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# Analysis of farmers' perceived and observed climate variability and change in Didessa sub-basin, Blue Nile River, Ethiopia

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**Farmers' local knowledge of how the climate is changing is crucial in anticipating the effects of climate change, as only farmers who know/perceive the impacts will develop coping and adaption measures. The study is designed to assess farmers' perception and understanding in climate variability and change and to establish the observed climate change parameters with farmer's perception and climate anomalies. Household survey, semi-structured interviews, focus group discussions and 30 years of climate data was employed. Non-parametric test using Mann-Kendall climate trend analysis and Sen's slope estimator was employed to test the variability of climate using MAKESENSE software. The findings revealed that farmers perceived climate change in terms of increase in temperature, decrease in rainfall, increase in drought conditions and change in seasonal rainfalls. Analysis of the observed climate data for the sub-basin showed that average annual temperature trends has exhibited a positive slope and increased by 1.4°C and above the national average (1.3°C) over the study periods (1986-2014) at mean temperature rise with an average rate of 0.181°C in the last decade. The observed climate variability was confirmed by farmer's perception. The Mann-Kendall rainfall trend analysis showed that annual and monthly precipitation variability in terms of intensity and distributions declined and vary across agro ecological zones. The analysis indicated that annual rainfall variability in Dega agro ecological zone (CV>63%) and in Kola agro ecological zone (CV>104%) was extremely variable. However, in terms of amount and distributions of precipitation, farmers understanding of precipitation in relation to observed precipitation data showed disparities. This disparity was due to understanding of agronomic drought (farmer's view) and metrological drought (scientific view). Therefore, based on the findings, scientist and policymakers has to integrate the metrological information into farmers' perception and knowledge of climate variability for future climate adaptations measure.**

**Key words:** Climate variability, farmers' perception, climatological normal, local knowledge.

## INTRODUCTION

Global climate variability and change threatens the life and livelihoods of people in the world (IPCC, 2014). Climate variability refers to variations in the mean state of the climate on both temporal and spatial scales beyond

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the individual weather events (IPCC, 2014). On the other hand, climate change is a change in climate over periods of time, which credited directly or indirectly to human activity and alters the composition of the global atmosphere (IPCC, 2014).

The changes in climate variability worsens the already existing vulnerabilities of the poorest peoples who depend on climate variables and sensitive sectors such as agriculture and natural resources for survival (Mubaya et al., 2012) and live with a range of livelihood risks (Gandure et al., 2013; IPCC, 2014). Studies showed that unless adequate measures are taken to adapt to the adverse effect of climate change, a decline of 2 to 7% of GDP by 2100 in some parts of sub-Sahara Africa; 2- 4% in West and Central Africa and 0.4 to 1.3% in Northern and Southern Africa is expected (IPCC, 2014).

There is considerable evidence that the climate in Ethiopia is changing and projections suggest that the rate of changes will increase in the future. Mean annual temperature has risen by 1.3°C with an average rate of 0.28°C per decade (CRGE, 2011). Climate models suggest that Ethiopia will see further warming in all seasons between 0.7 and 2.3°C by the 2020's and between 1.4 and 2.9°C by the 2050s (Conway and Schipper, 2010*)*. As a result, under moderate global warming, cereal production in Ethiopia is expected to decline by 10 to 12% (MOA, 2011).

The changes in climate and variability demand coping and adaptation mechanisms largely depending on farmer's perception and knowledge's of climate change, variability, along with its impacts on their livelihoods. About 95% of sub Saharan African agriculture was impacted by climate change and the consequence are significant particularly for those who directly depend on unreliable weather patterns for livelihood. Farm households that perceived climate variability and change can adapt to the change and hence less vulnerable compared to farmers who did not perceive it (UNFCCC, 2007; Tadross et al., 2009).

Perception of the long-term changes in climate by indigenous local people plays an important role in shaping their behavior toward climate variability and change (Abid et al., 2014). Perceptions of risk and cognitive process of primary decision makers are important for the employment of different adaptation options (Nyanga et al., 2011; Simelton et al., 2011; Amdu et al., 2013; Berman et al., 2014).

There are alternative ways to assess how climate is changing. These are the quantitative mode (relay on observed climate data) and the qualitative approach (participatory). The important difference between the qualitative (participatory) and top-down modeling (quantitative) approaches is that contextual analyses recognizes the experiences from and perceptions of past events by local people in influencing responses to future events. Metrological observation of changes in climatic patterns with perception of farming community about the climate variability and change as well as its impacts on agricultural activities are paramount to the importance of adaptation strategies/polices as "perception is a pre requisite for adaptation" (Madisson, 2007). Despite this, change in rainfall rarely produces the type of significant trends that climate data does (Boko et al., 2007).

Even though climate change may bring conditions beyond previous experience, local knowledge and perceptions remain the foundation for any local response. In this research contexts, indigenous, local, community or traditional knowledge were used synonymously. "Local perceptions" refers to the way local people identify and interpret observations and concepts (Byg and Salick, 2009; Vignola et al., 2010). Hence, rural societies have in-depth knowledge of local climate variability as part of their traditional ecological knowledge (TEK), that is, their knowledge acquired and transferred through generations (Berkes et al., 2000). However, the capacity to cope with climate change are often constrained by socio economic, policy/institutional integration which can further exacerbate existing problems and reduce adaptation options (Stringer et al., 2010).

Previous studies has dealt with perceptions of seasonality (Bryan et al., 2009), risks threats related to climate variability (Thomas et al., 2007; Adger et al., 2009; McCarthy, 2011; Deressa et al., 2009; Fisher et al., 2010) and local knowledge in predicting the climate variability and adaptation (Mertez et al., 2009) as well as agro ecological zones in influencing farmers perception of climate variability and change (Simane et al., 2012; Joshua et al., 2013).

Nevertheless, limited attention has been given to the knowledge and practice that emanated from the perception of smallholder farmers (Maddison, 2007; Deressa et al., 2011; Legesse et al., 2013). Assessments of meteorological data or refute is given in previous studies (Deressa et al., 2009; Fisher et al., 2010; Simane et al., 2012; Maddison, 2007). Besides, local perceptions and the resulting indigenous knowledge are location and site specific.

Consequently, this study was specifically designed to assess whether there is a common understanding among stakeholders of *what* aspect of climate (exposure) is changing, or *how* it is changing, along with its perceived impacts in the Didessa sub-basin in combination with meteorological evidence for over a 30-year period (1986 to 2015) from close proximity stations. Hence, local perspectives combined with scientific knowledge of climate data to generate useful policy-relevant information for future adaptation strategy.

**Descriptions of the study area**

The study was conducted in the Didessa sub-basin (DSB) of the Blue Nile Basin where agriculture is the major economic activity, among which coffee production, crop cultivation and livestock production are the dominant sectors, undertaken by smallholder farmers. It is the largest tributary of the Blue Nile Basin in terms of volume of water contributing roughly a quarter of the total flow as measured at the Sudan border (BCEOM, 1998).

