to identify the key factors important for coffee quality.

MATERIALS AND METHODS

Study sites

The study was conducted in the southwest (Berhane-Kontir, Bonga and Yayu) and the southeast (Harenna) natural coffee forest ecosystems of Ethiopia, geographically separated by the Great Rift Valley System. The sites were selected for their landscape diversity so as to study the effects of various environmental factors on coffee quality in these ecologically diverse natural coffee forest ecosystems. Sheko, Bonga and Yayu are located west of the Great Rift Valley System, whereas Harenna is located east of the Great Rift Valley System (Figure 1).

The Yayu Natural Coffee Forest is located in the Yayo District, Illubabor Zone of Oromia Regional State in the southwest Ethiopia. Yayu has got its name from the word Yayo, the name of the Oromo sub-clan living in the Illubabor Zone. The soils of the area are red or brownish Ferrisols derived from volcanic parent material (Tafesse, 1996). The total annual rainfall is about 1900 mm with mean temperature of 19.7°C (minimum temperature 7.6°C, maximum

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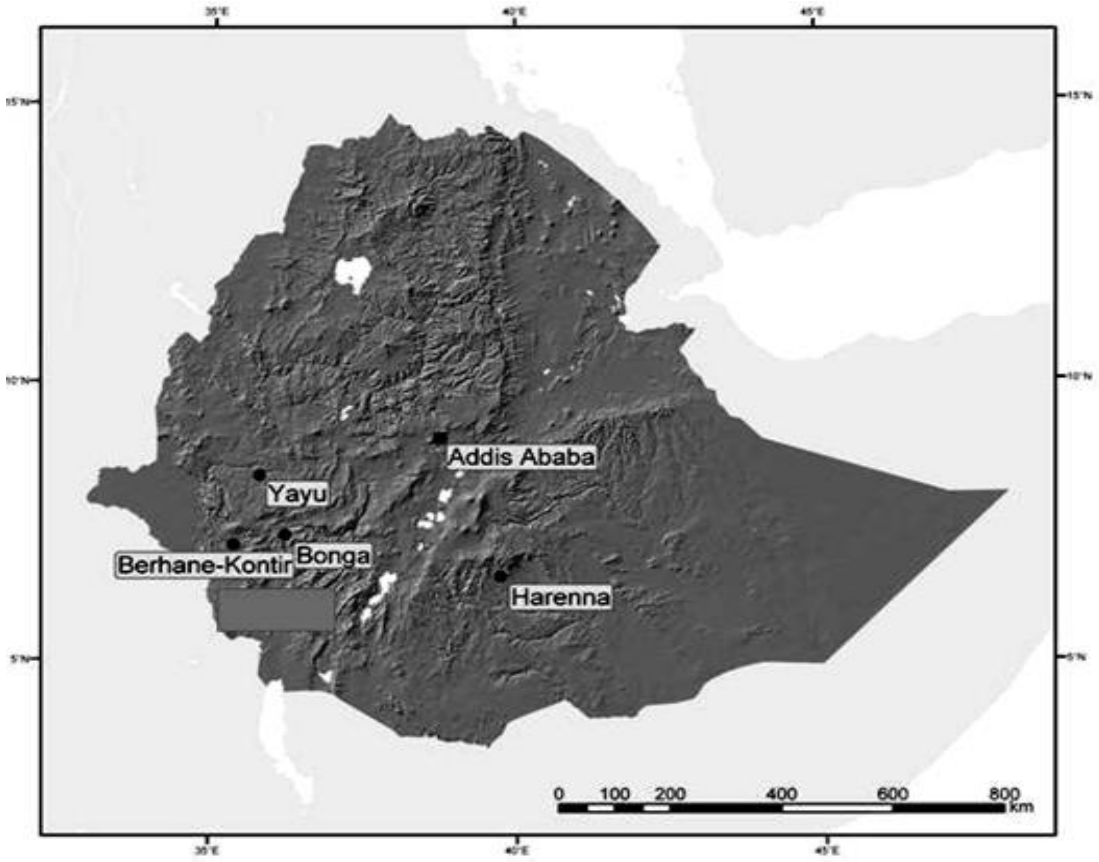

Figure 1. A map of Ethiopia showing the geographical location of the study sites.

temperature 34.7°C) and relative humidity of 80.9% (Kufa, 2006).

The Berhane-Kontir Natural Coffee Forest is also called Sheko forest. It is located in the Sheko District, Bench-Maji zone in the South Nations, Nationalities and Peoples Regional State, and hence the name Sheko forest. It represents the transition between the Afromontane moist forest and the lowland dry forest, located west of the Great Rift Valley (Senbeta, 2006). The total annual rainfall is about 2100 mm with mean temperature of 20.3°C (minimum temperature 13.8°C, maximum temperature 31.4°C) and relative humidity of 68.9% (Kufa, 2006).

The Bonga Natural Coffee Forest is located in Kaffa Zone of the Southern Nations, Nationalities and Peoples Regional State (SNNPRS) in the southwest Ethiopia. Bonga has got its name from Bonga, the king of Kaffa Kingdom. Nitisols are the most dominant soils in southwestern Ethiopia, prevailing mainly in coffee and tea growing areas such as the Bonga region (Schmitt, 2006). The total annual rainfall is about 1700 mm with mean temperature of 18.2°C (minimum value of 8.7°C, maximum value of 29.9°C) and relative humidity of 80.4% (Kufa, 2006).

The Harenna Natural Coffee Forest is located in Bale Zone of the Oromia Regional State in the south-eastern part of the country. It is a part of Bale Mountains, and the Bale Mountains include the northern plains, bush and woods, the Sannate Plateau, and the southern Harenna forest. The area is known for its floral and faunal diversity and endemicity (Friis, 1986; Hillan, 1988). It is located east of the Great Rift Valley. The total annual rainfall is about 950 mm with mean temperature of 22.2°C (minimum temperature 10.4°C, maximum temperature 34.4°C) and relative humidity of 63.2% (Kufa, 2006).

Sampling procedures and coffee cherry sampling

During site selection, preliminary information from the local people and key informants was collected to assess their perceptions on what factors might affect coffee quality. To assess the influence of environmental factors on coffee quality, coffee cherries were sampled from different plots located at different elevation ranges and geographic locations. Depending on the nature of the study site, the existing slope aspects, including valley bottoms or flat plots were also included in the sampling as described in Avelino et al. (2005). Transects were laid out systematically along the topographic aspects of the study coffee forest sites. Forty one samples from Berhane-Kontir, 19 from Bonga, 34 from Yayu and 20 from Harenna were studied. Coffee cherries were sampled from the respective plots. Elevation and geographic locations (latitude and longitude) were measured per plot. Garmin GPS was used for measuring geographic coordinates and elevation above sea level.

Coffee cherry harvesting, processing and cup tasting

Cherries were harvested at full maturity, which is usually during peak harvesting period. Coffee cherries mature and were harvested first in Berhane-Kontir (Sheko), followed by Bonga and Harenna, and lastly in Yayu according to their maturity order in the field. Red cherries were hand-picked from the coffee trees in the coffee forest and all the samples were then dry processed. The dried cherries were manually depulped and the beans were made ready for different analyses. Bean size distribution of wild Arabica coffee was

determined by conventional screen analysis, as described in Feria-Morales (2002) and Wintgens (2004a). Weight fractions retained on each sieve were recorded as described in Muschler (2001), and then converted into percentage basis. Cup tasting was conducted at the Coffee Quality Inspection and Auction Center in Addis Ababa, Ethiopia by a panel of 5 experienced cup tasters (three from Ethiopia and two from Germany). The major coffee quality attributes (fragrance, aroma, acidity, body, flavour, aftertaste and overall quality) were assessed using the beverage quality denominations ranging from 1 to 10, corresponding to the total absence (or presence) of the criterion in the coffee samples, respectively. The tasters first assessed the fragrance (dry aroma) by smelling the coffee powder before adding the hot water. After the coffee powder has been infused in hot water, the wet aroma of the brew was assessed. And next the resulting foam was removed before tasting started and then after the tasters assessed the acidity, flavour, body, aftertaste and finally the overall quality.

Soil sampling and analysis

Soil samples (0-20 cm) were collected from each plot. Five samples were collected per plot and then bulked to obtain a composite sample, and finally one representative sample was taken from the bulk per plot as described in Yadessa et al. (2001, 2009). Soil samples were analyzed for chemical and physical properties following the standard procedures at International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. Soil texture was determined by the Boucoucos hydrometer method (Day, 1965); soil pH by pH meter in a 1:2.5 (v/v) soil: water suspension; organic carbon (O.C.) by the wet oxidation method (Walkley and Black, 1934); available P following the procedures of Bray and Kurtz (1945); and total N by the Kjeldahl method (Jackson, 1958). Cation exchange capacity (CEC) was analyzed after extraction with 1 N ammonium acetate at pH 7 (ammonium acetate method). Micro-nutrients were extracted following the method of Lindsay and Norvell (1978), and the concentrations in the extract were determined using atomic absorption photometer.

Data analysis

Multivariate method (redundancy analysis) was used to assess the relationships between coffee quality traits and environmental variables. This is because correlation and regression analysis alone may not be suitable when large numbers of variables are involved, and thus different methods should be integrated for comprehensive analysis (Liebhold and Gurevitch, 2002; Zhang and Oxley, 1994). Multivariate analysis provides statistical methods for study of the joint relationships of variables (James and McCulloch, 1990). Moreover, the multivariate approach usually minimizes the problem of multicollinearity effects since the ordination axes are independent (Sokal and Rohlf, 1995), and multivariate analyses are therefore widely used to summarize large data sets (with many variables) by removing the influence of redundant or irrelevant variables in the data set (Dray, 2008; Guoqing et al., 2008).

Redundancy analysis (RDA) is a multivariate direct gradient analysis method appropriate where spatial environmental gradients are short (Jongman et al., 1987; Lepš and Šmilauer, 2003; van der Wollenberg, 1977). RDA can be best understood as methods for extending multiple regression that has a single response Y and multiple predictors X (e.g. several environmental predictors), to multiple regression involving multiple response variables Y (e.g., several species, traits, etc.) and a common matrix of predictors X (Peres-Neto et al., 2006). Ordination analysis was conducted using CANOCO for windows version 4.5 computer program (terBraak and Smilauer, 2002).

RESULTS AND DISCUSSION

The present study clearly demonstrated that the quality of wild Arabica coffee was considerably influenced by the environmental conditions and anthropogenic factors prevailing in the natural coffee forest ecosystems from where the samples were collected.

Influence of soil characteristics

Results revealed that there were significant relationships between soil properties and coffee quality traits. The cup quality of wild Arabica coffee was considerably influenced by soil properties, especially by available P (positive relationship) and soil texture (positive relationship with fine particles, but negative relationship with sand) (Table 1). This means higher levels of soil available P and clay or silt were associated with better cup quality, and vice versa. The probable reason for better cup quality of coffee associated with higher available P concentration of the soil might be due to the fact that phosphorus is vital to plant growth and it is involved in several key plant functions. Phosphorus is a structural element in nucleic acids (Hawkesford et al., 2012), and it plays an important role in energy storage and transfer in crop plants. Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are compounds with high-energy phosphate groups (Fageria, 2009; Hawkesford et al., 2012), and energy is released when a terminal phosphate is split from ADP or ATP (Sanchez, 2007). Both flowering and fruiting are reduced by P deficiency (Pallardy, 2008), and thus available P is a very important soil nutrient for cup quality of coffee.Seed quality improves with P nutrition (Roy et al., 2006), which is in agreement with the findings of the present study. A similar trend was previously reported by Yadessa et al. (2008a) for Sheko and Yayu sites (n=74). Nutrient concentration of the soil and that of coffee leaves in the study sites are presented in Appendix Tables 1 and 2, respectively.

According to the present findings, bean size distribution was also influenced by soil characteristics of the coffee plots, especially soil pH, Mn, organic matter and soil texture. There was significant positive relationship between soil pH or Mn and the proportion of bold beans (proportion of beans retained on screen 17). Generally, higher concentrations of soil organic matter, Mn, pH and sand content were associated with higher proportions of larger/bolder beans, and vice versa (Figure 2 and Appendix Table 3). This could be because mineral nutrients are essential for plant growth and development (Roy et al., 2006; Barker and Pilbeam, 2007). The developing beans normally act as priority sinks for assimilates and minerals (Cannell, 1985), which affects endosperm development and dry matter accumulation and this in turn affects bean size and weight.

A study by Mintesnot et al. (2015) showed that coffee quality attributes increased with increase in the levels of

Table 1. Pearson correlation matrix showing relationships between cup quality traits and soil properties in the four natural coffee forests of Ethiopia (n=102).

Soil parameter	Cup quality traits						
	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall
OM	-0.17	-0.089	0.045	-0.06	-0.072	-0.134	-0.039
Total N	-0.168	-0.073	0.076	-0.054	-0.05	-0.103	-0.024
Available P	**0.229***	**0.284****	0.115	**0.257****	0.192	**0.301****	**0.239***
OC	-0.170	-0.089	0.045	-0.06	-0.072	-0.134	-0.039
Na	-0.120	-0.021	0.005	-0.008	-0.076	-0.083	-0.065
K	0.117	0.177	0.012	0.054	0.11	0.148	0.103
Ca	-0.089	0.085	0.185	0.118	0.105	0.09	0.135
Mg	0.057	**0.219***	0.133	0.119	0.117	0.132	0.125
CEC	-0.113	0.039	0.127	0.09	0.069	0.037	0.078
pH	-0.077	0.127	0.175	0.136	0.052	0.098	0.119
PBS	0.046	0.169	0.121	0.070	0.099	0.113	0.126
Sand	**-0.297****	**-0.321****	-0.076	**-0.216***	-0.148	**-0.298****	-0.192
Silt	**0.333****	**0.398****	0.189	**0.251***	**0.219***	**0.320****	**0.264****
Clay	**0.214***	**0.203***	-0.02	0.149	0.067	**0.224***	0.102
Fe	-0.054	-0.067	-0.007	-0.024	0.027	-0.033	-0.020
Mn	-0.151	-0.007	0.130	0.103	0.014	0.021	0.048
Zn	0.123	0.19	0.029	0.046	-0.001	0.099	0.027

*, ** = correlations are significant at 0.05 and 0.01 level of significance, respectively.