Topographically, the sub-basin has an area of 27,000 $km^2$. It is located at 36° 02' and 36° 46' East longitude and between 7° 43' and 8° 13' North latitude. The altitude in the sub-basin ranges approximately between 630 to 3130 m above sea level excluding mountains of height greater than 3500 m above sea level (Bizuneh, 2011). The sub-basin is divided into three agro-ecological zones, namely Dega [1] (7% of the sub-basin), Woyna-dega [2] (45.8% of the sub-basin) and Kola[3] (47% of sub-basin) (USGS, 2016). The southern highland parts of the basin are higher in altitude greater than 127 m above sea level. The lowlands have lower altitude less than 1100 m above sea level in the Northern parts of the sub-basin. Mean annual rainfall is 1675 mm with only one summer rainy season. The mean max-min temperature is 29 and 11.47°C respectively (Figure 1) (NMA, 2015).

## MATERIALS AND METHODS

The study primarily relies on climate data and cross sectional household surveys. The primary data were collected from household surveys, semi-structured interviews, focus group discussions and field observations. Survey was conducted in three agro ecological zones (Dega, Woyina-Dega and Kola) of Didessa sub-basin. The selection and classification of the agro-ecology was based on three specific criteria: (1) climate variables, temperature and rainfall (2) soil and (3) farming system. A total of 450 household heads in three agro ecologies (100, 200 and 150 from Dega, Woyna-Dega and Kola respectively) in 18 villages in four districts were asked about local perception of change in climate variability and its impact on people and livelihoods. Systematic random sampling (SRS) was the technique used to get the final respondents.

In addition, six (6) focus group discussions, 2 from each agro ecology and key informant interviews were also used. Moreover, secondary source of observational climate data of 30 years obtained from National Meteorological agency (NMA).

World Metrological Organization (1992) recommends a minimum of 30-year climatological normal data series for a study to detect trends in hydro-climatic time series. As a result, rainfall and temporal records of four stations (Agaro, Arjo, Anger and Kemashi) representing the three agro ecologies were assembled from National Meteorological Agency of Ethiopia (NMA) (Table 1).

The climate data was broken and there are missing observations. However, the missing values were filled by averaging the nearby records and proxy station records considering the maximum flexible thresholds of 10% missing values adopted by Ngongondo et al. (2011). This categorization helps to have a better comparative analysis at agro ecological levels and highlights how climate characteristics were spatially distributed in the study area.

[1] Dega is an agro ecological classification characterized by tepid to cool climatic zone

[2] Woyina-dega agro ecological classification characterized by mid highlands

[3] Kola is an agro ecological classification characterized by hot to warm moist lowland

The research employed qualitative and quantitative data analysis methods. Qualitative information from farmers in each agro ecological zone (AEZ) along with their views on climate variables were organized, coded, themed and processed qualitatively whereas quantitative climate data was analyzed using a non-parametric test of climate data series called Mann-Kendall trend analysis and Sen's slope estimators with the Macro software MAKESENS (Olofintoye and Sule, 2010; Kendall, 1975; Mann, 1945; Hirch et al., 1991; Loftis et al., 1991).

In climate study, non-parametric test called Mann-Kendall trend analysis and Sen's slope estimator was employed to study the spatial and temporal trends climatic series. These are because of the skewed nature of the marginal distribution of climate data, prevalence of non-normality with too large sample size (Hirsch et al. 1991; Loftis et al. 1991; Kendall, 1975) as well as difficulty in identifying the normality distribution of the data beforehand (Hirsch et al. 1991). Mann-Kendall test is relatively robust against missing value and not greatly affected by gross data error or outliers along with truncated observation (Sonali and Nagesh, 2013; Berryman et al., 1988). The model was used when more than one station was tested in a single study (Hirch et al., 1991). The model has two phases in climate analysis. First, Mann-Kendall trend tests for the presence of monotonic increase or decrease in climate data. Second whereas Sen's slopes estimator tests for the magnitudes of the trends. Correlation coefficient of the meteorological variables was computed to determine the better strength and understanding of the linear relationship between variables (Hirsch et al., 1991).

The Man-Kendall test assumed that a value can always be declared less than, greater than, or equal to another value that are independent; and that the distribution of data remains constant in either the original or transformed units (Hirsch et al., 1991). The null hypothesis in the Mann-Kendall test is that the data are independent and randomly ordered. In this study, let for instance, X1, X2, X3…Xn represents n data points where Xj represent the data point at time series j. Thereafter, the Mann-Kendall test was used to compute the difference between the later measured value and all early measured values $(Xj - Xi)$ where *j*>*i*, and test statistics S is calculated using the formula

$$S = \sum_{i=1}^{n-1} \sum_{i=1j-1+1}^{n} sign(Xj - Xi) \quad (1)$$

$$sign\ (Xj - Xi) = [+1, \text{if } (xj-xi)>0| \quad ][0, \text{if } (xj-xi)=0| \quad ] \quad (2)$$

Where, S is Mann-Kendell test statistics, Xi and Xj are climate data values of year i and j (j>i), and 'n' is the length of time (Motiee and Mcbeen, 2009; Abrha and Simhadri, 2015). A positive/negative value of 'S' indicates an upward/downward trend (Drapela and Drapelova, 2011). A large positive number of 'S' reveals that the later-measured values tend to be larger than earlier values and an upwards trend is indicated. When S is a large negative number, later values tend to be smaller than earlier values and a downward trend was indicated. When the absolute value of S is small, no trend was shown.

Kendall (1975) assumes that for a data series n ≥ 10, the statistic S is approximately normally distributed with the mean and variance and then computed (Yenigun et al., 2008) as follows: E(s) = 0 The variance ($s^2$ ) for S-statistics, in a situation where there may be ties (same values) in the x value, is given by:

$$Var(s) = \frac{n(n-1)(2n+1)-)=\sum_{i=1}^{m} ti(ti-1)(2ti+5)}{18} \quad (3)$$

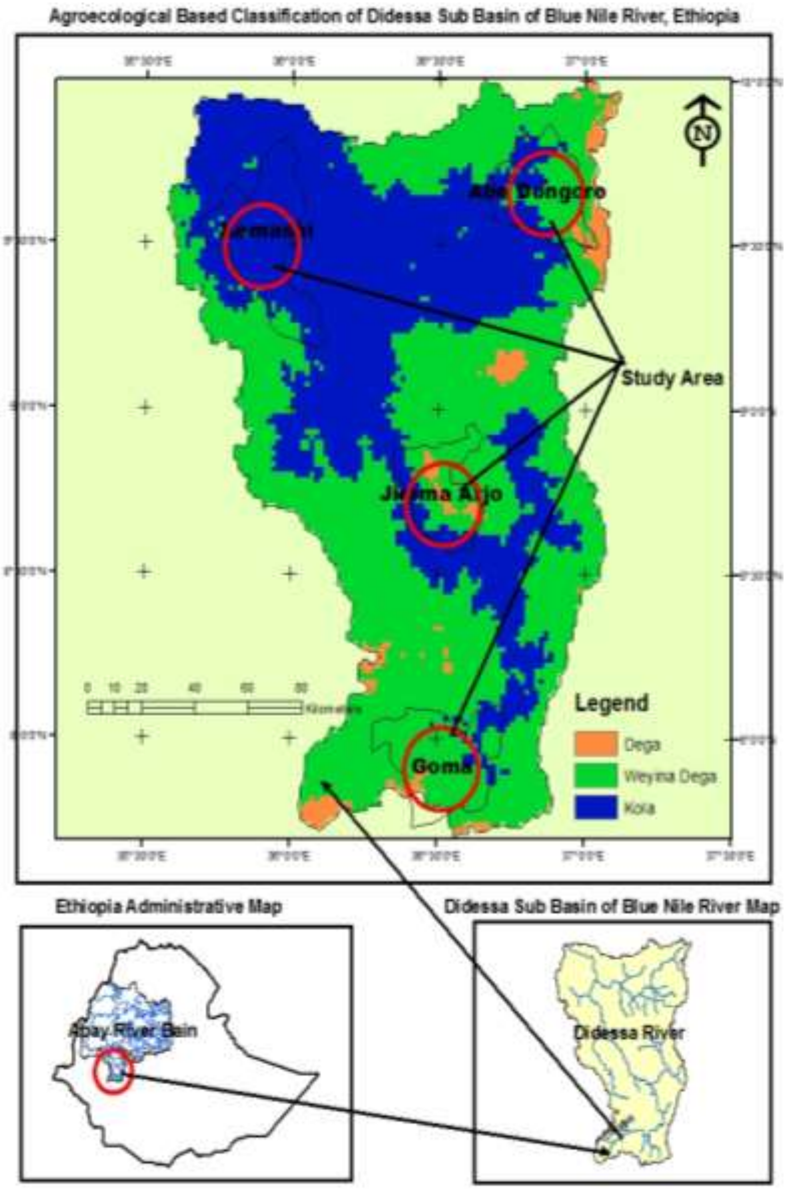



**Figure 1.** Location of the study area, boundary, agro-ecological classification. Source: USGS, 2016.

Where the summation term in the numerator is used only if the data series contains tied values, m is the number of tied groups, and $t_i$ is the number of data points in the tied group. The standard test $Z_s$ statistic is calculated as follows:

$$\frac{S-1}{\sqrt{var(S)}} \text{ if } s>0 \qquad 0 \text{ if } s=0 \tag{4}$$

$$\frac{S+1}{\sqrt{var(S)}} \text{ if } s<0$$

Z score follows a normal distribution. The presence of a statistically significant trend was evaluated using the Mann-K test. In a two-sided test for trend, the null hypothesis ($H_0$) should be accepted if Mann-Kendall is at a choice of α=0.05 (95%) level of significance and has a two-sided alternative. For instance, for 5% significance level, value of Z1-a/2 is equal to -1.96 to 1.96 (from the standard normal table). The trend was said to be decreasing if Z is negative and the absolute value is greater than the level of significance while it is increasing if Z is positive and greater than the level of significance. If the absolute value of Z is less than the level of significance, there is no trend (Refat and Uddin, 2013, Abrha and Simhadri, 2015). An EXCEL template MAKESENS tested the Z score significance level at α: 0.001, 0.01, 0.05 and 0.1 (Refat and Uddin, 2013). However, when n is 9 or less, the absolute value of S is compared directly to the theoretical distribution of S derived by Mann-Kendall (Gilbert, 1987).

Many climate data exhibit a marked right skeweness partly due to fluctuation and deviation from a normal distribution and do not follow a normal distribution (Refat and Uddin, 2013). Consequently,

**Table 1.** Selected metrological station and their characteristics in the selected agro ecologies**.**

| | Station name | | | |
|---|---|---|---|---|
| **Climate station** | **Jimma Arjo** | **Gomma** | **Anger** | **Kemashi** |
| **Field work carried** | | **April - May 2015** | | |
| FGD,HH interviews | 2Focus groups (n=12) HHinterviews (n=100) | 2Focus groups (n=12) HH interviews (n=200) | 2Focus groups (n=12) HH interviews (n=150) | |
| Altitude (m) | 2300-3200 m | 1500-2300 m | 500-1500 m | |
| Farming system | Wheat, Neug, Pulses Barley (2 Crops/Year in the high lands of the basin, Livestock Sheep, Cattle, Goats, horses, Bees, Poultry Cattle,shoats, chicken. Honey | Tef, Maize, Enset Neug, Barley Maize, Sorghum, Tef, Enset, (Rare) Wheat, Finger millet, Barley, Coffee, chat. Livestock: Cattle, goats, sheep, horses, donkeys, bees, poultry Cattle, goats, mules | maize, sorghum, finger millet, sesame, groundnuts and mangoes Taro, Sugar, Coffee, Orange Sorghum, Teff (Rare), Neug, Finger, Millet, Groundnuts, sesame Livestock: Cattle, goats, donkeys, bees, poultry | |
| **Growing season** | | **June/July to October (*kiremt*)** | | |
| **Years of Obsn.** | | **1986-2015** | | |
| Latitude | 8° 33’ -8° 55N | 7° 40-8° 04 N | 9°25’&10° 05’N | 9°30’N 11°39’N |
| Longitude | 36°22’-36° 44’E | 36° 17’-36° 46’ E | 36° 85 -37° 22’E | 34°20’E 36°30’E |
| Annual Rainfall | 1850-2750mm | 1500-1850mm | 900-1500mm | |
| agro ecology | Dega | Woyina-Dega | kola | |

Source: Authors Own Construction, 2015.

Theil-Sen slope (Sen, 1968) also known as “Kendall’s slope” or “nonparametric linear regression slope” estimator was used in this study due to its relative insensitivity to extreme values and better performance even for normally distributed data (Karpouzos et al., 2010).

The slope was estimated following the procedure of Sen (1968). This means that linear model $f$ (t) can be described as if a

$$f(t) = Qt + B) \quad (5)$$

Where Q is the slope, B is a constant and t is time. To derive an estimate of the slope of Q, the slopes of all data pairs was calculated using the equation

$$\frac{Xj-Xi}{j-i}, \text{ i= 1, 2, 3.......N} \quad \text{j>I} \quad (6)$$

If there are $n$ values $X_j$ in the time series there will be as many as $N = \frac{n(n-1)}{2}$) slope estimates $Q_l$. To obtain estimates of B in the equation, the $n$ values of differences $Xi - Qti$ are calculated. The median of these values gives an estimate of B. The estimates for the constant B of lines of the 99% and 95% confidence intervals were calculated by a similar procedure. Data was processed using an EXCEL macro names MAKESENS created by Salmi et al. (2002) for detecting and estimating trends.