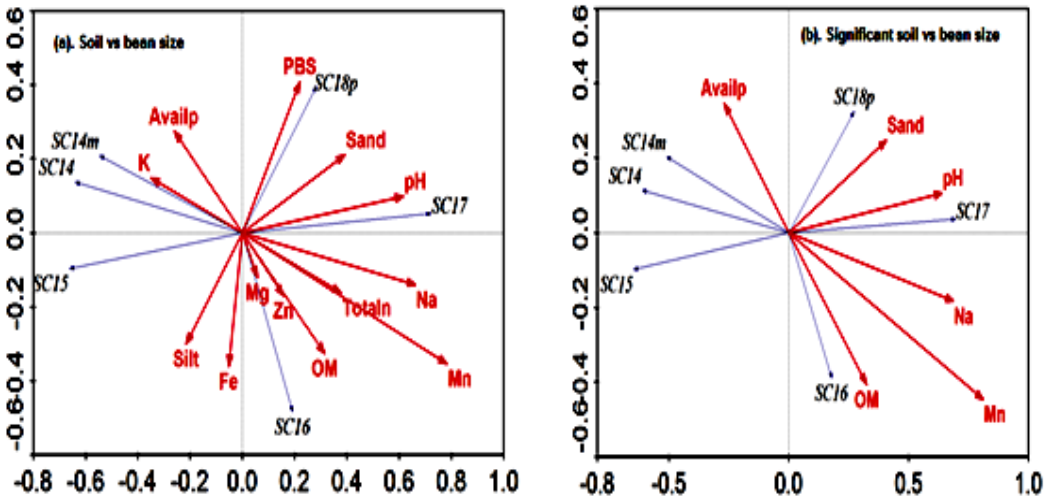

Figure 2. Redundancy analysis (RDA) biplot of bean size distribution versus soil properties; soil versus bean size (a) and significant soil versus bean size (b). Arrows represent the directions of maximum variation of the variables; arrows pointing in the same direction indicate a high positive correlation, arrows crossing at right angles indicate near-zero correlation, whereas arrows pointing in opposite directions indicate high negative correlation; the location of coffee quality scores near environmental vectors suggests the environmental affinities of the trait; p=0.002 for Mn, p=0.004 for sand, p=0.012 for pH, p=0.016 for Na, p=0.018 for available P and p=0.032 for organic matter. Appendix Table 3 shows the description of screen sizes.

soil Mg, but decreased with the increase in the levels of soil total N. A study by Kilambo et al. (2015) reported positive correlation between cup quality and some soil parameters (Ca, Mg, and K), and they also reported that soils with excessive calcium and potassium produce coffees with hard and bitter tasting liquor. A study by Ngugi et al. (2016) showed that Mn and Zn were important elements in the determination of organoleptic

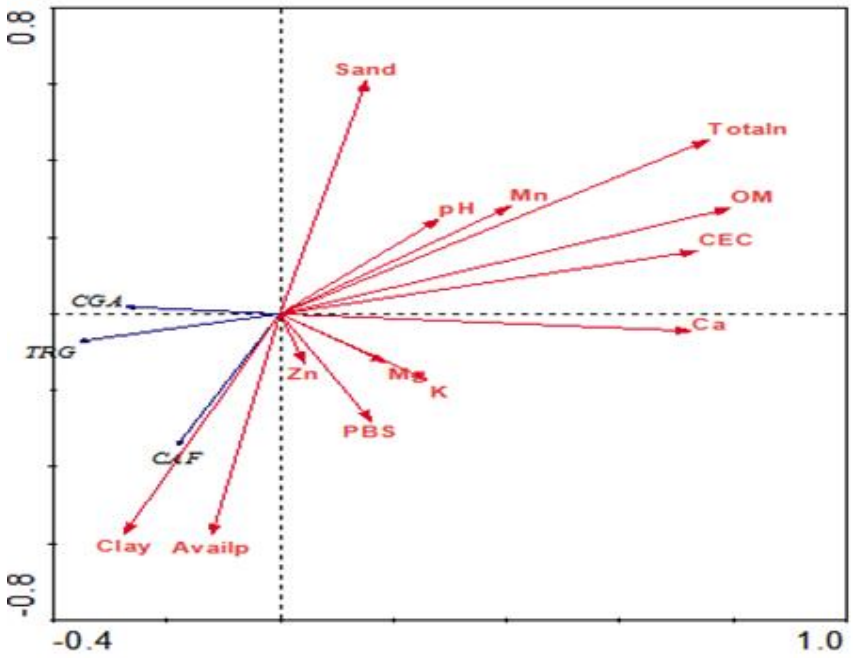

Figure 3. RDA biplot showing the relationships between soil properties and biochemical contents of wild Arabica coffee in the natural coffee forests of Ethiopia. CGA= chlorogenic acid, TRIG= trigonelline, CAF= caffeine.

cup quality in Robusta coffee.

Results also revealed that the biochemical contents of wild Arabica green coffee beans were also influenced by soil properties. Higher concentrations of soil organic matter, total nitrogen and sand content were associated with lower caffeine content; but the higher the clay content, the higher the caffeine content. There was a positive relationship between available P and bean caffeine content. Trigonelline was inversely correlated with most soil parameters. But chlorogenic acid was less influenced by soil properties (Figure 3). The probable reason for low caffeine content under P limited ecosystem or positive correlation of caffeine with available P status of soil and its negative correlation with nitrogen could be due to nutrient interaction or antagonism between N and P. In P limited ecosystem, N uptake is reduced and subsequently N concentration in plant tissue is decreased. The decrease in N concentration with increasing P limitation may be mediated by a decrease in leaf cytokinin levels (de Groot et al., 2003). Cytokinins regulate cell division in shoots and roots and promote movement of nutrients (Taiz and Zeiger, 2002; Hopkins and Hüner, 2009). A study conducted in Brazil by Mazzafera (1999) to investigate the influence of mineral nutrition of coffee on its caffeine contents showed that the omission of P induced the lowest caffeine content.

Generally, both physical and chemical properties of the soil were very important factors for the quality of Arabica coffee in its natural habitat of southwest and southeast coffee forests of Ethiopia, as they influenced every aspect of coffee quality traits (bean physical quality, cup quality and biochemical contents). Thus, soil is a very important factor of quality in coffee production. This may be because soil property is an output of different soil-forming factors (topography, climate, parent material, living organisms, time) and hence factors that influence soil property most likely influence coffee plant growth and hence its quality.

Influence of elevation above sea level

The effect of elevation much depended on other factors, such as geographic location (latitude, longitude), soil, etc. This is because elevation is an indirect environmental gradient (no direct effect on plant physiology), and the major variable that changes with elevation is temperature, which also changes with latitude and longitude (Austin et al., 1984). Increase in elevation led to an increase in bean size and soil organic matter up to the elevation of about 1600 m above sea level, but thereafter no significant increase (that is, hump-shaped relationship, not monotonic) (Figure 4). This might be attributed to decrease in soil organic matter decomposition and mineralization (organic matter accumulation), which arise due to decrease in temperature with increasing elevation. A study by Alpizar and Bertrand (2004) also showed that

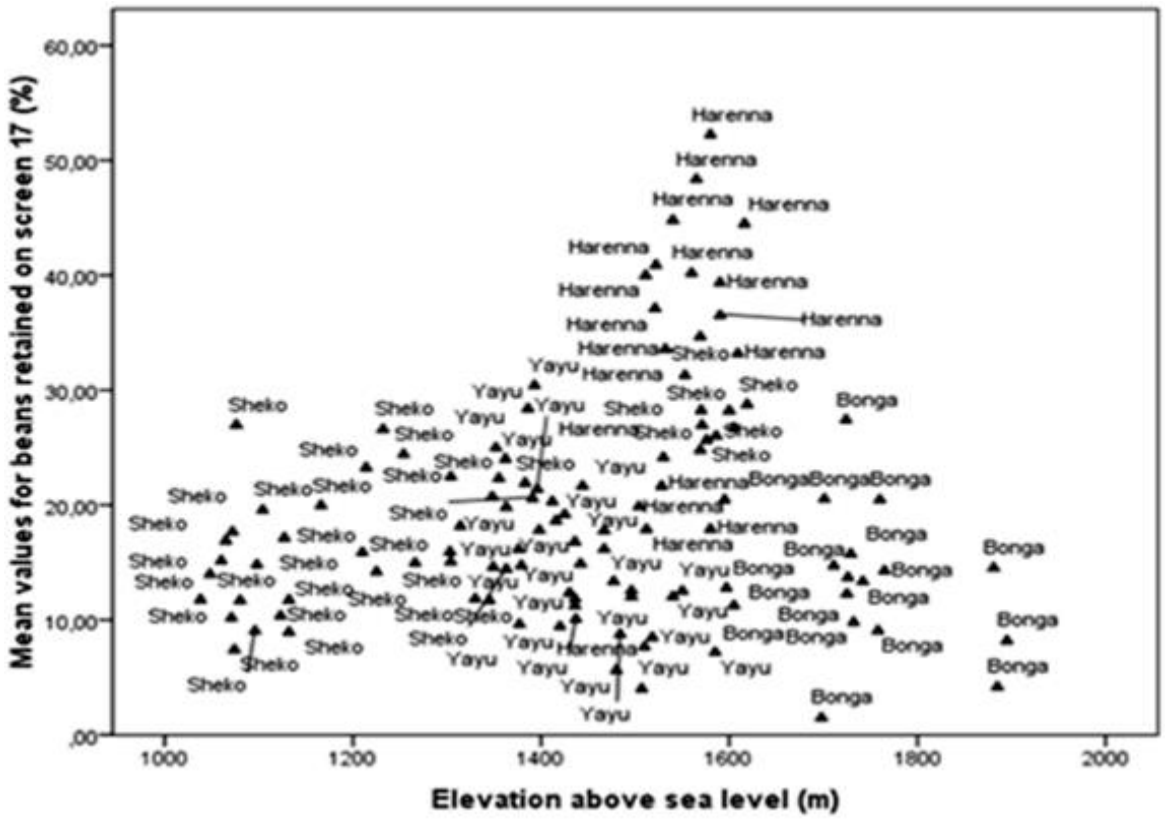

Figure 4. Variability in the proportion of beans retained on screen 17 (bold beans) across the elevation gradients in the natural coffee forests of Ethiopia.

the higher the elevation, higher the proportion of large size beans; and this relation was observed up to an elevation of 1400 m above sea level and then started to decline thereafter. This could be due to reduced nutrient availability, which is characterized by higher carbon-to-nutrient ratios, such as C:N, C:P, etc. (Wilcke et al., 2003, 2008). In the present study, C:P and C:N ratios were significantly higher at higher elevations than at lower elevations (data not shown). Thus, decreasing nutrient availability at higher elevations might be the probable reason for decreasing bean size after an elevation gradient of 1600 m.

As shown in Figure 5, coffee bean weight was significantly varied across the elevation ranges, being highest in the elevation range 1300-1600 m above sea level (asl). Values of 100 bean weight varied from 13.53 to 18.79 g (mean 15.62 g) in the elevation range <1300 m asl, from 14.52 to 20.51 g (17.28 g) in the elevation range 1300-1600 m asl, and from 14.43 to 19.57 g (mean 16.47 g) in the elevation range >1600 m asl.

As a general trend, bean size showed a hump-shaped relationship with elevation; that is, it increased with increase in elevation at low elevation levels, reached a peak at intermediate elevation levels, but declined at high elevation levels. This may be because increase in elevation in already a highland area and to higher ranges may lead to decrease in temperature below the optimal range for coffee. The optimum temperature for Arabica coffee is between 15-24°C year round, and photosynthesis is reduced above these temperatures (Willson, 1999; CRI, 2001). And this also leads to decrease in decomposition rate and subsequent accumulation of soil organic matter. Temperature has a significant impact on coffee trees (Descroix and Snoeck, 2004), and it is generally agreed that every 100 m of elevation corresponds to a decrease in temperature of 0.6°C (Wintgens, 2004b; CRI, 2001).

Influence of topographic aspect

The effect of topographic aspect was rather more important than elevation for coffee quality in the natural coffee forests. Generally, beans on the southern and western facing aspects were bolder in size as opposed to those on the northern and eastern aspects (Figure 6). This could be due to difference in environmental factors, especially soil properties, which is evidenced by higher soil organic matter and nutrients on the south-and west-facing aspects as compared to the north- and east-facing

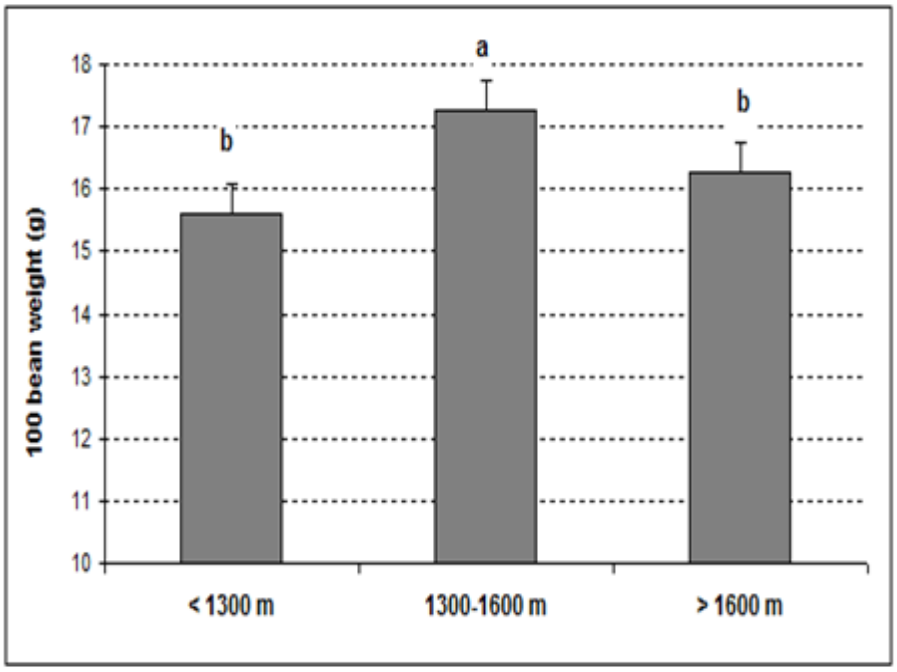

Figure 5. Hundred bean weight across the elevation gradient in the natural coffee forests of Ethiopia; mean values followed by similar letters are not significantly different by Tukey's significant test.

aspects. The reason why coffee beans from the south- and west-facing aspects are bolder than those from the north-and east-facing aspects could be due to variability in environmental factors like soil characteristics. This argument was supported by significantly higher soil organic matter, pH, and nutrients (e.g. Mn, Ca, Na, etc.) on the south-and west-facing aspects as compared to the north- and east-facing aspects, as shown in Figure 7. Soil Mn (important soil parameter for bean size) was 339.07, 258.29, 251.12 and 84.07 ppm, under west, south, east and north facing aspects, respectively. A study in China also revealed that high fertility plots often exist on south-facing slopes and soil organic matter is an important indicator to soil fertility (Fu et al., 2004). Variation in coffee quality with respect slope aspect may be related to differences in availability of light, moisture, etc. Slopes facing the equator (south-facing slopes in the northern hemisphere and north-facing slopes in the southern hemisphere) receive more radiation than opposing slopes and thus have warmer and drier conditions (Chapin et al., 2002). A study by Avelino et al. (2005) in Costa Rica showed that coffees from east-facing slopes had better quality.

Influence of geographical location

Latitude was inversely correlated with bean size; that is, as one moves from north-south gradient within the study sites (decrease in latitude), the proportion of larger/bolder beans increased, and vice versa. But for the case of longitude, the opposite trend was observed. Generally, samples that were collected from plots with higher longitude readings had relatively higher proportions of larger/bolder beans. This means the proportion of larger beans decreased from east-west gradient (decrease in longitude).

In general, the proportion of bold beans decreased as latitude increased, but it increased as longitude increases, and vice versa. The relationship is shown as follows:

The proportion of bold beans (% of beans retained on screen 17) = -3.042 latitude readings (in decimal degrees) + 3.064 longitude readings (in decimal degrees) – 69.788

From geographical location point of view, coffee beans from the SE Afromontane rainforests were bolder in size as compared to those from the SW Afromontane rainforests. This is because soil organic matter, pH, Mn and sand in the SE were higher than in the SW, and also their distribution followed an increasing trend along the west-east longitudinal gradient in the study sites. This variation in soil characteristics imparts variability in coffee quality. The soils in the southeast are more sandy and less weathered (Yimer et al., 2006) compared to the more clay dominated and highly weathered soils in the

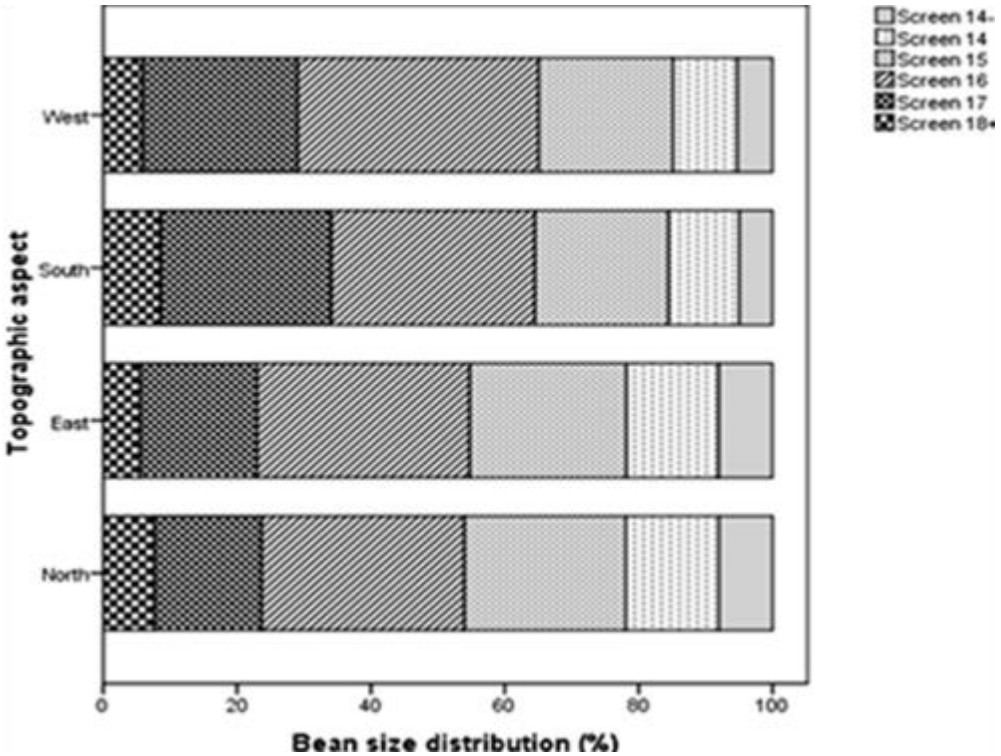

Figure 6. Bean size distribution as influenced by topographic aspect in the natural coffee forests of Ethiopia.