## RESULTS AND DISCUSSION

### Local perception and observed climate variability

Agro ecology based classifications were strongly influenced by prevailing climate conditions, which is in many ways a proxy for soil type or slope as well as farming systems. Precipitation, temperature and altitude are also the most influential variables (Simane et al., 2013) in climate study. Consequently, the study area was agro-climatically characterized by tepid to cool (Dega), sub humid and tropical mid highlands (Woyna-Dega), and hot to warm moist lowland (Kola). These differences in classification lead to differences in socio-economic development, which actually vary in local farmer’s perceptions to climate variables and adaptation strategies.

### Local perceptions about temperature and precipitation changes

From the survey data within the Didessa sub-basin, a farmer perception on climate change is shown in Table 2. Out of the respondents interviewed on perception of long-term changes in temperature in the agro-ecologies, most of the farmers (82% of Dega, 92% of Woyina-Dega and 83.33% of Kola) perceived that temperatures have been increasing. It was only 18, 5 and 12% of Dega, Woyina-Dega and Kola farmers respectively that noticed a decrease in temperature while 3% of farmers in Woyina-Dega and 4.67% of Kola noticed that temperature had stayed the same throughout the years.

As indicated in Table 2, farmers’ perceptions regarding temperature change varied across agro-ecologies due to variation in altitude, socio economic, ethnic-cultural

**Table 2.** Farmers perception of towards change in temperature.

| Perceived change in temperature | HL (N=100) | ML (N=200) | LL (N=150) |
|---|---|---|---|
| Increased | 82 | 92 | 83.33 |
| Decreased | 18 | 5 | 12 |
| No change | 0 | 3 | 4.67 |

*All values in the table presented in percent (%) in a given agro-ecology).
Source: Survey Result, 2015

**Table 3.** Climatic zones and their characteristics in the study area.

| AEZ | Altitude | Area (%) | Properties | AMRF (mm) | Rainfall (mm) | MaxTemp (°C ) | MinTemp (°C) |
|---|---|---|---|---|---|---|---|
| | | | Mean | | 1956.69 | 21.41 | 11.47 |
| Dega | 2300-3200 | 7 | Std Dev | 163.06 | 69.59 | 2.03 | 0.52 |
| | | | CV (%) | | 63 | 9 | 5 |
| | | | Mean | | 1565.88 | 28.35 | 12.12 |
| Woyina-Dega | 1500-2300 | 45.8 | Std Dev | 130.49 | 56.87 | 1.48 | 0.89 |
| | | | CV (%) | | 61.4 | 5 | 7 |
| | | | Mean | | 1502.94 | 29.6 | 16.07 |
| Kola | 500-1,500 | 47.2 | Std Dev | 125.25 | 64.8 | 2.87 | 1.13 |
| | | | CV (%) | | 104 | 10 | 7 |
| Didessa | 500-3200 | 100 | Mean | 139.59 | 1675.17 | 26.45 | 13.22 |

Source: Authors Calculation from NMA, 2015

setting, farming system, and soil characteristics. Accordingly, farmers living in Woyina-Dega had perceived increasing temperatures trend as compared to the farmers in the Kola areas (Table 2). These variations in perception lead to differences in production philosophy, farming systems and adaptation strategies toward climate changes. Evidences showed that the variations in perceptions of climate change leads to difference in production systems, overall socio-economic profile, adaptation measures and interventions towards building adaptive capacity to climate change (Simane et al., 2013; Gutu et al., 2012; Maddison, 2007).

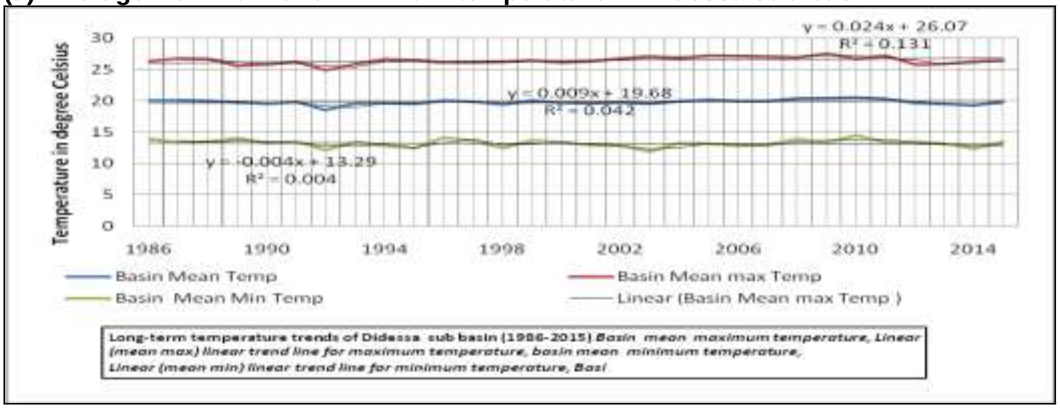
## Temperature trends in DSB (1986-2015)

To verify the farmers' perception of long-term change in temperatures, climate data was statistically analyzed to depict its trend over the decades. The results indicated that the annual temperature for the basin has shown increasing trends for the last three decades (1986 to 2015). The mean annual temperature over the study period was 19.84°C with average maximum and minimum temperature of the basin measured at 26.45 and 13.22°C respectively (Table 3 and Figure 2). The lowest and the highest of the mean annual temperatures within the periods were 11.47 and 29.6°C in the Dega and Kola agroecology respectively. This result was similar to Yilma and Awlachew (2009) where annual mean maximum–minimum temperatures in the Blue Nile Basin varies between 20 - 33°C and 6.5 - 19°C, respectively.

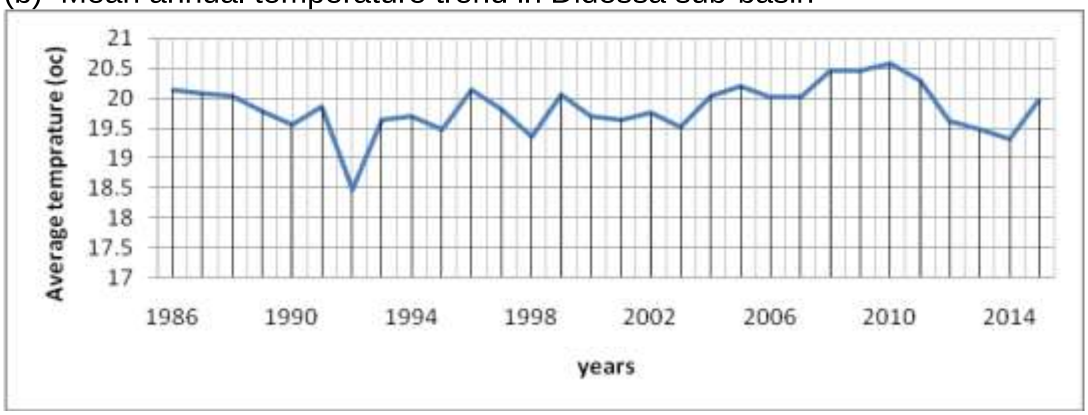
The study projected the average annual temperature of the study area using linear trend ($y = 0.009x + 19.68$) and showed that there was clear rising trends of temperatures during the past three decades (Figure 2). The standard deviation of annual mean maximum temperature is 2.03°C in Dega, 1.48°C in Woyina-Dega and 2.87°C in Kola whereas the standard deviation of mean minimum temperature of the study period is 0.52, 0.89 and 1.13°C in Dega, Woyina-Dega and Kola AEZs respectively. High temperatures were exhibited in the Kola agro ecology with maximum of 35°C. The average annual temperature trend line (minimum, maximum and mean) has exhibited a positive slope indicating that the average temperature has increased by 1.40°C in the past 30 years (Figure 2a) which is greater than the national average annual level temperature (1.3°C) (MOA, 2011).