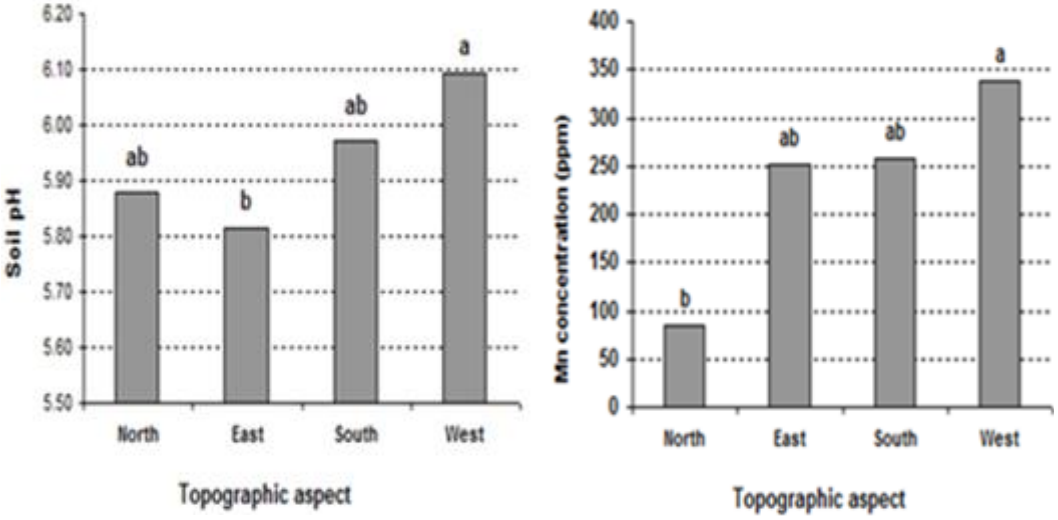

Figure 7. Soil pH and Mn concentrations across the different topographic aspects in the Afromontane rainforests of Ethiopia.

southwest (Dubale and Mikiru, 1994). As latitude increases, temperature decreases. Latitude influences the climate by influencing the amount of solar radiation received (MacMahon et al., 2007). Areas near the equator receive more incoming solar radiation than areas near the poles. At the equator, the sun's rays are almost perpendicular to the surface at solar noon. At lower sun angles experienced at high latitudes, the sun's rays are spread over a larger surface area, resulting in less radiation received per unit ground area (Chapin et al.,

Table 2. Pearson correlation coefficients showing the relationship between climate and coffee quality traits; climatic data from mobile weather station was used.

Traits	Rainfall	Tmin	Tmax
Sensory characteristics			
Fragrance	0.227*	0.348**	-0.267**
Aroma	0.116	0.262**	-0.100
Acidity	-0.071	0.024	0.103
Flavor	-0.009	0.135	0.028
Body	0.071	0.158	-0.032
Aftertaste	0.093	0.234*	-0.091
Bean charactersitics			
Screen 18^{+}	-0.077	-0.091	0.175
Screen 17	-0.584**	-0.446**	0.623**
Screen 16	-0.240*	-0.144	0.186
Screen 15	0.484*	0.365*	-0.559**
Screen 14	0.527**	0.406**	-0.533**
Screen 14^{-}	0.475**	0.335**	-0.460**
100 bean weight	-0.404**	-0.421**	0.502**
Bean length	-0.361**	-0.397**	0.309**
Bean width	-0.080	0.046	0.254**
Bean thichness	0.109	0.015	-0.085
Bean shape index	-0.313**	-0.385**	0.207*

Tmin = minimum temperature, Tmax = maximum temperature.

2002; Raven et al., 2010). For each degree of latitude away from the equator, the corresponding reduction in temperature is estimated at 0.5 °C (Descroix and Wintgens, 2004).

Influence of climate

There was a positive and significant relationship between minimum temperature and coffee aroma (Table 2). This may be because climate is an important factor with considerable effect on soil properties and thus important factor for coffee quality. It affects the rate of soil formation especially temperature and rainfall and the type of soil that is ultimately formed by influencing weathering processes.

The climate also affects the type of vegetation it supports by influencing the physiology of plants such as photosynthesis, flowering, maturity, etc., which has implications on coffee quality. This is very interesting in the context of current global climate change. The influence of rainfall on cup quality was not as apparent as that of its effect on bean size distribution. In general, the higher the rainfall, the higher the proportion of smaller beans and vice versa (Table 2). High temperature accelerates fruit maturation in coffee (Descroix and Snoeck, 2004). But at lower temperature coffee fruits undergo a slower maturation process, allowing the full manifestation of all biochemical steps necessary for the development of beverage quality (da Silva et al., 2005). A study by Camargo et al. (1992), cited in da Silva et al. (2005) also suggested that regions with a relatively high temperature tend to produce low quality coffee.

Influence of forest management on coffee quality

Anthropogenic factors (human activities) are one of the causes for spatial heterogeneity of ecosystems. According to the present study, the level of forest management influenced both the cup quality and the bean physical characteristics of wild Arabica coffee, but not the biochemical contents. The level of forest management (level of human interference) considerably influenced the bean size distribution and cup quality traits. When the level of human interference was relatively higher, the cup quality of coffee was better, the proportion of larger beans increased, and available phosphorus increased but soil organic matter decreased. ‘Managed (slashed) plots had relatively better cup quality and higher proportion of larger beans. This could be due to improved micro-environmental conditions such as light/temperature and subsequent decomposition and soil mineralization and reduction of weed competition (Table 3).

Forest coffee management modifies the forest ecosystem by changing the microclimate (e.g. light, soil, etc.) and the forest conditions (e.g. species composition,

Table 3. Cup quality and bean size distribution of wild Arabica coffee as influenced by forest management in the natural coffee forests of Ethiopia.

Level of forest management	Cup quality traits						
	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall
Little	5.54[b]	5.22[b]	5.48[b]	4.75[b]	5.53	4.64[b]	5.20[b]
Medium	5.93[ab]	5.79[a]	6.00[a]	5.58[a]	6.04	5.37[a]	6.02[a]
High	6.09[a]	5.94[a]	5.96[ab]	5.51[a]	6.06	5.33[a]	5.96[a]
P value	0.025	0.013	0.049	0.003	NS	0.018	0.004

	Bean size distribution (%)					
	Screen 18+	Screen 17	Screen 16	Screen 15	Screen14	Screen 14-
Little	5.12[b]	17.31[b]	32.93	24.45[a]	12.65	7.56
Medium	5.62[b]	17.81[ab]	32.56	23.47[a]	13.21	7.34
High	9.40[a]	23.03[a]	30.72	19.61[b]	11.14	6.11
P value	0.000	0.028	NS	0.005	NS	NS

Means followed by similar letters are not significantly different by Tukey's significant test.

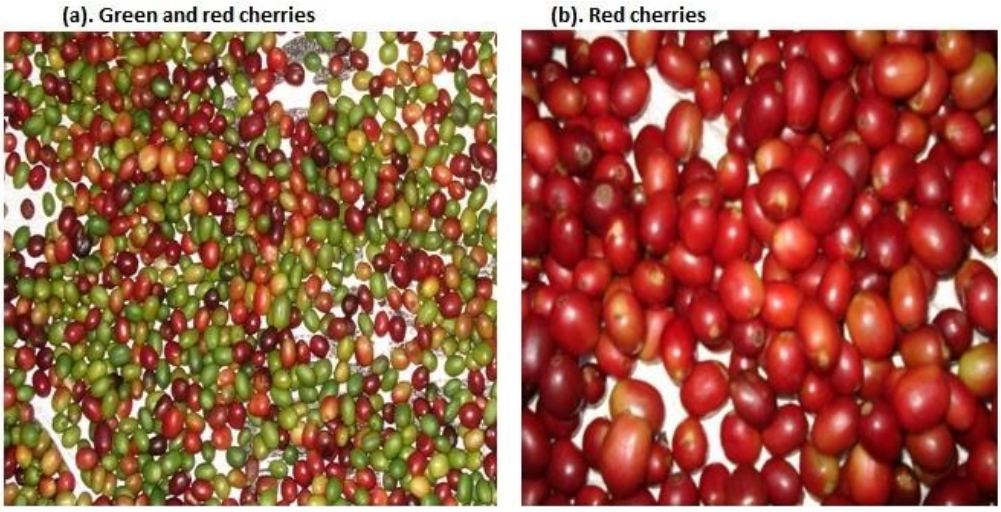

Figure 8. Theft affects coffee quality by affecting cherry harvesting quality.
Source: Photo by Abebe Yadessa.

vegetation structure, space availability, etc.), due to tree thinning and slashing of undergrowth (Senbeta and Denich, 2006; Hundera et al., 2013). Forest management also influences soil nutrient ratios, which impart variations in coffee quality (Yadessa et al., 2019). But in contrast to the present findings, a study by Geeraert et al. (2019) reported a decreasing trend in cup quality of Arabica coffee with increasing intensity of coffee forest management.

Other important factors affecting coffee quality

Theft problem

The effect of theft on coffee quality is indirect. Because of the problem of cherry thievery in the field during harvesting, farmers in some coffee growing areas are forced to pick green and red cherries together (that is, early harvest) as an escape strategy, and this has a considerable impact on coffee quality (Figure 8a, b). Harvesting quality is essential for better cup quality of coffee (Perroit et al., 2006). The problem of coffee cherry theft during harvesting is also reported by Schmitt (2006), which is in agreement with the present findings. But this forced early harvest due to thievery should not be confused with the early harvest of red cherries, which may give even better cup quality. For instance, Läderach (2007) reported relatively better beverage quality for early harvest as compared to late harvest. Unintentional or intentional harvesting of cherries at several stages of maturation may have adverse impacts on coffee quality if

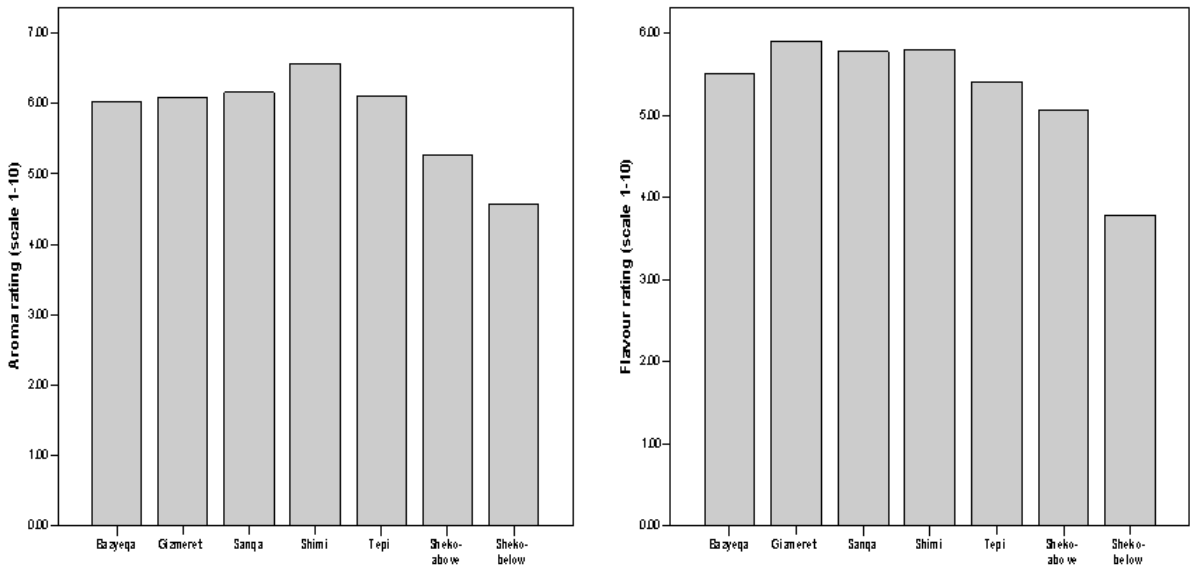

Figure 9. Selected cup quality traits of wild Arabica coffee collected from below the trees as compared to hand-picked coffees in Sheko coffee forest site. Sheko-above refers to coffee samples from farmers but collected from the coffee trees (not from the ground), whereas Sheko-below refers to coffee samples from farmers with coffees collected from below the trees (fallen berries from the ground floor).

these materials are processed together (Brando, 2004). Thus, coffee quality, especially its cup quality is directly correlated with optimal cherry maturity, and theft thus plays a considerable role in coffee quality by influencing cherry harvesting quality.

Heavy rainfall during peak harvesting period

Rainfall influences coffee quality in both direct and indirect ways. On one hand, heavy rainfall can lead to falling (drop) of cherries from coffee trees to the ground before harvest. Fallen cherries from the ground (below coffee trees) can be a major constraint to coffee quality unless care is taken (Figure 9). On the other hand, heavy rainfall during harvesting and/or processing can lead to mould development due to inappropriate drying. Coffee quality is highly dependent on post-harvest processing (Menon, 1992; Perroit et al., 2006). Because of heavy rainfall during cherry maturity or during post-harvest processing, coffee cherries and/or beans can be contaminated by soil when dropped to the ground giving earthy or moldy taste. A study by Tagliaferro et al. (2007) also showed that the impurities from the soil can reach the cup and spoil the beverage quality, and this supports the present findings. Falling of cherries from the coffee trees to the ground due to heavy rainfall was more apparent in Sheko area as compared to other forest coffee sites, due to heavy rainfall and local tradition of cherry handling prevalent in the area. Therefore, harvesting of green cherries, over-ripe cherries, and picking of fallen cherries from the ground are the major harvesting-related factors influencing coffee quality in the study sites.

Lack of differential price for coffees of different quality

Traders usually pay the same price for coffees of different quality, and bulking of coffees of different qualities or origins is not uncommon practice in the study areas and elsewhere as well with no consideration for quality harvesting and processing (Figure 10). And payment is effected on the basis of quantity, not on quality; that is, there is no market segmentation according to coffee quality. This inevitably leads to reluctance by producers for the coffee quality if this problem is not taken into consideration in the future. This is almost common in most of the study sites or elsewhere. A study by Kodama (2007) also showed that farmers sell a better quality coffee to cooperatives as they expect dividends, but they are less concerned about the quality of coffee for private traders. A study in Jimma zone by Tolessa et al. (2018) revealed that coffee beans managed by cooperatives had better quality scores than those managed by private traders. A study by Bacha (2007) in Bonga (Kaffa zone), for instance, showed that the share of forest coffee producers is only 3% of the retail price.