Temperature rises with an average rate of 0.181°C in the last decade showing that the basin was warming slower and/or temperature had risen in 10 years compared to the national level increase (0.23 to 0.25°C per decade) over the past 55 years (NMA, 2007; Gebrehiwot and Anne, 2013). Similar study by Asamirew

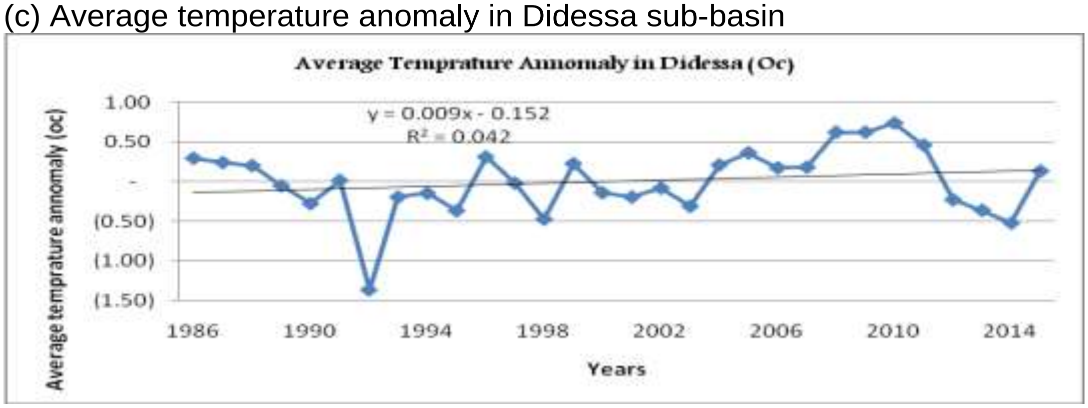
**(a) Average Maximum and Minimum temperature in Didessa sub-basin**



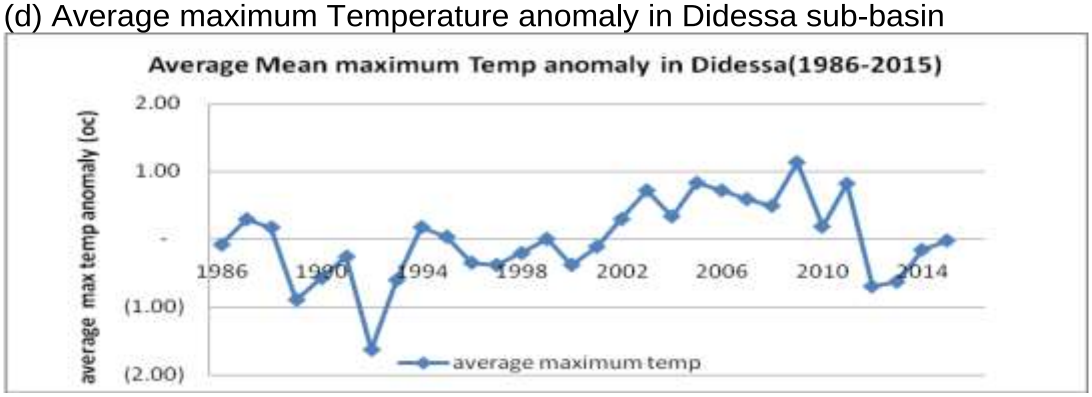

(b) Mean annual temperature trend in Didessa sub-basin




(c) Average temperature anomaly in Didessa sub-basin




(d) Average maximum Temperature anomaly in Didessa sub-basin




(e) Average minimum Temperature anomaly in Dudes' sub-basin

**Figure 2.** Trends in temperature in the DSB (1986-2015). Source Based on NMA Climate Data, 2015.

**Table 4.** Mann-Kendall Trend Analysis and Sen's Slope estimator across Agroecology (1986-2016).

| Month | Dega–Max | | Dega Min | | Woyina-dega–Max | | Woyina-dega Min | | Kola – Max | | kola Min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test Z | Sen's slope | Test Z | Sen's slope | Test Z | Sen's slope | Test Z | Sen's slope | Test Z | Sen's slope | Test Z | Sen's slope |
| Jan | 3.07 | 0.048** | 1.07 | 0.003 | -1.16 | -0.029 | 1.11 | 0.02 | 1.29 | 0.039 | -1.28 | -0.021 |
| Feb | 3.61 | 0.090*** | 0.13 | 0.000 | 0.00 | 0.00 | 0.2 | 0.00 | 4.34 | 0.081*** | -1.54 | -0.052 |
| Mar | 3.82 | 0.045*** | -0.58 | 0.000 | -0.34 | -0.011 | 1.29 | 0.048 | 3.54 | 0.080*** | 0.59 | 0.019 |
| Apr | 2.9 | 0.093** | 0.58 | 0.000 | 0.41 | 0.011 | 1.33 | 0.038 | 2.85 | 0.067** | -0.9 | -0.015 |
| May | 3.02 | 0.068** | -0.02 | 0.000 | 0.39 | 0.011 | 0.95 | 0.044 | 1.57 | 0.062 | -0.61 | -0.006 |
| Jun | 1.9 | 0.047+ | 1.04 | 0.000 | -1.27 | -0.075 | 1.2 | 0.027 | 3.22 | 0.038** | -1.51 | -0.034 |
| Jul | 1.26 | 0.046 | -0.02 | 0.000 | -0.3 | -0.016 | 1.04 | 0.029 | 4.63 | 0.089*** | -0.9 | -0.033 |
| Aug | 3.35 | 0.042*** | 0.23 | 0.000 | 2.02 | 0.045* | 1.27 | 0.035 | 2.18 | 0.038* | -1.31 | -0.023 |
| Sep | 2.99 | 0.033** | -0.18 | 0.000 | 0.88 | 0.025 | 0.59 | 0.015 | 1.58 | 0.015 | -1.36 | -0.027 |
| Oct | 3.35 | 0.072*** | 0.81 | 0.008 | -1.59 | -0.025 | -0.64 | -0.017 | 1.77 | 0.022$^{+}$ | -1.61 | -0.042 |
| Nov | 3.15 | 0.068** | 0.32 | 0.000 | -1.59 | -0.046 | -1.54 | -0.05 | 2.5 | 0.025* | -2.51 | -0.068* |
| Dec | 3.58 | 0.049*** | 0.16 | 0.000 | -2.18 | -0.050* | -1.02 | -0.038 | 1.88 | 0.021$^{+}$ | -3.28 | -0.098** |

***, ** and * indicate that the trends are significant at 99, 95 and 90% level of confidence, respectively; S- Sen's slope Z- Mann-kendall test of trend.
Source Based on NMA climate data, 2015

and Dirba (2015) confirmed the study results based on 30 years of temperature data revealing that there was high variability from year to year in the maximum temperatures over North Shoa, which increases between 0.5 to 2°C for the last three decades. This certainly varies across agro ecology (Table 3 and Figure 2f).

The Mann–Kendall test of climate trend analysis of the study areas climate also confirmed that the temperature showed an increasing trend over the study periods with a confidence level of 99, 95 and 90% (Table 4) across agro ecology. The analysis result showed that the rise in maximum temperature was significant in almost all the months (p<0.01 and p<0.05) in the Dega and Kola AEZs except for the months of June and July (Table 4). The Sen's slope estimate showed 0.048 (P<0.05) in January, 0.090 (P<0.01) in February, 0.045 (P<0.01) in March, 0.093 (P<0.05) in April, 0.068 (P<0.05) in May, 0.047+ in June, 0.046 in July, 0.042 (P<0.01) in August, 0.033 (P<0.05) in September, 0.072 (P<0.01) in October, 0.068 (P<0.05) in November and 0.049 (P<0.01) in December.

The Mann–Kendall test further showed that, Woyina-Dega mean maximum temperature increased in the month of August with statistical significance at p<0.1. Decreasing slopes were shown in the month of December at 0.050°C/month for highest maximum temperature. These results coincided with MOA (2011) finding where an increase in temperature in Ethiopia was shown in the months of June, August and September at the rate of 0.32˚C. In the case of lowest minimum temperature, decreasing trends were found in the Dega and Kola agro ecologies in almost all the months with increasing trends in Woyina-Dega agro ecology.

Statistically significant decreasing trends were found in November and December with Sen's Slope estimates of 0.068 and 0.098°C, 0.068°C/month and 0.098°C/month for lowest minimum temperature in November and December respectively in the Kola agro ecology. Climate prediction in Ethiopia depicted that the mean annual temperature in the country would increase by 1.1 to 3.1°C by the year 2060s and 1.5 to 5°C by the year 2090s and will reduce the GDP by about 3-10% by 2025 (MOA, 2011).

## Farmers' perception about precipitation changes

Regarding perceived precipitation changes across the agro-ecology, about 81% of Dega, 54.5% of Woyina-Dega and 73.33% of Kola farmers perceived that rainfall was decreasing. It was only 10, 38.5 and 13.33% of Dega, Woyina-Dega and Kola farmers respectively that noticed an increase in rainfall. About 9% of farmers in Dega, 7% in Woyina-Dega and 13.33% of Kola farmers, respectively noticed seasonality change in rainfall as well as increase in drought frequency (Table 5).

## Meteorological rainfall trend analysis (1986-2015)

To verify the farmers' perceptions of long-term changes in precipitation, the historical monthly rainfall data for the period 1986 to 2014 was analyzed. The analysis result indicated that the overall rainfall amount and distributions were highly varied from time to time especially in the last few years with an annual average precipitation of 1675.17 mm in the sub-basin and varies with 1956.70, 1565.88 and 1502.94 mm in Dega, Woyina-Dega and Kola agro-ecologies respectively. These were quite

**Table 5.** Perception of farmers towards rainfalls.

| Perceived change in precipitation | Dega (N=100) | Woyina-Dega (N=200) | Kola (N=150) |
|---|---|---|---|
| Increased | 10 | 38.5 | 13.33 |
| Decreased | 81 | 54.5 | 73.33 |
| Change in season of rainfall | 7 | 6.5 | 12 |
| Increase in frequency of drought | 2 | 0.5 | 1.33 |

*All values in the table presented in percent (%) in a given agro-ecology).Source Survey result, 2015.

emphasized by positive and negative anomalies (Figures. 3a to d and Table 3) and are similar to Yilma and Awlachew (2009) in the upper Didessa Basin which showed annual rainfall ranging from 1200 to 2200 mm. The annual temporal precipitation data across AEZs showed high rainfall variability and deficiencies in Woyina-Dega and Kola compared to their long-term mean for most years.

There was variation in the monthly amount of rainfalls in terms of intensity and distribution (Table 3) across the agro-ecologies. Climate analysis of the study showed that the mean monthly rainfall was 163, 130.49 and 125.24 mm in Dega, Woyina-Dega and Kola, respectively. The coefficient of variation in most stations revealed that rainfall in the basin has high inter-annual variability. The result indicated that annual rainfall variability at Dega (CV>63%) and at Kola (CV>104%) were extremely variable. Similarly, high monthly coefficient of variation was found in Kola (96%), in Dega (77%) and in Woyina-Dega (64%) agro-ecology (Table 3 and Figures 3b, c and d and 4).

The Mann-Kendall trend and Sen's slope estimators showed that, precipitation in the month of March was significantly decreasing at $p<0.1$ level in the Woyina-Dega AEZ while the months of May, June, July, August and September rainfalls trend was positive and significantly increasing at $p<0.01$ level in the Dega agro-ecology (Table 6). Precipitation in the Kola agro ecology has an increasing and positive trend in all the months and is significant at $P<0.05$ (January, April, May, September and November) and $p<0.01$ (in February, March August, October and December). In Blue Nile Basin in general as well as in the sub-basin in particular, the two main crop seasons are Belg and Kiremt seasons. *Kiremt (*June-September) is the main rainy season with highest rainfall records in the months of June, July and August preceded by a small rainy season *Belg* (*February-May*) (Figure 4). This finding confirms that of CSA (2012) which showed dry spell months in the Southwestern parts are few and receive rainfall for about 8 months.

The Mann-Kendall trend analysis of monthly rainfall results showed variation in the spatiotemporal distribution and gradual concentration of rainfall in a few months to increase the prevalence of lengthy dry-spells across agro-ecologies.

The declining trends in March and May (in *Belg* season) and June and July (in *Kiremt* season) in Woyina-Dega agro-ecology is an indication of the common place nature of late start and early ending rainfall to affect the number of rainy days (and the growing season) and crop production during the two rainy seasons. Rainfall during Kiremt season exhibited a significantly growing trend in the Dega, whereas that of Belg rainfall had a significant declining trend in the Woyina-Dega AEZs (Table 6) due to the recent afforestation program by the government.