The lack of differential price has two negative effects, namely: (i) discourages farmers to invest in coffee quality improvement (resources like money, labor, time, etc.),

Figure 10. Coffees of different quality, but sold with the same price; (a) well dried coffee, and (b) mould developed coffee.
Source: Photo by Abebe Yadessa.

better quality scores than those managed by private traders. A study by Bacha (2007) in Bonga (Kaffa zone), for instance, showed that the share of forest coffee producers is only 3% of the retail price.

The lack of differential price has two negative effects, namely: (i) discourages farmers to invest in coffee quality improvement (resources like money, labor, time, etc.), and (ii) bulking of coffees of different quality (sources) considerably influences coffee quality. Generally, if there is no or little difference in the price of a high and a low quality coffee, farmers will show reluctance for investing in coffee quality improvement activities because such investment cannot justify the incurred cost.

Poor share-harvesting arrangements

The use of hired labour from the neighbouring non-coffee growing areas (e.g. Adaba, Ganale, Bidire, etc.) is also an important factor of coffee quality since payment for labourers is based on the quantity harvested, not on the quality of coffee harvested. One third of the harvest is usually for the collector and two third is for the owner, without due consideration to the quality of cherry harvested. This problem is more common in Harenna area (Yadessa et al., 2008b). It is also a problem in Sheko area.

Bulking of coffees from different sources

In many coffee producing areas, coffees from different sources, from forest or plantation, red or green harvested, well dried or mould developed, etc., are sold with the same price. There is usually little or no market differentiation, if any, for coffees of different quality, which leads to reluctance of farmers to give more attention to quality. High quality coffee requires special care and thus coffees with better quality represents good differentials of product price (Pereira et al., 2010).

Limited research capacity

Although Ethiopia is the birthplace of Arabica coffee, factors influencing coffee quality are less studied in the country as compared to other Arabica coffee producing countries. Limited trained manpower and institutional capacity are the major bottlenecks in the country. But this is not the case at the present time, and this should be given due attention in the future.

Poor cooperation between the coffee stakeholders in quality control

Collectors/traders in most cases buy coffees without due consideration to quality, no differential price for different quality coffees. They also buy coffees from anybody, including thieves. In the case of cooperatives, on-farm supervision is a common practice, and production, harvesting and processing are usually supervised with the technical staff of the cooperatives. They also participate in development works such as schools, clinics,

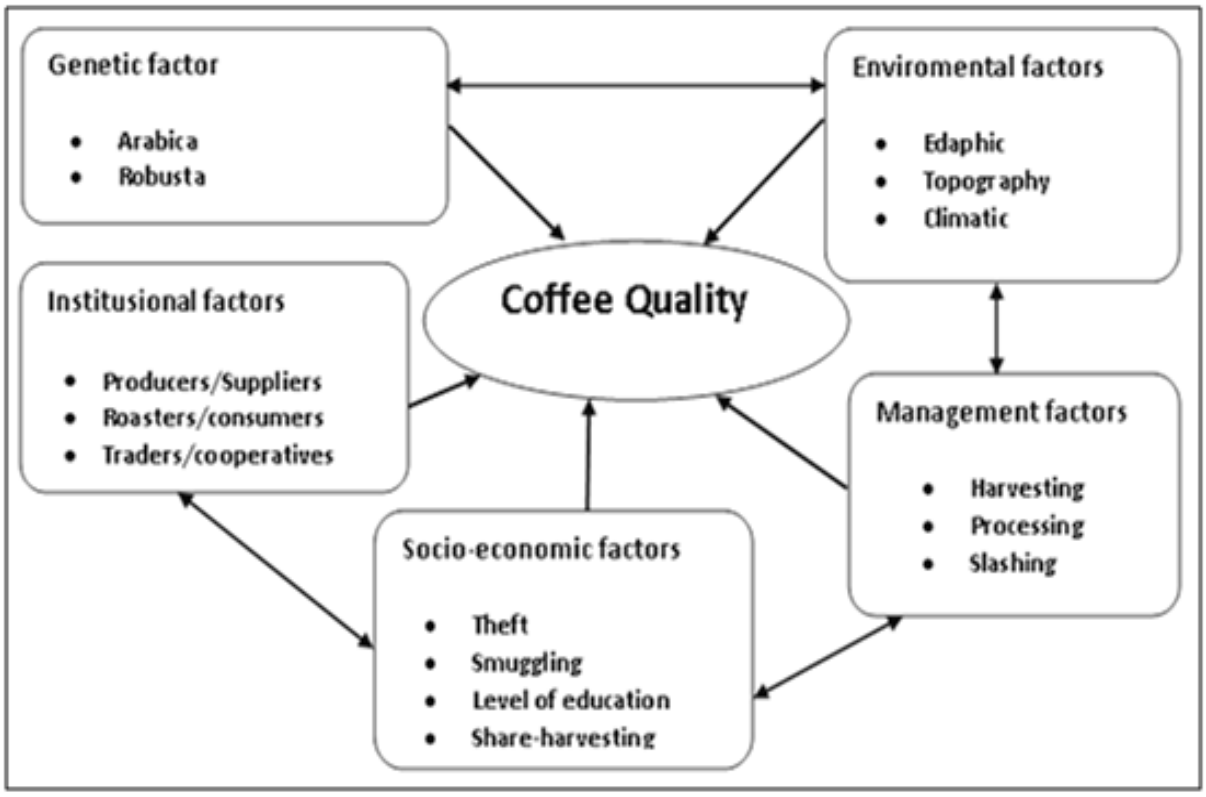

Figure 11. Schematic presentation of the major factors influencing coffee quality and their interactions in Ethiopia.

feeder roads, etc. But in the case of traders, participation in development activities are less experienced. But traders in some cases slightly increase some cents for coffee price to relieve from the competition with cooperatives or farmers, but still these are not as such significant. Apart from the price, handling coffee lots from different sources or farmers differently is not an easy task as well, which is one constraint for coffee quality improvement.

In Ethiopia, coffee cooperatives have brought benefits to coffee farmers by providing a new marketing channel. The dividends are being appreciated by farmers and have encouraged farmers to improve the quality of their coffees. Although the actual volume purchased by cooperatives is limited due to financial constraints, the existence of cooperatives in the coffee market has improved the purchasing price offered by private traders because of competition with the cooperatives. Since the late 1990s, in Ethiopia cooperative activities have been encouraged again, despite bad experiences during the socialist regime (Kodama, 2007). Cooperatives are being appreciated or recognized as business and marketing organization in Ethiopia and as one means of protecting farmers as opposed to its past notion. This is because, in union there is strength. Thus, different stakeholders in coffee sector better promote quality and the sustainability of coffee production rather than competing with each other. Therefore, the role of agricultural marketing cooperatives is very crucial for smallholder farmers.

Poverty and illiteracy

Apart from the above factors, one of the main challenges facing small-scale coffee producers is their lack of access to physical, economic, and educational resources. Many farmers lack the knowledge and resources (financial or material) to ensure efficient and high quality coffee production. Moreover, traders or suppliers should also be experts in coffee quality themselves to alleviate these problems. Another problem is that those who have information often lack the resources for quality improvement (ITC. 2011a).

In general, coffee quality is the result of interaction of both natural/environmental (soil, climate, elevation, aspect, latitude, longitude) and human (coffee management, theft, harvesting, processing) factors, as summarized in Figure 11. For any system to function properly, there are naturally interactions between the different components of the system. One element of the system can't exist on its own (Yadessa et al., 1999). Similarly, there is an inevitable interdependence and interrelationships among the different factors affecting coffee quality in one way or another. As presented in

As presented in Figure 11, different factors affect coffee quality:

i) Environmental factors (soil, topography, climate, geography),
ii) Management factors (cherry harvesting and processing, slashing/forest management),
iii) Socio-economic factors (theft problem, poor-share arrangement, lack of differential price, poverty and illiteracy etc.),
iv) Institutional factors (cooperatives, traders, etc.).

As to the genetic factors, the study was conducted in natural coffee forest ecosystem (the gene pool for other Arabica coffee varieties) and Robusta coffee is not common in Ethiopia, and hence the issue of coffee species/variety in natural coffee forests harbouring wild coffee Arabica populations is less relevant here. Of course, the genetic factor might have contributed to the quality of wild Arabica coffee in its natural habitat of southwest and southeast Afromontane rainforests of Ethiopia, but further study on genotype and environment (G*E) interaction is required.

As the importance of quality and origin is increasing in coffee market, the research that deals with factors that influence coffee quality should be a priority in coffee research. Generally, high-quality coffee arises from maintaining close control over a multitude of factors in the field, in the plant and in the cup across the value chain (Prodolliet, 2004; Perroit et al., 2006).

Conclusion

The study demonstrated that the quality of wild Arabica coffee was influenced by different factors -environmental factors (soil, topography, climate, geography), management factors (cherry harvesting and processing, slashing/forest management), socio-economic factors (theft problem, poor-share arrangement, lack of differential price, poverty and illiteracy etc.), institutional factors (cooperatives, traders, etc.) and others. Coffee quality was influenced by complex interactions of different factors. In Ethiopia, wide range of climatic conditions, soil characteristics, topographic features, geographic locations, socio-economic factors, etc. inevitably have led to high diversity of coffee production systems managed with different intensities, which impart diversity in coffees with diverse quality attributes.Consequently, coffee quality is the product of many environmental and anthropogenic factors acting together and hence yielding coffees of different quality with their own unique characteristics. The study tested the traditional theory that states coffee quality is affected by different factors. The results presented here under different sections in this article were highlighted to support the framework outlined in Figure 11 (factors influencing coffee quality), which is the nucleus or focus of the current study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX

Table 1. Mean values (±standard deviation) for the considered soil parameters from the four Afromontane rainforests in the SW and SE Ethiopia.

Statistic	SW soils			SE soils	
	B. Kontir (n=41)	Bonga (n=16)	Yayu (n=34)	Harenna (n=20)	P value
SOM (% DM)	4.64±1.34^{c}	6.52±1.25^{b}	7.21±2.20^{b}	8.49±1.00^{a}	0.000
Total N (% DM	0.32±0.07^{c}	0.41±0.05^{b}	0.41±0.13^{b}	0.52±0.005^{a}	0.000
Avail. P (ppm)	39.99±34.48^{a}	3.44±7.52^{b}	11.22±12.56^{b}	1.94±2.09^{b}	0.000
Na (meq/100 g)	0.05±0.06^{c}	0.10±0.06^{b}	0.04±0.02	0.16±0.07^{a}	0.000
K (meq/100 g)	1.23±0.68^{a}	1.34±0.80^{a}	1.07±0.74^{a}	0.56±0.40^{b}	0.002
Ca (meq/100 g)	11.88±4.87^{bc}	9.40±3.52^{c}	13.15±5.74^{b}	19.18±3.89^{a}	0.000
Mg (meq/100 g)	3.70±1.77	2.91±1.09	3.04±1.56	3.73±0.58	NS
CEC (meq/100 g)	29.08±7.39^{b}	34.96±5.05^{b}	32.22±12.33^{b}	43.77±4.69^{a}	0.000
BS (%)	56.58±12.57^{a}	39.01±13.68^{b}	53.89±11.83^{a}	54.44±10.23^{a}	0.000
pH	5.90±0.24^{b}	5.47±0.43^{c}	5.82±0.22^{b}	6.42±0.18^{a}	0.000
Sand (% DM)	20.18±9.07^{c}	29.13±6.37^{b}	43.82±11.14^{a}	46.70±5.92^{a}	0.000
Silt (% DM)	37.76±4.76^{a}	34.57±3.37^{a}	28.88±7.76^{b}	27.86±2.70^{b}	0.000
Clay (% DM)	42.06±8.02^{a}	36.31±5.49^{b}	27.30±4.69^{c}	25.44±5.95^{c}	0.000
Fe (ppm)	57.39±34.98^{b}	246.36±313.99^{a}	50.93±40.78^{b}	82.61±50.44^{b}	0.000
Mn (ppm)	136.91±45.96^{ab}	212.10±158.79^{b}	66.29±28.11^{b}	738.74±179.06^{a}	0.000
Zn (ppm)	2.97±1.72^{a}	3.26±01.85^{a}	1.41±0.60^{b}	2.38±0.55^{ab}	0.000

Means followed by similar letters within a row are not significantly different, DM = dry matter, BS= base saturation, SOM = soil organic matter, 1 ppm=mg/L (liquid substance) or 1 mg/kg (solid substance). In terms of percents, 1 ppm equals 0.0001 percent.
Source: Yadessa et al. (2019).

Table 2. Leaf nutrient content in the Afrontane rainforests of southwest and southeast Ethiopia harbouring wild *C. crabica* populations.

Nutrient	Harenna	Bonga	Yayu	B.-Kontir
P (%)	0.07^{c}	0.12^{b}	0.11^{c}	0.15^{a}
K (%)	1.80^{c}	2.2^{b}	2.1^{b}	2.7^{a}
Ca (%)	1.60^{a}	1.2^{b}	0.33^{c}	0.02^{c}
Mg (%)	0.35^{a}	0.33^{b}	0.26^{bc}	0.28^{ac}
Zn (ppm)	13.9^{b}	11.5^{b}	9.7^{c}	11.1^{b}
Mn (ppm)	61.0^{b}	67^{b}	59^{b}	98^{a}

Means followed by similar letters within a row are not significantly different.
Source: Beining (2007).

Table 3. Screen sizes and descriptions.

Screen no.	Screen diameter (mm)	ISO norm	Bean size description
20	7.94	8.00	Very large
19	7.54	7.50	Extra large
18	7.14	7.10	Large
17	6.75	6.70	Bold
16	6.35	6.30	Good
15	5.95	6.0	Medium
14	5.55	5.6	Small
13	5.16	5.0	
12	4.76	4.75	

Source: Wintgens (2004b).

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Combining ability of maize inbred lines (*Zea mays* L.) for yields in mid altitude sub-humid Agroecology of Ethiopia

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Maize is an important staple food for most Ethiopians, but the national average productivity of maize is below that of the world. Development and cultivation of high yielding maize hybrids can improve maize productivity and production. Having information on combining ability and heterosis of maize inbred lines is important for the development of high yielding maize hybrids. The objectives of this study were to identify good hybrids based on grain yield and yield related traits, to estimate the general combining ability (GCA) and specific combining ability (SCA). Thirteen inbred lines were crossed in 2017 with two line testers using a line by tester mating design. The resulting 26 crosses were evaluated in a randomized incomplete block design (RCBD) with three replications during the main rainy seasons between June and November, 2018 at Bako, Ethiopia. In addition, the 13 parental lines including the two tester lines were evaluated using RCBD with three replications in a separate trial. Analysis of variance (ANOVA) showed that mean squares due to crosses were highly significant (P≤0.01) for most of the traits studied, except ear aspect. Also mean square due to line was significantly different (P < 0.01 or P < 0.05) in all studied traits except days to anthesis (AD) and ear aspect (EA). The overall mean grain yields (GY) of the hybrids were 6.32 t/ha ranging from 5.21 to 8.19 t/ha. L7 had the lowest negative GCA for grain yield whereas L6 had the highest positive GCA. Among the crosses with high positive SCA, estimates showed high mean grain yield, which implied good correspondence between SCA effects and mean GY. The result obtained in this study could be useful to design for developing high yielding hybrids and synthetics adapted to the mid altitude sub humid agro ecologies of Ethiopia.

Key words: Grain yield, maize inbred lines, line by tester, general combining ability, specific combining ability.

INTRODUCTION

Maize (*Zea mays* L) is a diverse species, with rich morphological and biological variability. It is a monoecious plant, bearing distinct male and female flowers. It is predominantly cross pollinated through wind.

Maize is grown in many countries of the world, across a wide range of climates and ecological zones (Dowswell, 1996).