The onset of rainfall for the Dega and Woyina-Dega agro-ecologies are in March and cessations are in October while the Kola rain starts in the months of April with monthly rainfall variability between 83.16 to 125.07 mm. Rainfall in Kola and Dega AEZ has an extreme inter-annual variability (Table 3). The results coincided with a study in Ethiopia where a declining trend was noticed in monthly rainfall with variability ranging from 23.5-146.16 mm (Asamirew and Dirba, 2015) as well as decreasing rainfall trends over the past several decades in South Africa and in the Sahel region of Africa (Gbetibouo, 2009; Mertez et al., 2009). Additionally, in Tanzania, a finding indicated a decreasing trend of rainfall for the last 35 seasons from 1973/74-2007/08 (Mongi et al., 2010). However, the result contradicts a study conducted by MOA (2011) where rainfall projection in South West of Ethiopia showed an increasing trend in the months of October, November and December.

## Comparison observation climate variability and climate perceptions

Climate change was assessed by using meteorological observations and/or farmers perceptions. The analysis of climate variables (temperature and precipitation) for the study sub-basin has shown that there is high variability of temperature and rainfall both spatially and temporally. The study tried to compare farmers' observations and perception with historical meteorological trends in the study area. Farmer's observation and perception related to temperature were consistent with the meteorological records indicating a clear increase in temperature across agro-ecologies with slight variations (Tables 2 and 3 and Figure 2a to d). Hence, farmers can accurately perceive climate variability and change as well as its impacts on

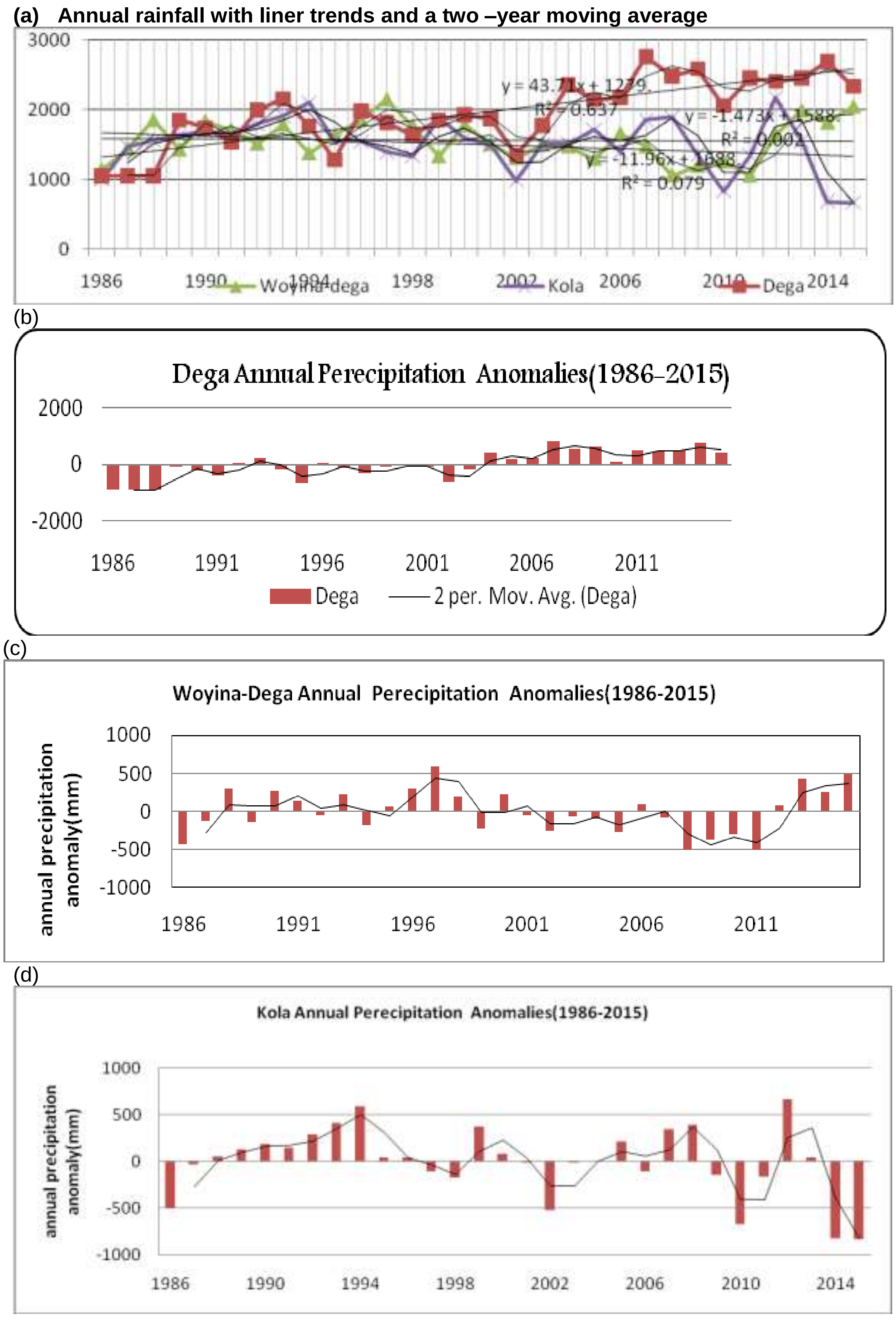



**Figure 3.** Trends in Rainfall in the DSB (1986-2015). Source Based on NMA Climate Data, 2015.

**Table 6.** Mann-Kendall and Sen's slope estimator for monthly Precipitation.

| Months | Kola –RF | | Woyina-Dega –RF | | Dega – RF | |
|---|---|---|---|---|---|---|
| | Test Z | Sen's slope | Test Z | Sen's slope | Test Z | Sen's slope |
| Jan | 3.07 | 0.048** | 0.21 | 0.045 | 0.83 | 0.253 |
| Feb | 3.61 | 0.090*** | 0.00 | 0.000 | 0.92 | 0.2 |
| Mar | 3.82 | 0.045*** | -1.96 | -2.007* | 0.93 | 1.64 |
| Apr | 2.9 | 0.093** | 0.16 | 0.306 | 1.13 | 0.7 |
| May | 3.02 | 0.068** | -0.02 | 0 | 3.83 | 9.028*** |
| Jun | 1.9 | 0.047+ | -1.21 | -1.465 | 4.15 | 7.369*** |
| Jul | 1.26 | 0.046 | -1.77 | -2.413+ | 4.83 | 8.447*** |
| Aug | 3.35 | 0.042*** | 0.75 | 2.093 | 3.29 | 5.815*** |
| Sep | 2.99 | 0.033** | 1.46 | 2.052 | 4.43 | 8.714*** |
| Oct | 3.35 | 0.072*** | 0.63 | 1.358 | -0.68 | -0.638 |
| Nov | 3.15 | 0.068** | 1.3 | 0.614 | 1.52 | 1.295 |
| Dec | 3.58 | 0.049*** | -0.05 | 0 | -1.56 | -0.465 |

***, ** and *trends are significant at 99, 95 and 90% level of confidence, respectively, S- Sen's slope estimate Z- Mann-Kendall test of trend. Source Based on NMA climate data, 2015.

their livelihoods for short term periods. However, the observed and perceived pattern in precipitation showed variability in terms of amount and distributions and does not fit the metrological data across the agro-ecologies (Table 3, Figure 3 and Appendix Box 4.1).