This crop is one of the most important staples crops in

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the world. Maize, wheat, and rice are among the stable crops supporting over 75% of the world's population (Ji and Wang, 2013). Maize is first among cereal crops of 41.56% followed by wheat with 29.39% and rice with 29.05% interms of production (FAOSTAT, 2018).

Maize is one of the most crucial field crops in Ethiopia, with respect to production, yield, area under cultivation and economic importance. Its production has increased five folds over the past two decades (from 1.45 in 1993 to 7.84 million tons in 2017), while the area coverage increased two folds over the same period (FAOSTAT, 2018). In the main cropping season of 2017, maize production accounted for the highest share in food security and economy of Ethiopia (CSA, 2017). Among the cereal crops, maize was first in terms of production (26.8%) and productivity (3.38 t/ha). However, the national average maize productivity of the country is very low in comparison to the average yield per hectare of the world (5.2 t/ha) and that of the developed countries (7.2 t/ha).

Hybrid development involves the evaluation and selection of new locally developed maize inbred lines based on their hybrid performance through combining ability studies (Hallauer et al., 2010; Amiruzzaman et al., 2011). Combining ability studies provide vital information on the genetic mechanisms that control the pattern of inheritance of quantitative traits. This illuminating information enables the breeder to select suitable parents for further improvement or use in hybrid breeding for commercial release (Abuali et al., 2012). Combining ability indicates the potential breeding values of parental lines to produce hybrids through determining the general combining ability (GCA) and specific combining ability (SCA) of the inbred lines. GCA is the mean performance of a line in a hybrid combination. On the other hand, SCA refers to cases in which some hybrid combinations perform comparatively better or less than expected based on the average performance of the parental lines involved. GCA and SCA effects are important indicators of the level of usefulness of the inbred lines in hybrid combinations and in categorizing materials into heterotic groups (Tolera et al., 2017). Combining ability analysis and collection of correct genetic information in breeding materials is necessary at the beginning of the development of the inbred line. It provides reliable estimates of genetic components and gene action governing complex traits.

Details of combining ability of maize germplasm are necessary for maximizing the benefits of hybrid development. Many researchers have reported on the combining ability and heterosis for maize grain yield (Dagne, 2008; Berhanu et al., 2009, Amiruzzaman et al., 2011; Abuali et al., 2012; Mohammad et al., 2016; Dar et al., 2017; Tolera et al., 2017; Tulu et al., 2018). Combining ability analysis can be very useful if properly conducted and the results are correctly interpreted. If such studies reveal the required genetic information, inbred lines with poor combining abilities are jettisoned and only promising inbred lines are advanced in subsequent cycles of selection (Sadat and Khalil, 2011). Therefore, evaluation of inbred lines for combining ability and heterosis is an important component of maize hybrid breeding program.

Justification of the study

This research was informed by the need to determine the combining ability of inbred lines and hybrids for yield and yield components so as to ascertain their possible value in current and future maize improvement programmes in Ethiopia, a country where the crop is a foremost staple.

Aim of the study

The purpose of the research was to study and evaluate the combining ability of maize inbred lines and hybrids for yield and yield components.

This study was under-taken with the following objectives:

(1) To estimate general combining ability for yield and yield components.
(2) To estimate specific combining ability for early generation white maize inbred lines for yield and yield components.

MATERIALS AND METHODS

Descriptions of experimental site

The experiment was conducted at Bako National Maize Research Center (BNMRC) during 2018 main cropping season. BNMRC is located in East Wollega Zone of the Oromia National Regional State, Western Ethiopia. The center is 250 km from Addis Ababa, the capital city of the country, and lies between 9°6' North latitude and 37°09' East longitude in the sub-humid agro-ecology and average altitude of 1650 m above sea level (Figure 1). The mean annual rainfall of the previous 56 years was 1239.4 mm and the mean annual rain fall during the season, in 2017 was 1316.7 mm according to metrological data from Bako Agricultural Research. The minimum, mean and maximum air temperature is 13.3, 28.0, and 20.6°C, respectively and the relative humidity was 63.55%. The soil is reddish brown in color and clay loam in texture (Wakene, 2001). According to USDA soil classification, the soil is alfisols developed from basalt parent materials, and is deeply weathered and slightly acidic in reaction (Wakene et al., 2001).

Experimental materials

Twenty-eight entries composed of 26 test crosses and two standard checks (BH546 and BH547) were used for this study. The test crosses were made by using line by tester mating design which involved crossing 13 white maize inbred lines with testers (referred to as tester A and tester B) in 2017 main season. F1 hybrids were evaluated in the rainy season of 2018. In addition, the testers and

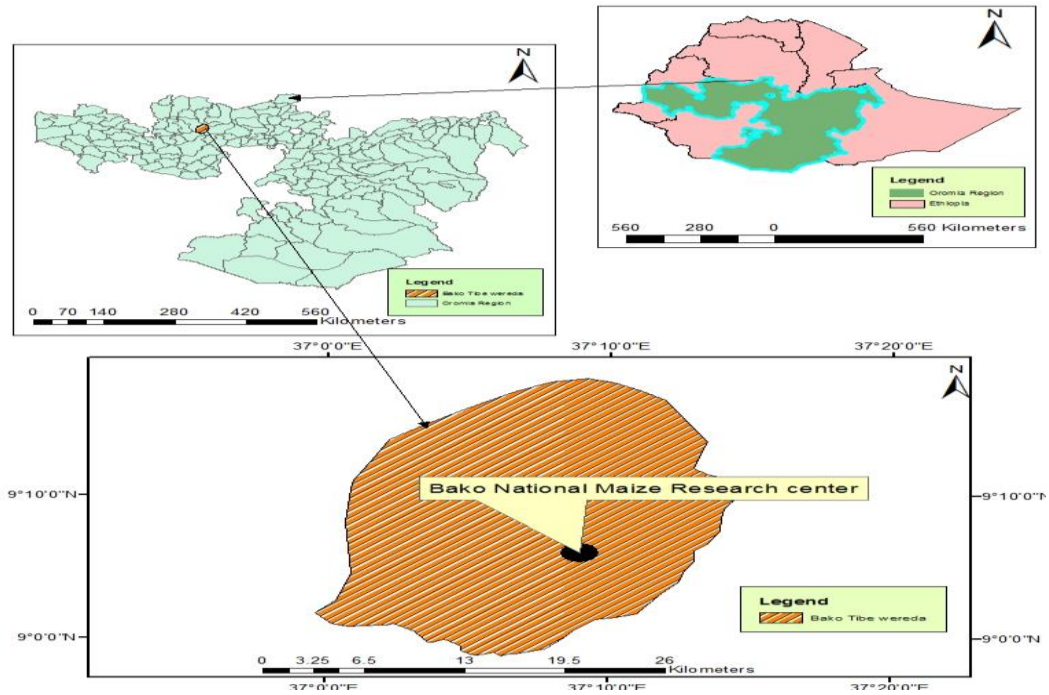

Figure 1. Map of study area Bako National Maize Research Center (ARC).

Table 1. List of maize inbred lines and testers used for test cross formation.

Inbred line code	Pedigree of maize inbred lines	Source
L1	BKINT2012F2-1-1-1-1	BNMRC
L2	BKINT2012F2-1-1-2-1	>>
L3	BKINT2012F2-1-1-2-2	>>
L4	BKINT2012F2-1-1-2-3	>>
L5	BKINT2012F2-7-1-1-1	>>
L6	BKINT2012F2-16-2-1-1	>>
L7	BKINT2012F2-26-2-1-1	>>
L8	BKINT2012F2-26-2-2-1	>>
L9	BKINT2012F2-44-1-1-1	>>
L10	BKINT2012F2-48-1-1-1	>>
L11	BKINT2012F2-69-1-1-1	>>
L12	BKINT2012F2-79-1-1-1	>>
L13	BKINT2012F2-1-2-1-1	>>
TB	PO'00E-3-2-1-2-1	>>
TA	ILO'00E-1-9-1-1-1-1-1	>>

L= Inbred line, TA=Tester B, TB=Tester A.

the inbred line parents evaluated in a separate trial along with the hybrid trial for estimation of the magnitudes of heterosis for each test cross during the same season. The inbred lines and testers were developed at BNMRC through pedigree breeding technique. The list and the pedigrees of the inbred lines and testers used in the line × tester crosses are shown in Table 1. The standard checks BH546 and BH547 are high yielding (8.5 to 11.5 t/ha) medium maturing conventional white commercial maize hybrids released by BNMRC for the mid-altitude sub-humid maize growing agro-ecology of Ethiopia, the high potential maize production belt.

Experimental design

The experimental design was (0, 1) alpha lattice design (Patterson and Williams, 1976) with 4 plots and 7 incomplete blocks with three replicates for hybrids. For the parental inbred lines, experimental design was randomized complete block design with three replications. Each entry was planted in one row by 5.1 m long plot

Figure 2. Ear length and ear diameter for L6 × TA.

with spacing of 0.75 m between rows and 0.30 m between plants within a row.

Trial management

The experimental materials were hand planted with two seeds per hole, which were later thinned down to one plant to get a planting density equivalent to 44,444 plant population per hectare. Planting was conducted at the onset of the rainy season (June 3, 2018) after ample amount of moisture level had been attained to ensure good germination and seedling development. Pre-emergence herbicide, ®Primagram-Gold was applied at the rate of 3 L/ha after planting to control weeds. Hand weeding and slashing was used to control weeds throughout the growing season. Di-ammonium phosphate (Nitrirogen, phosphorous, Sulfar) and urea fertilizers were applied at the rate of 180 and 200 kg/ha, respectively. NPS fertilizer was applied once at sowing time, while urea was applied in split, half at planting and the remaining half at 10 to 12 leave or (35 to 40) days after planting. Based on recommendations for the areas, other agronomic practices were carried out.

Data collection

Data on grain yield and other important agronomic traits were collected as follows:

(1) Days to anthesis: The number of days from emergence to when 50% of the plants in a plot started shedding pollens
(2) Days to silking: The number of days from plant emergence to when 50% of the plants in a plot have grown 2 to 3 cm long silks.
(3) Anthesis-silking interval (ASI): The number of days betweendays to silking and days to anthesis (this is calculated data).
(4) Days to physiological maturity: The number of days from planting to when 50% of the plants in a plot form black layer at the tip of the kernel.
(5) Stand count at harvest: The number of plants on each plot.
(6) Number of ears per plant: The ratio of total number of ears harvested from a plot to the total number of plants in that particular plot (calculated data).
(7) Grain weight (t/ha): The weight of the ears per plot adjust to 12.5% moisture level and 80% shelling percentage to estimate grain yield in tons (t/ha) for each genotype (Carangal et al., 1970)
(8) Actual moisture content: Moisture content of samples from the bulk of shelled grain in each plot measured using a handheld moisture tester. Recorded at the same time as the measurement of grain weight per plot and 1000 kernels weight.
(9) 1000 kernel weight (g): Randomly selected 1000 kernels from the bulk of shelled grain of each experimental unit were counted and weighed by adjusting to 12.5% moisture content of the grain.
(10) Plant aspects: Recorded on a 1 to 5 scale, where 1 means the best variety (considering general appeal of the plants per row: plant vigor, ear size, good ear placement or position, husk cover, uniformity, disease infestation, and so on), while 5 means the worst plant aspect
(11) Ear aspects: Recorded on a 1 to 5 scale where 1 refers to the best ear aspect (considering general appeal of the ears: ear size, uniformity, bare tipness (whether the grain filled up to the tip of the ear), kernel row arrangement, ear rot infection and other acceptable characters), while 5 refers to the poorest ear aspect with undesirable characteristics (Figure 3).
(12) Ear height (cm): Average height in cm of five randomly selected plants per plot measured from the ground level to the upper most ear bearing node.
(13) Plant height (cm): Average height in centimeter of five randomly selected plants measured from the ground level to the first tassel branch.
(14) Ear cob length (cm): Average length in cm of ten randomly taken ears from each experimental unit measured from the base to tip of the ear at the time of harvest (Figure 2).
(15) Ear cob diameter (cm): Average diameter in cm of ten randomly taken ears of a plot measured using digital caliper at harvest time (Figure 2).
(16) Number of rows per ear: Average number of kernel rows of five randomly taken ears of a plot.
(17) Number of kernels per row: Average number of kernels per row of five randomly sampled ears per plot.

Figure 3. Ear aspect evaluation for inbred lines.

Statistical analysis

Analysis of variance

Analysis of variance (ANOVA) was carried out using the PROC MIXED procedure in SAS (SAS Institute, 2014) to determine the differences among the genotypes. Genotypes were considered as fixed effects while replications and blocks within replications were considered random. Significant differences were further subjected to least significant difference (LSD) to separate treatment means.

Line by tester analysis

Line × tester analysis was done for traits that showed significant differences among crosses to partition the mean square due to crosses into lines (GCAm), tester (GCAf) and line × tester interactions (SCAmf) Table 2 (Singh and Chaudhary, 1985).

Contribution of lines (L) = SS (l) × 100 / SS (Crosses), Contribution of testers (T) = SS (t) × 100 / SS (Crosses) and Contribution of line by tester (L × T) = SS (L × T) × 100 / SS.

Estimation of GCA Effects:

a) Lines: $g_i = \frac{x_{i...}}{tr} - \frac{x...}{ltr}$

b) Testers: $g_j = \frac{x_{j...}}{tr} - \frac{x...}{ltr}$

where g_i = GCA effect for ith line, g_j= GCA effect for jth tester, $X_{.j.}$ = sum of the jth tester in hybrids, $X_{i..}$ = Sum of the ith line in hybrids, X… = grand sum, l= number of inbred lines, t= number of testers and r =number of replications.

$$\sum gi = \sum gj = 0$$

Estimation of SCA effects:

$$sij = \frac{Xij\ ...}{r} - \frac{Xi\ ...}{tr} - \frac{Xj\ ...}{rl} + \frac{X..}{rlt}$$

where Si SCA effect of the ijth cross, X…= Grand mean, Xij... = i x j cross sum,
Xi**…=** ithinbred line sum in hybrids, Xj...= jth tester sum in hybrids, l = number of inbred lines,
t= number of testers and r = number of replications.
Standard errors for combining ability effects were calculated as:

(1) Standard error for general combining ability effects

$$a)\ InbredLine: SE(GCA\ for\ line) = \frac{\sqrt{Mse}}{rt}$$

$$b)\ Tester: SE(GCA\ for\ tester) = \frac{\sqrt{Mse}}{rl}$$

(2) Standard error for specific combining ability effects

$$SE(SCAeffects) = \frac{\sqrt{Mse}}{r}$$

(3) T-tests for GCA and SCA

The significance of the GCA effects was tested using the formula described by Cox and Frey (1984):

$$tcal = \frac{GCA}{SE\ gca(male)}, whereas\ SE(gca\ male) = \frac{\sqrt{Me}}{rt}$$

$$tcal = \frac{GCA}{SE\ gca(female)}, whereas\ SE(gca\ female) = \frac{\sqrt{Me}}{rt}$$

$$tcal = \frac{SCA}{SE\ sca(Line\ by\ tester)}, whereas\ SE(sca\ female) = \frac{\sqrt{Me}}{r}$$

Table 2. Skeleton of ANOVA line by tester as suggested by Singh and Chaudhary (1985).

Source of variance	Degree of freedom	Mean square (MS)
Replication (r)	r-1	
Genotype (g)	g-1	
Parents(p)	p-1	
parents vs crosses	1	
Line(L)	L-1	MS_l
Testers(T)	T-1	MS_t
Lines × Testers (L×T)	(L-1)(T-1)	MS_{lxt}
Error	(LT-1)(r-1)	MSe

r=Replication, g=genotype, p=parent, l= inbred lines, t=tester, MS=mean square and MSe=mean square error

Table 3. Analysis of variance for grain yield and yield components of test crosses generated using 13 lines and two testers evaluated at Bako in 2018 cropping season.

Source of variance	DF	GY	DA	DS	ASI	DM	PH	EH
Replication	2	2.64*	2.68^{ns}	2.58^{ns}	0.62^{ns}	10.71^{ns}	484.82^{ns}	850.30^{ns}
Block (Rep)	18	1.2^{ns}	0.73^{ns}	0.78^{ns}	0.37^{ns}	2.91^{ns}	150.66^{ns}	126.67^{ns}
Genotype	27	1.98**	11.27**	11.02**	1.26**	45.09**	699.32**	447.12**
Error	36	0.71	1.3	1.74	0.54	5.21	129.68	183.41
CV%		13.34	1.48	1.71	237.43	1.48	3.99	8.7
Mean		6.32	77.03	77.34	0.31	154.07	285.71	155.59

Source of variance	DF	PA	EA	NRPE	NKPR	EL	ED	TSW
Replication	2	0.24^{ns}	0.12^{ns}	0.06^{ns}	5.63^{ns}	10.35^{ns}	0.1**	569.61^{ns}
Block (Rep)	18	0.2*	0.19^{ns}	0.44^{ns}	15.77^{ns}	1.74^{ns}	0.03*	1127.64^{ns}
Genotype	27	0.26**	0.24^{ns}	2.03**	34.89*	5.65**	0.16**	1512.46**
Error	36	0.1	0.14	0.47	18.43	0.98	0.02	739.11
CV%		13.27	15.29	4.89	11.26	5.16	2.72	10.2
Mean		2.34	2.47	13.97	38.31	19.16	4.77	266.46

*=0.05 and **= 0.01 significant probability level. GY=Grain yield, AD = days to anthesis, DS = days to silking, ASI = anthesis silking interval, DM = days to maturity, PH = plant height, EH = ear height, PA=plant aspect, EA=ear aspect, RPE = number of rows per ear, KPR = number of kernels per row, EL = ear length, ED = ear diameter, TKW=thousand kernel weight, DF = degrees of freedom.

where Me is the error mean sum of squares; *r, t, l* are numbers of replications, testers and lines, respectively; SE is standard error.

RESULTS

Analysis of variance (ANOVA)

The analysis of variance revealed that there were highly significant differences among the genotypes ($P \leq 0.01$) for all the traits except for ear aspect which was non-significant at $p < 0.5$ (Table 3). The ANOVA for the parental inbred lines also revealed significant variation (at $p < 0.01$ or 0.05) for all traits studied except anthesis silking interval, ear height, number of row per ear, ear diameter and thousand seed weight (Table 4).

Estimation of combining ability for yield and yield traits

The variances for the crosses were partitioned into GCA (lines or testers) effects and SCA (lines by tester) effects. The ANOVA table for line by tester mating design for grain yield and yield related traits are presented in Table 5. Mean square due to line by tester was significantly different at (P<0.01 or P<0.05) for all the traits except days to anthesis, days to maturity, ear height, number of kernels per row, and number of row per ear. Mean squares due to lines were also significant at (p<0.01 or0.05) for all traits studied except anthesis silking interval, ear height, number of row per ear, ear diameter and thousand seed weight. Similarly, mean square due to

Table 4. Analysis of variance for grain yield and yield components of parental inbred lines composed of 13 lines and 2 testers evaluated at Bako in 2018 cropping season.

Source of variance	DF	GY	AD	SD	ASI	DM	PH	EH
Replication	2	0.26^{ns}	4.55^{ns}	17.84**	1.31^{ns}	14.78^{ns}	0.27^{ns}	551.72^{ns}
Genotype	13	4.99**	18.66**	21.09**	1.69^{ns}	71.79**	0.0004**	517.12^{ns}
Error	25	29.92	80.06	2.9457	3.34	13.18	403.39	325.2
CV %		29.93	2.07	1.96	257.92	2.1	1135	20.93
Mean		2.18	86.36	87.39	0.7	172.5	176.95	86.13

Source of variance	DF	PA	EA	NRPE	NKPR	EL	ED	TSW
Replication	2	0.62^{ns}	0.08^{ns}	9.87	12^{ns}	8.95^{ns}	1553.63^{ns}	8775.09*
Genotype	13	0.45*	0.6*	10.51^{ns}	26.28*	13**	1430.02^{ns}	3571.12^{ns}
Error	25	0.2	0.25	12.61^{ns}	12.41	4.39	1501.44	2531.06
CV%		18.1	21.6	25.84	13.08	14.43	389.63	23.26
Mean		2.35	2.31	13.74	26.92	14.52	9.94	216.25

*=0.05 and **= 0.01 significant probability level. GY=Grain yield, AD = days to anthesis, DS = days to silking, ASI = anthesis silking interval, DM = days to maturity, PH = plant height, EH = ear height, PA=plant aspect, EA=ear aspect, NRPE = number of rows per ear, NKPR = number of kernels per row, EL = ear length, ED = ear diameter, TKW=thousand kernel weight, DF = degrees of freedom.

tester was significant for days to anthesis, days to silking, plant height, ear height and ear diameter. However, mean square due to tester showed insignificant difference for GY, AD, DM, NRPE, EL and ED. Proportion of variance for general combining ability was greater than that of specific combining ability for all traits studied except GY which showed almost equivalent proportions of GCA and SCA variances.

General combining ability effect (GCA)

General combining ability effects of 13 newly developed inbred lines were estimated to determine their breeding values for use in hybrid formation. Estimates of GCA effects for line (GCA_L) and GCA for tester (GCA_T) are presented in Table 6. All inbred lines showed significant positive or negative GCA effects (P<0.01 or P<0.05) for GY except two inbred lines. Four inbred lines displayed highly positive significant GCA_L for GY whereas seven inbred lines showed negative significant GCA_L for the same trait. Significant positive GCA effects on GY were recorded for L2, L4, L6 and L12. Inbred line 6 (1.5) had the highest significant positive GCA effect while inbred lines 2 (0.24) had the lowest significant positive GCA_L.

Four inbred lines (L1, L2, L4 and L3) showed highly significant positive GCA_L for ear length (EL). The highly significant positive GCA_L (P<0.01) for this trait was 2.2 for L1 whereas the lowest was 1.07** for L3. Four inbred lines showed highly significant GCA_L. L6 (0.35) and L5 (0.23) showed positive significant GCA_L and L4, L1 showed negative significance for ear diameter. Only L5 (-1.36) exhibited highly negative significant differences GCA_L for number of kernel per row. None inbred line displayed significant GCA_L for DM, TKW and NRPE. Except L1 all inbred lines displayed highly significant GCA_L for AD, SD and ASI. L6 (2.68), L11 (2.05) and L8 (0.87) displayed the highest positive GCA_L for AD, SD and ASI, respectively. L9 (-2.65**), L9 (-2.28**) and L6 (-0.79**) had the lowest negative GCA_L for AD, SD and ASI, respectively. L1 showed the highest positive significant GCA_L for plant height (19.94 cm).

Specific combining ability effects

Estimates of SCA effects of the twenty-six crosses for various traits are shown in Table 7. The crosses showed considerable variation in SCA effects for the different traits. All crosses showed significant SCA effects for grain yield. The highest positive SCA value for GY was obtained from L1 × TA (0.77*) whereas the lowest SCA value for the same trait was from L4 × TB (0.03**). L4 × TA expressed the highest negative SCA value for GY whereas the lowest value for the same trait was detected from L1 × TA. Only four crosses exhibited significant SCA effects for ear length in both directions. L4 × TB and L13 × TA showed positive and significant SCA for this trait with values of 0.79 and 0.80, respectively. Whereas L4 × TA and L13 × TB showed significant negative SCA effects. L1 × TB, L4 × TB, L6 × TA, L9 × TA, L11 × TB, L12 × TA and L13 × TB showed significant positive SCA effects for ED. However, crosses L1 × TA, L4 × TA, L6 × TB, L9 × TB, L11 × TA, L12 × TB and L13 × TA showed significant negative SCA effects for this trait.

Two crosses exhibited significant SCA effects for NKPR. L9 × TB (5.19) displayed significant positive SCA for NKPR, while L9 × TA (-5.19) showed negative and is significant for this trait. For DS, crosses L2 × TB, L5 × TB, L10 × TB, L8 × TA, L11 × TA and L13 × TA showed positive and significant SCA effect with value of 1.53**,

Table 5. Analyses of variance line by tester for grain yield and yield related traits of 26 crosses and 13 inbred lines evaluated in 2018 main season at Bako, Ethiopia.

Source	Df	Mean square						
		GY	DA	SD	ASI	DM	PH	EH
REP	2	0.67^{ns}	381.05**	2.51^{ns}	10.05^{ns}	0.16^{ns}	0.06**	638.78*
CROSSES	25	1.85**	1477.92**	15.90**	63.60**	0.39**	0.11**	596.51**
LINE (GCA)	12	1.89**	889.10^{ns}	13.85**	55.38**	0.56**	0.12**	730.24**
TESTER (GCA)	1	1.04^{ns}	23796.8^{ns}	197.13**	788.51**	0.00^{ns}	0.81**	2832.05*
LINE × TESTER (SCA)	12	1.87**	206.82^{ns}	2.85*	11.4*	0.25^{ns}	0.05**	276.5^{ns}
ERROR	32	0.49	550.4751	1.14	4.56	0.15	0.02	144.78
GCA%		50.27	81.13	82.93	82.93	69.14	70.59	72.54
SCA%		49.73	18.87	17.07	17.07	30.86	29.41	27.46

Source	Df	PA	EA	NRPE	NKPR	EL	ED	TKW
REP	2	4.30**	0.63^{ns}	3.85^{ns}	0.05^{ns}	0.09^{ns}	329.17^{ns}	2.46^{ns}
CROSSES	25	6.13**	0.46^{ns}	37.78*	2.34**	0.25**	861.65**	15.07**
LINE(GCA)	12	9.85**	0.54^{ns}	56.73**	2.57**	0.42**	1437.82**	12.46**
TESTER (GCA)	1	20.28**	1.67*	20.10^{ns}	23.47**	0.15^{ns}	169.55^{ns}	169.55**
LINE × TESTER (SCA)	12	1.23*	0.29^{ns}	20.29^{ns}	0.36^{ns}	0.10*	343.16**	4.8**
ERROR	32	1.01	0.29	18.3	0.41	0.08	125.50	1.37
GCA%		88.90	65.06	73.66	87.71	80.77	80.73	72.19
SCA%		11.10	34.94	26.34	12.29	19.23	19.27	27.81

*P=0.05 and **P = 0.01 significant probability level, ns =Non-significant, DF = degrees of freedom, MS = mean square, GY=grain yield, DA = days to anthesis, DS = days to silking, ASI = anthesis silking interval, DM = days to maturity, PA = plant aspect, EA = ear aspect EH = ear height, PH = plant height, EL = ear length, ED = ear diameter, NRPE = number of rows per ear, NKPR = number of kernel per rows, TKW=thousand kernels weight, GCA %= general combining ability percentage, SCA %=specific combining ability percentage.

1.19**, 1.19**, 0.97* and 0.97*, respectively. While, crosses L2 × TA, L5 × TA, L10 × TA, L8 × TB, L11 × TB and L13 × TB showed negative and significant SCA effects.

Similarly, for AD crosses L2 ×TB, L5 × TB, L7 × TB and L12 × TA showed significant and positive SCA estimate, while L2 ×TA, L5 × TA, L7 × TA and L12 × TB showed significant SCA estimate for days to anthesis. Traits like NRPE, TKW, DM, PH and EH showed non-significant SCA effects.

DISCUSSION

Estimate of GCA and SCA for grain yield and yield related traits

The significance of the mean squares due to lines and testers indicated considerable variation among the inbred lines variation and the testers in their performance. Meanwhile, significant line × tester interaction suggests that inbred lines performed uniquely according to the testers they were crossed to. These results are consistent with the findings of Makumbi et al. (2011) and Bullo and Dagn (2016). This result revealed that the mean squares of GCA and SCA were highly significant for grain yield, days to anthesis, days to silking, anthesis-silking interval, days to maturity, plant height, plant aspect, number of row per ear (NRPE), ear length, ear diameter and a thousand seed weight. Only the mean square of number of kernel per row (NKPR) was significant (P ≤0.05) whereas the mean square of the GCA and SCA of the ear traits were insignificant. The magnitude of GCA_L is a measure of the level of the performances of the inbred lines in hybrid combinations. Positive significant GCA_L indicates that the performance of the inbred lines is higher than the collective mean of all crosses. Thus, significant positive GCA_L is desirable for yield and yield components. Significant GCA and SCA mean squares indicate the complementary roles of both additive and non-additive gene actions in determining grain yield. Hadji (2004), Mohamed et al. (2016) and Silvestro et al. (2018) also found highly significant GCA and SCA for grain yield in diallel study of maize inbred lines. Moreover, researchers like Dagne (2008), Mokenen (2015) and Tulu et al. (2018) reported the parts played by additive and non-additive gene actions in controlling grain yield in maize. In this study, the contribution of GCA variance was much greater than the contribution of SCA for all the characters studied. The higher percentage relative contribution of GCA of mean square over SCA showed the predominant role of additive gene action over non-additive action in the inheritance of all traits studied. Two

Table 6. Estimates of general combining ability (GCA) effects for grain yield and other agronomic traits of 13 maize inbred lines crossed using line × tester mating design and evaluated at Bako in 2018 main cropping season.

Line	Traits											
	GY	AD	SD	ASI	DM	PH	EH	NRPE	NKPR	EL	ED	TKW
L1	0.94	0.51	0.72	0.21	1.03	19.94**	2.82	-0.09	5.04	2.2**	-0.19**	4.82
L2	0.24**	-0.82**	-1.45**	-0.63*	-1.64	15.77**	8.65	0.31	2.04	1.51**	0.01	19.76
L3	-0.41	0.85**	0.05**	-0.79*	1.69	11.60	6.99	0.51	1.61	1.07**	-0.08	-5.66
L4	0.50**	0.01**	-0.45**	-0.46*	0.03	11.60	1.99	-0.23	1.61	1.11**	-0.12**	9.78
L5	-0.35**	-0.99**	-1.12**	-0.13*	-1.97	-7.56	-1.35	1.31	-1.36**	-0.93	0.23**	4.11
L6	1.50**	2.68**	1.88**	-0.79*	5.36	5.77	15.32**	0.51	0.54	-0.64	0.35**	2.47
L7	-0.58**	-2.65**	-1.95**	0.71*	-5.31	-35.90	-18.01	-0.43	-0.39	-0.34	-0.01	-2.05
L8	-0.90**	0.51**	1.38**	0.87*	1.03	-5.90	-1.35	-0.36	2.61	0.51	0.08	-6.20
L9	-0.62**	-2.65**	-2.28**	0.37*	-5.31	-17.56	-18.85	-0.49	-7.26	-0.63	-0.10	2.13
L10	-0.26**	-0.32**	-0.45**	-0.13	-0.64	-11.73	-15.51	0.37	-1.13	-0.21	0.03	-19.11
L11	-0.44**	1.51**	2.05**	0.54*	3.03	4.10	8.65	-1.49	0.71	-1.27	-0.07	-21.4
L12	0.60**	0.35**	0.22**	-0.13*	0.69	0.77	1.15	-0.09	-3.89	-3.14	-0.03	-5.86
L13	-0.21**	1.01**	1.38**	0.37*	2.03	9.10	9.49	0.17	-0.13	0.75	-0.09	17.21
SE	0.34	0.43	0.47	0.28	0.86	4.48	5.02	0.25	1.65	0.39	0.05	9.23
SE (gi-gj) line	1.62	3.51	3.33	1.30	7.02	35.75	25.48	1.54	7.10	3.24	0.34	28.01
TB	-0.11	1.59	1.47	-0.12	3.18	1.47	-6.03	0.56*	0.51	-0.53	0.11	-17.89
TA	0.11	-1.59	-1.47	0.12	-3.18	-1.47	6.03	-0.56	-0.51	0.53	-0.11	17.89
SE(GCA)	0.10	0.12	0.13	0.08	0.25	1.29	1.45	0.07	0.48	0.11*	0.01	2.66
SE (gi-gj) tester	5.89	86.04	79.80	6.24	172.08	79.80	326.13	30.25	27.48	28.78	5.93	968.05

*P=0.05 and **P= 0.01 significant probability level. GY=Grain yield, AD = days to anthesis, S D= days to silking, ASI = anthesis silking interval, DM = days to maturity, EH = ear height, PH = plant height, EL = ear length, ED = ear diameter, NRPE = number of rows per ear, NKPR = number of kernel per rows, TKW=thousand kernels weight, SE (gi) = standard error of general combining ability effects of lines.

factors are given priority consideration in the evaluation of an inbred line for possible use in the production of maize hybrid: characteristics of the line itself and behavior of the line in a given hybrid combination. As the present study results illustrates, the inbred lines displayed superior performance in their GCA effects, especially for grain yield and other prominent traits that contribute to grain yield, that is, PH, EH, PA EA, ER, NRPE, NKPR, EL, ED, and TSW. Numerous researchers have reported similar results in their works on GCA for yield and yield related traits in maize (Hadji, 2004; Bayisa et al., 2008; Chandel and Mankotia, 2014; Dagne et al., 2008; Shushay, 2014; Amare et al., 2016). Amare et al. (2016), inparticular, found highly significant mean squares due to GCA and SCA for GY in L × T crosses of 17 maize inbred lines. Similarly, they observed highly significant GCA and SCA mean squares for days to anthesis, days to silking and days to maturity, indicating the significance of additive and non-additive gene actions in controlling the inheritance of the concerned traits. Amiruzzaman et al. (2011), Aminu and Izge (2013) and Tolera (2013) reported highly significant GCA and SCA mean squares for AD and SD in combining ability analysis of maize inbred lines. Other breeders also reported consonant results of mean squares due to GCA of lines and SCA of crosses for AD, SD and DM (Gudata, 2007).

General combining ability

General combining ability is estimated by the additive effects of genes and it can differentiate the genetic component of an inbred line and indicate its potential for utilization in breeding. The magnitude of GCA_L is an indication of the level of the performances of the inbred lines in hybrid combinations. Consequently, significant positive GCA_L is needed for grain yield and yield components. BKINT2012F2-16-2-1-1(L6) had the highest positive GCA which indicates that the line had a higher favorable allele frequency for grain yield. This fact is substantiated by the mean yields obtained from its crosses (the second highest yielding hybrid) which is L6 × TB had one of these lines as its parent. Concerning grain yield, L6 was the best combiner, followed by L1 and L12 while L5, L7, L8, L9, L10, L11 and L13 showed negative GCA effects and were poor combiners for GY. Pavan et al. (2011), Ram et al. (2015), Amare et al. (2016), Ju-lin et al. (2018) reported similar results for yield and yield related traits.

Inbred lines that show the highest and positive significant GCA for the traits like TKW, ED, EL, NRPE and NKPR can be used to improve grain yield. For ear length, ear diameter, number of row per ear, number of kernel per ear and thousand kernel weights, L1, L6, L5, L1 and L2 were the best combiners of GCA_L, respectively.

Table 7. Estimates of SCA effects for yield and yield components of 13 maize inbred lines crossed with two testers in line × tester mating design and evaluated at Bako in 2018 main cropping season.

Crosses	Traits							Crosses	Traits				
	GY	AD	SD	ASI	DM	PH	EH		NRPE	NKPR	EL	ED	TKW
L1×T1	-0.77*	-0.26	-0.64	-0.38	-0.51	-1.47	-0.64	L1×TB	0.17	-0.71	0.32	0.09*	10.92
L1×T2	0.77*	0.26	0.64	0.38	0.51	1.47	0.64	L1×TA	-0.17	0.71	-0.32	-0.09*	-10.92
L2×T1	-0.2**	1.41**	1.53**	0.12	2.82	-0.64	-8.14	L2×TB	-0.09	-0.04	0.13	0.06	7.06
L2×T2	0.2**	-1.41**	-1.53**	-0.12	-2.82	0.64	8.14	L2×TA	0.09	0.04	-0.13	-0.06	-7.06
L3×T1	-0.14**	0.08	0.69	0.62	0.15	11.86	3.53	L3×TB	0.37	-0.47	0.11	0.01	1.34
L3×T2	0.14**	-0.08	-0.69	-0.62	-0.15	-11.86	-3.53	L3×TA	-0.37	0.47	-0.11	-0.01	-1.34
L4×T1	0.03**	-0.09	-0.14	-0.05	-0.18	10.19	3.53	L4×TB	0.17	0.39	0.79*	0.19**	-7.89
L4×T2	-0.03**	0.09	0.14	0.05	0.18	-10.19	-3.53	L4×TA	-0.17	-0.39	-0.79*	-0.19**	7.89
L5×T1	0.70*	0.91*	1.19**	0.28	1.82	11.03	15.19	L5×TB	-0.16	-0.44	0.28	-0.18	-3.73
L5×T2	-0.70*	-0.91*	-1.19**	-0.28	-1.82	-11.03	-15.19	L5×TA	0.16	0.44	-0.28	0.18	3.73
L6×T1	0.32**	-0.42	0.19	0.62	-0.85	-7.31	-4.81	L6×TB	-0.29	-0.01	0.02	-0.10*	6.88
L6×T2	-0.32**	0.42	-0.19	-0.62	0.85	7.31	4.81	L6×TA	0.29	0.01	-0.02	0.10*	-6.88
L7×T1	-0.06**	0.91*	0.03	-0.88	1.82	-7.31	-1.47	L7×TB	0.24	-2.21	-0.25	0.02	-3.31
L7×T2	0.06**	-0.91*	-0.03	0.88	-1.82	7.31	1.47	L7×TA	-0.24	2.21	0.25	-0.02	3.31
L8×T1	0.37**	-0.26	-0.97*	-0.72	-0.51	-7.31	-1.47	L8×TB	0.17	-0.34	-0.66	0.01	-4.69
L8×T2	-0.37**	0.26	0.97*	0.72	0.51	7.31	1.47	L8×TA	-0.17	0.34	0.66	-0.01	4.69
L9×T1	-0.08**	-0.76	-0.64	0.12	-1.51	-7.31	-5.64	L9×TB	-0.36	5.19*	-0.11	-0.10*	-2.06
L9×T2	0.08**	0.76	0.64	-0.12	1.51	7.31	5.64	L9×TA	0.36	-5.19*	0.11	0.10*	2.06
L10×T1	-0.38**	0.24	1.19*	0.95	0.49	-8.14	-5.64	L10×TB	-0.43	0.19	0.66	-0.07	-4.88
L10×T2	0.38**	-0.24	-1.19*	-0.95	-0.49	8.14	5.64	L10×TA	0.43	-0.19	-0.66	0.07	4.88
L11×T1	0.26**	-0.59	-0.97*	-0.38	-1.18	1.03	5.19	L11×TB	-0.03	1.16	-0.58	0.10*	-5.26
L11×T2	-0.26**	0.59	0.97*	0.38	1.18	-1.03	-5.19	L11×TA	0.03	-1.16	0.58	-0.10*	5.26
L12×T1	-0.17**	-0.76*	-0.47	0.28	-1.51	-0.64	-7.31	L12×TB	0.17	-0.24	0.12	-0.13*	5.96
L12×T2	0.17**	0.76*	0.47	-0.28	1.51	0.64	7.31	L12×TA	-0.17	0.24	-0.12	0.13*	-5.96
L13×T1	0.12**	-0.42	-0.97*	-0.55	-0.85	6.03	7.69	L13×TB	0.04	-2.47	-0.80*	0.10*	-0.34
L13×T2	-0.12**	0.42	0.97*	0.55	0.85	-6.03	-7.69	L13×TA	-0.04	2.47	0.80*	-0.10*	0.34
SE(sca)	0.34	0.43	0.47	0.28	0.86	4.48	5.02	SE(sca)	0.25	1.65	0.39	0.05	9.23
SE (Sji-Skl)	0.33	11.06	0.50	1.01	0.18	0.07	5.67	SE (Sji-Skl)	2.02	0.30	0.13	5.28	0.55

	Traits						Traits				
Crosses	NRPE	NKPR	EL	ED	TKW	Crosses	NRPE	NKPR	EL	ED	TKW
L1XTB	0.17	-0.71	0.32	0.09*	10.92	L8XTB	0.17	-0.34	-0.66	0.01	-4.69
L1XTA	-0.17	0.71	-0.32	-0.09*	-10.92	L8XTA	-0.17	0.34	0.66	-0.01	4.69
L2XTB	-0.09	-0.04	0.13	0.06	7.06	L9XTB	-0.36	5.19*	-0.11	-0.10*	-2.06
L2XTA	0.09	0.04	-0.13	-0.06	-7.06	L9XTA	0.36	-5.19*	0.11	0.10*	2.06
L3XTB	0.37	-0.47	0.11	0.01	1.34	L10XTB	-0.43	0.19	0.66	-0.07	-4.88
L3XTA	-0.37	0.47	-0.11	-0.01	-1.34	L10XTA	0.43	-0.19	-0.66	0.07	4.88
L4XTB	0.17	0.39	0.79*	0.19**	-7.89	L11XTB	-0.03	1.16	-0.58	0.10*	-5.26
L4XTA	-0.17	-0.39	-0.79*	-0.19**	7.89	L11XTA	0.03	-1.16	0.58	-0.10*	5.26
L5XTB	-0.16	-0.44	0.28	-0.18	-3.73	L12XTB	0.17	-0.24	0.12	-0.13*	5.96
L5XTA	0.16	0.44	-0.28	0.18	3.73	L12XTA	-0.17	0.24	-0.12	0.13*	-5.96
L6XTB	-0.29	-0.01	0.02	-0.10*	6.88	L13XTB	0.04	-2.47	-0.80*	0.10*	-0.34
L6XTA	0.29	0.01	-0.02	0.10*	-6.88	L13XTA	-0.04	2.47	0.80*	-0.10*	0.34
L7XTB	0.24	-2.21	-0.25	0.02	-3.31	SE(sca)	0.25	1.65	0.39	0.05	9.23
L7XTA	-0.24	2.21	0.25	-0.02	3.31	SE (Sji-Skl)	2.02	0.30	0.13	5.28	0.55

*P=0.05 and **P= 0.01 significant probability level. SE (L × T) = standard error of specific combining ability effects ofline by testers, SE (sji-Skl) =standard error of the difference of specific combining ability GY=Grain yield, AD = Days to anthesis, SD = Days to silking, ASI = Anthesis silking interval, DM = days to maturity, EH = ear height, PH = plant height, EL = ear length, ED = ear diameter, NRPE = number of rows per ear, NKPR = number of kernel per rows, TKW=thousand kernels weight.

However, L12, L1, L11, L9 and L10 are the poorest combiner of GCA_L, respectively. Minimum and negative significant of GCA for AD, SD, and DM were obtained from L7, L9 and L7, L9, respectively. The minimum negative significant GCA effects for such traits suggests for their potential usefulness in the development of early maturing hybrid which can be planted for drought escape. These findings corroborate the prior results of Amiruzzaman et al. (2011), Khan et al. (2014) and Alamerew and Warsi (2015).

For morphological traits, L1, L2 showed positive and significant of GCA for plant height and L2 showed positive and significant GCA for ear height. These two traits are desirable for developing tall hybrids. Inbred lines that show significantly high negative GCA for PH and EH are useful in developing dwarf hybrids. Inbred lines 7, 9 and 10 showed negative significant GCA; therefore, they may be suitable for developing short hybrid. Also, short inbred lines produce hybrids that are amenable to mechanized harvest, contributing traits of shorter plants with low ears. Other researchers have reported similar results (Aminu et al., 2014; Alamerew and Warsi, 2015; Ju-lin et al., 2018; Sun et al., 2018).

Specific combining ability effects

Specific combining ability is determined by the non-additive effect of genes, which is influenced by the environment and cannot be inherited sustainably. It is used as a reference when shifting through hybrid combinations (Ju-lin et al., 2018). The crosses showed considerable variation in SCA effects for the different traits. All crosses showed highly significant SCA for grain yield. This indicates that the inbred lines involved in these hybrids are genetically divergent and hence could be regarded as discrete heterotic groups. Among the crosses with good SCA, estimates showed high mean grain yield, which implied good correspondence between SCA effects and mean GY. Many researchers reported significant positive and negative SCA for GY (Bayisa et al., 2008; Dagne, 2008; Kamara et al., 2014; Girma et al., 2015; Ram et al., 2015).

Only four crosses exhibited significant SCA effects for ear length in both directions. Seven crosses showed positive and significant SCA for ear diameter. Crosses that showed positive SCA for ear diameter can be used to improve width of ear cob. Two crosses exhibited significant SCA for number of kernel per row for both directions. Cross L4 × TB showed positive and significant SCA for both ear length and ear diameter, which could be used for the improvement of grain yield through recycling breeding method. The hybrids with negative SCA for AD and SD are desirable for early maturity that would be expected based on GCA of their parents. This finding agrees with the reports of Berhanu (2009); Kanagarasu et al. (2010), Aminu and Izge (2013), and Aminu et al. (2014).

Conclusion

The combining ability of new parental inbred lines and their performance in hybrid combinations are crucial information for a breeding program. The present study was conducted to estimate the combining ability of 13 newly developed normal white inbred lines for grain yield and yield related traits and to classify them into heterotic groups. Highly significant differences were observed among the inbred lines and line by testers for all traits which indicates their suitability for selection for the improvement of yield and yield related traits.

GCA mean squares component was greater than SCA sum of squares for all of the studied traits, suggesting that variations among the crosses were mainly due to additive rather than non-additive gene effects. Hence, selection would be effective in improving grain yield and other agronomic traits. Among parents, BKINT2012F2-16-2-1-1(L6) showed highly desirable GCA effects for grain yield which suggests that it could serve well if selected for the development of hybrids. Inbred lines L1, L2, L4, L6 and L12 were the best general combiners for grain yield.

The results obtained in this study could be helpful for the design of appropriate breeding strategy for developing hybrids and synthetics adapted to the mid altitude sub-humid agro-ecologies of Ethiopia.

Recommendation

From this research study, the following were recommended:

(1) Crosses L6 × TB and L1 × TA which had yield advantage and better in plant aspect and ear aspect than best standard check BH 547 can be evaluated again and released as new hybrids for mid altitude of Ethiopia.
(2) Identified inbred lines with desirable positive GCA effects for grain yield and other grain yield related traits will be used for breeding to develop hybrid.
(3) However, the present study was conducted at one location and the result is only an indication and definite conclusion cannot be reached. Therefore, we recommend that further research should be conducted.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Antibacterial activity of endophytic fungi isolated from mangroves of Jaffna Peninsula, Sri Lanka

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Mangroves are plant communities growing in the intertidal zone of tropical to subtropical coastal rivers. Some endophytic fungi which live in the tissues of mangrove plants produce some biologically active substances. By screening these biologically active substances some researchers have found that these substances have antimicrobial activity. This research is aimed to determine the antibacterial activity of endophytic fungi isolated from leaves of mangrove plants *Excoecaria agallocha, Avicennia marina, Rhizophora mucronata* and *Lumnitzera racemosa* in Sarasalai area in Jaffna Peninsula in Sri Lanka. Various species of endophytic fungi were isolated from the leaves of mangrove plants and identified based on morphological characteristics. Five fungal species were isolated from *E. agallocha* four from *R. mucronata, A. marina* and two from *L. racemosa.* Fifteen endophytic fungi were tested against six selected bacteria for their antagonistic effect. Antibacterial activity was tested against *Escherichia coli, Bacillus* sp*, Klebsiella* sp*, Pseudomonas* sp*, Staphylococcus* sp. and *Proteus* sp. using disc diffusion assay. Almost all endophytic fungi inhibited the growth of bacteria. *Aspergillus flavus* had the highest amount of inhibition against *E. coli*, *Pseudomonas* and *Staphylococcus* sp. *Aspergillus tamari* had higher amount of inhibition against *Klebsiella* sp. Few other species of *Aspergillus* also showed higher inhibitory activity against different bacteria when compared to other endophytic fungi.

Key words: Mangrove, endophytic fungi, bacteria.

INTRODUCTION

Mangrove plants grow well in between sea and terrestrial ecosystem that contain brackish water. Mangroves live in wide range of salinities, tidal amplitudes, changes in sea level, winds, high temperatures, muddy and anaerobic soil conditions. They are well adapted for their extreme environmental conditions. In addition, most mangrove species are used as medicinal plants and also they have antimicrobial properties. These mangroves contain bioactive compounds that have potential antimicrobial, antiviral, anticancer, antidiabetic, antimalarial and antioxidant compounds (Zhang et al., 2009). Previous studies showed that most of the bioactive compounds were derived from the interaction between plants and microbes such as bacteria and endophytic fungi (Rossiana et al., 2016). Endophytic microorganisms grow within tissues of higher plants as facultative saprophytic, parasitic, mutualistic and commensalistic symbioses. These microorganisms grow intracellularly or intercelullarly in the tissues of higher plants without causing any symptoms on the host plants in which they live (Molina et al., 2012). Endophytic microorganisms are generally capable of producing bioactive compounds

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similar to their host plants (Nurhajati, 2011). Several studies have found that the endophytic fungi are one of the main sources of producing new antibiotics (Zhang et al., 2009). Endophytic fungi have been widely investigated as source of bioactive compounds (Bills et al., 1991). Most of these bioactive compounds have antimicrobial activity. The objective of this study was to isolate and identify endophytic fungi from selected mangroves and to test the biological activity of fungal isolates.

METHODOLOGY

The mangroves which are common in Sarasalai area in the Northern part of Sri Lanka were selected for this study. Leaves were collected from mangroves namely *Excoecaria agallocha, Avicennia marina, Rhizophora mucronata* and *Lumnitzera racemosa* at five random sites in the area during July before the rainy season. Three plants were selected from each mangrove species. The identification was based on the herbarium specimens (M 23/1900) available in the Department of Botany University of Jaffna Sri Lanka and assistance from a taxonomist. The mangrove specimens collected for this study were preserved as herbarium and maintained in the laboratory for future reference.

Mature leaves (3-4) from the selected mangrove plants were collected into sterile polythene bags in the field. After reaching the laboratory, the leaves were immersed in Sodium hypochloride for 1-2 min for sterilization. The leaves were washed with sterile water 3 times. Four small segments (about 4 mm x 4 mm size) from each leaf in between the mid rib and periphery were cut using a sterile razor. Potato Dextrose Agar (PDA 39 g/L) medium was prepared in Petri dishes with the addition of streptomycin to prevent the growth of bacteria. Leaf segments were placed on the surface of PDA. The plates were incubated at room temperature for 2-3 days to observe fungal growth. The individual fungi were sub cultured until pure fungal isolates were obtained. Microscopic morphology and macroscopic characters were used for the examination of fungal cultures in the laboratory. Fungi were identified using standard keys available for fungi (Pitt and Hocking, 1997).

Discs (5 mm diameter) of 5 days old fungal culture were inoculated into 250-mL conical flasks containing 50 mL of Potato Dextrose Broth medium (PDB). The conical flasks were placed on a thermostatic shaker at 180 rpm at 28°C for 7 days for fermentation.

The cultured fungal mats were filtered through cheese cloth. The filtrate (50 mL) was transferred into separating flask. The crude metabolites were extracted using ethyl acetate at room temperature. The extracted ethyl acetate fractions were pooled into a conical flask, dried over anhydrous $MgSO_4$ and evaporated by using rotary evaporator. The crude extract was dissolved in Dimethyl Sulphoxide (DMSO).

The bacterial cultures used in this study were obtained from the bacterial culture collection available in the laboratory in the Department of Botany University of Jaffna. The choice of bacteria was based on the previous studies made on the antibacterial activity. Bacteria (*Escherichia coli, Bacillus* sp*, Klebsiella* sp*, Pseudomonas* sp*, Staphylococcus* sp and *Proteus* sp) were streaked on the surface of the Nutrient Agar (NA) (28 g/L) medium. The plates were incubated at 37°C for overnight. After the incubation, a loop full of young bacterial culture (16-24 h old) from the isolated colony was transferred into the universal bottle containing sterile distilled water. It was stirred well by using vortex stirrer. Suspension was prepared which contain 10^5 CFU/ml from each bacterial culture.

Nutrient agar (NA) medium was prepared. 0.1 ml of bacterial suspension was transferred into the centre of the NA plates separately by using sterile pipette. Thereafter, the suspension was spread all over the surface of the NA medium by using sterile glass spreader.

A 50 µL of fungal metabolite extract was added into a sterile paper disc (5 mm diameter, Whatman No. 1). The paper disc was placed on NA plates which were surface inoculated with bacterial cultures. The antibacterial agent ampicillin (60 µg/mL) was used as a positive control and DMSO was used as a negative control. The plates were incubated at 37°C for 24-48 h and inhibition zones were measured. The experiment was carried out in triplicates.

Fifteen endophytic fungi were tested against six selected bacteria for their antagonistic effect. Positive and negative controls were also maintained. The obtained results were analyzed by 2-way ANOVA.

RESULTS AND DISCUSSION

A total of 15 different species of endophytic fungi were isolated from the leaves of mangrove plants. Five different fungal species were isolated from *Excoecaria* sp., four from *Rhizophora* sp and *Avicennia* sp and two from *Lumnitzera* sp (Figures 1 and 2). The features of identification are given in Table 1. The mean values of the inhibition zones are given in the Table 2.

Almost all fungi inhibited the growth of *E. coli*. Endophytic fungus *Aspergillus flavus* (F1) has the higher amount of inhibition against *E. coli*. Endophytic fungus *Aspergillus* sp (F5) has the lowest amount of inhibition against *E. coli*. Most of the fungus inhibited the growth of *Bacillus* sp. Endophytic fungus *Aspergillus* sp (F3) has the higher amount of inhibition against *Bacillus* sp. Endophytic fungus *Aspergillus* sp (F5) and *Epicoccum nigrum* (F14) had the lowest amount of inhibition against *Bacillus* sp. Almost all fungus inhibited the growth of *Klebsiella* sp. Endophytic fungus *Aspergillus tamarii* (F2) has the higher amount of inhibition against *Klebsiella* sp. Endophytic fungus *Chaetomium* sp (F7) has the lowest amount of inhibition against *Klebsiella* sp. Almost all fungus inhibited the growth of *Proteus* sp. Endophytic fungus *Aspergillus* sp (F4) has the higher amount of inhibition against *Proteus* sp. Endophytic fungus *Mucor* sp (F6) has the lowest amount of inhibition against *Proteus* sp. Almost all fungus inhibited the growth of *Pseudomoas* sp. Endophytic fungus *A. flavus* (F1) has the higher amount of inhibition against *Pseudomoas* sp. Endophytic fungus *E. nigrum* (F15) has the lowest amount of inhibition against *Pseudomoas* sp. Almost all fungus inhibited the growth of *Staphylococcus* sp. Endophytic fungus *A. flavus* (F1) has the higher amount of inhibition against *Staphylococcus* sp. Endophytic fungus *E. nigrum* (F14) has the lowest amount of inhibition against *Staphylococcus* sp.

At P=0.05, statistical analysis showed that, there is no interaction between mean values of diameter of fungus. Also, there is no significant difference (P=0.05) in the diameters of clear zones of different fungi tested in this study. Highest antagonistic activity was shown by *Aspergillus* sp. (F3) against *Pseudomonas* sp. whereas

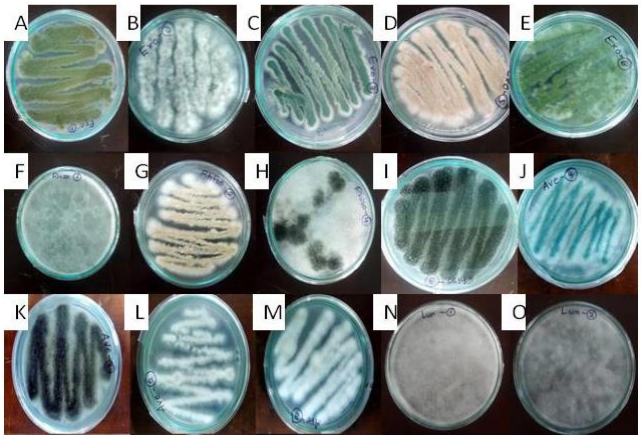

Figure 1. (A-E) Fungus isolated from *Excoecaria* in PDA medium A- *Aspergillus flavus ;* B- *Aspergillus tamari ;* C- *Aspergillus* sp. 1; D- *Aspergillus* sp. 2; E- *Aspergillus* sp. 3; (F-I) Fungus isolated from *Rhizophora* F- Unknown G- *Aspergillus* sp. 4; H- *Aspergillus niger* I- *Mucor* sp 1(J-M); Fungus isolated from *Avicennia* J- *Aspergillus* sp 5; K- *Mucor* sp 2; L- *Chaetomium* sp M- *Aspergillus* sp 6 (N-O) Fungus isolated from *Lumnitzera* N- *Epicoccum nigrum* O- *Epicoccum nigrum.*

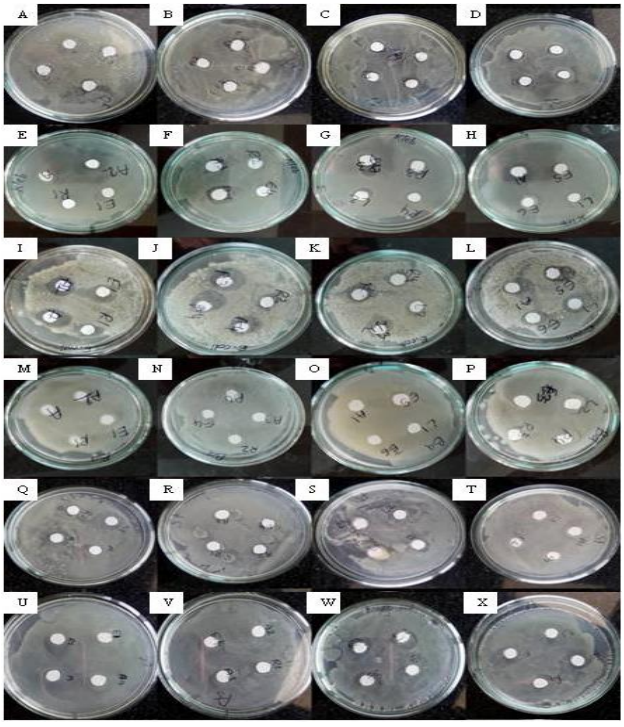

Figure 2. Antibacterial activity of endophytic fungi. (A-D) Inhibition zones of all fungi on NA plate surface inoculated by *Proteus* sp.; (E-H) Inhibition zones of all fungi on NA plate surface inoculated by *Klebsiella* sp.; (I-L) Inhibition zones of all fungi on NA plate surface inoculated by *Escherichia coli.;* (M-P) Inhibition zones of all fungi on NA plate surface inoculated by *Bacillus* sp.; (Q-T) Inhibition zones of all fungi on NA plate surface inoculated by *Staphylococcus* sp.; (U-X) Inhibition zones of all fungi on NA plate surface inoculated by *Pseudomonas* sp.

Table 1. The features of Identification of endophytic fungi.

Fungus	Colony character	Microscopic character	Name of the fungus
A	Colony Lime green in colour. Cottony, powdery appearance. Edge is white colour. Middle of the colony is raised. Backside of the colony is white in colour.	Conidia are round shape. Loosely radiate phialiodes on most of the vesicle. Hyphae are septate and branched	*Aspergillus flavus*
B	Colony is green in colour. No mycelium. Powdery appearance and irregular form. Middle of the colony is raised. Backside of the colony contains black colour spots.	Hyphae are septate and branched	*Aspergillus tamari*
C	Colony is bluish green in colour. Edge of the colony is white in colour. Yellow colour spots present. Backside of the colony is white colour and black colour spots present.	Conidia are round shape. Hyphae are aseptate and unbranched	*Aspergillus* sp. 1
D	Light brown colour. Margin of the colony is white colour. Backside of the colony is yellow colour	Hyphae are septate and branched	*Aspergillus* sp. 2
E	Colony is green in colour.Backside of the colony is white colour. Middle raised.	Hyphae are septate and branched	*Aspergillus* sp. 3
F	Whitish colour colonies present. Later it changes in to blackish colour. Backside of the colony is black colour. Black colour spores are present.	Hyphae are aseptate and unbranched	Unknown
G	Light brown colour. Margin of the colony is white colour. Backside of the colony is yellow colour	Hyphae are septate and branched	*Aspergillus* sp. 4
H	White colour mycelium with black colour spores. Backside of the colony is white colour.	Hyphae is branched and septate.conidia are globose shape	*Aspergillus niger*
I	Colonies are black colour. Margin is white colour. Backside of the colony is white colour.	Aseptate broad hyphae, sporangiophore round slightly elongated	*Mucor* sp.
J	Colonies are bluish green in colour. Margin is white colour. Edges of the colonies are smooth. Backside of the colony is white colour	Hyphae are septate and branched.	*Aspergillus* sp. 5
K	Colonies are black colour. Margin is white colour. Backside of the colony is white colour.	Aseptate broad hyphae, sporangiophore round slightly elongated	*Mucor* sp.
L	Colonies are white in colour. Middle raised. Filamentous mycelia present. Backside of the colony is white colour.	Hyphae are septate and branched	*Chaetomium* sp.
M	Colonies are milky white colour. Middle raised. Backside of the colony is white colour.	Hyphae are septate and branched	*Aspergillus* sp. 6
N	White colour mycelium present. Cottany appearance. Backside of the colony is black colour.	Conidia are black colour warted and spherical Hyphae of the mycelium are septate and branched.	*Epicoccum nigrum*
O	White colour mycelium present. Cottany appearance. Backside of the colony is black colour.	Conidia are black colour warted and spherical. Hyphae of the mycelium are septate and branched.	*Epicoccum nigrum*

lowest antagonistic activity was shown by *E. nigrum* (F14) against *Bacillus* sp. The interaction between fungi and bacteria is shown in Figures 1 and 2.

Conclusion

Different mangroves used in this research study had different endophytic fungi or different strains of the same endophytic fungus. This study was done during the dry period or just before the rainy season. Future studies should be carried out to correlate the seasonal variations and the presence

Table 2. Mean values of the inhibition zones of endophytic fungi (mm).

Fungi	*E. coli*	*Bacillus*	*Klebsiella*	*Pseudomonas*	*Proteus*	*Staphylococcus*
F1	2.0333	0.5250	1.1583	2.5833	1.2583	1.6667
F2	1.8083	0.9750	1.8083	2.0417	1.1833	0.7917
F3	1.5750	1.3250	1.5417	2.7250	1.1583	1.2083
F4	1.7250	0.9583	1.5917	1.7250	1.4750	1.4667
F5	0.5583	0.0000	1.4583	1.2917	1.2917	0.9167
F6	1.7167	0.5083	1.5583	2.0417	1.0667	1.3167
F7	1.9250	0.5167	0.9167	2.3917	1.1917	1.1104
F8	1.7833	0.4500	1.5833	2.4750	1.2667	0.6917
F9	1.7500	0.9250	1.6833	2.5333	1.2333	1.3750
F10	1.9833	0.5917	1.5583	1.9083	1.2333	1.4917
F11	1.4750	0.8917	1.4750	2.1083	1.2083	1.2500

of endophytic fungi. It can be concluded that endophytes are rich sources of bioactive natural products with promising applications in development of pharmaceutical and industrial compounds. The fungal metabolites in the crude form have been used in this study. Further research should be carried out with the purified extracts. The follow up study would be the identification of the endophytic fungi at the molecular level to confirm the species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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