In the focus group discussion (FGD), the farmers revealed that: *"the rain does not come on time anymore. After we plant, the rain stops just as our crops start to grow. And it begins to rain after the crops have already been ruined."* Most farmers felt that the rainy season commenced later and stopped earlier in recent past as compared to a long time in the past. They are aware of climatic changes related to the rainy seasons than other seasons. Farmers in all of the agro-ecology said that rainfall was becoming 'increasingly unpredictable' with temporal variations.

The late start and early stop of rainfalls does not only cause failure in harvest but also a delay in farm preparation for the next productive season and prevents planting of long cycle high-yielding crops (maize and sorghum) during *Belg* season. The decreased trends for *Belg* rainfall in Woyina-Dega means a lot for local farmers who used to rely on *Belg* rains for food production (Table 6 and Appendix Box 4.2).

Conversely, availability of Belg rain water assist in the regeneration of grazing lands, land preparation and for planting of long cycle high yielding crops (maize and sorghum). The significant increased rainfall in Kola (in both Kiremt and Belg) is crucial for the production of long cycle high yielding lowland crops (maize, sorghum and millet).

Kiremt rainfall in the Dega agro ecology (90-95% national total cereal output) is paramountly important for the production of highland crops such as tef, barley and wheat respectively. Of course, the smallholder farmers' perceptions of decrease in rainfalls might have been influenced by other factors such as distribution of rainfall, farm inputs and farming system. Study by Simelton et al. (2011) on African farmers' perceptions of erratic rainfall showed that farmers financial, physical, social status, and access to inputs influences how they are affected by rainfall changes and how they perceive those changes, as well as how they can respond to or adapt to those changes.

According to the study, key informants perceptions about precipitation are influenced by farmers own beliefs and expectations as well as experience related to their agronomic practices. Consequently, the same amount of rainfall can result in a good year for some and a bad year for others; perceptions therefore are closely associated with (expected and previously experienced) impacts, not only the actual rainfall levels. Besides, the farming practices and farmers farm calendar also influences the perception of rain in one season or another that could lead to a mismatch in the actual meteorological data and farmer's perceptions.

There are reasons for discrepancies: The study offers a few conceivable explanations below. Farmers can not accurately track probabilistic changes in climate and are more likely to recall recent years of unusual extreme events in rainfalls and classic drought, rather than the rainfall and long-term climate events in intermediate years. This is because memory can be faulty, with unique events attributed to climate change while incremental change goes unnoticed.

The study asked participants of FGD about their feelings and compelled them to offer a story in line with a dominant narrative of climate change where their perceptions viewed as expectations of change or stability can influence one's capacity to detect probabilistic changes. Farmers' accounts and meteorological data differ in climate-related phenomena. Hence, there are

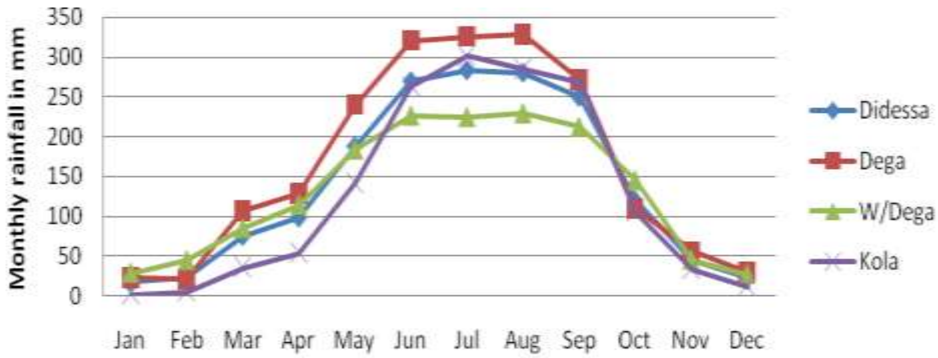



**Figure 4.** Monthly average rainfalls and trends across agro-ecology. Source Based on NMA climate data, 2015.

disparities among farmers in the study area and statistical trends detected in meteorological records. Farmers understand the *agronomic distinctiveness* but not *meteorological drought.* For instance, rising temperatures in the months of summer may result in reduced soil moisture during planting, even without a decrease in summer rainfall. It may be a change as this that farmers interpret as decrease in summer rainfall.

### *Perceived causes of climate variability and change*

Focus group discussion (FGD) of the study indicated that, a significant portion of smallholder farmers consider that humanity activities (anthropogenic) and super natural forces are the primary causes of climate changes. Accordingly, disobedience and unfaithfulness to religious following, failure to glorify God and alteration from the age-old study area tradition believed led to “divine intervention” type punishments, like rainfall variability in terms of amount and distribution as well as crop failure. This spiritual perspective is widespread throughout Africa (Patt et al., 2009; Gandure et al., 2013; Tumbo and Abdoulaye, 2013).

Teka et al. (2013) reported that farmers in Benin partly attributed climate variation to failure in observance of traditional customs and indigenous laws by the indigenous community. Besides, farmers in FGD also associated the variations in climate change with environmental explanation that identifies cause of deforestation and pollution from industries. These anthropogenic causes of climate change have a number of implications for agricultural productivity although the aggregate impact of these is not yet known or quantified, especially at the farm scale (Gornall et al., 2010).

### Perception of the effect of climate variability and change

Climate change is perceived to have adverse ecological, social and economic impacts. Consequently, farming practices in a particular location are strongly influenced by the long-term mean climate state, the experience and infrastructure of local farming communities. The existing climate trends and irregularities in the amount and pattern of rainfall over the study periods in Didessa Basin together with traditional backward farming system results in an unsustainable agricultural production that threaten the lives and livelihoods of many poor families. Accordingly, the study showed that smallholder farmers in the study area face numerous risks/vulnerabilities to climate variability and changes (Figure 5).

According to many of the respondents, for instance, about 29, 38 and 38.34% of Dega, Woyina-Dega and Kola agro-ecology respondents’ view of extent of vulnerability level to climate variability and change was rated as highly vulnerable. They claim that due to lack of feed and shortage of water for animals, increased incidence of plant, animal and human diseases and shortage of food for the households were indicated as major consequences of climate change in Didessa Sub-basin.

Moreover, majority of farmers within the study area are directing their livelihoods into mixed farming that involves both crop (95%) and livestock production. However, their farming practices in the Didessa sub-basin affected by the increased temperature and decrease in rainfall as well as the shift in timing, amount and distribution of rainfall, changes in the length and quality of the growing season. According to the key informant interview, changes in climate variables exacerbate shortage of feeds and water for animals, which causes frequent death of animals, delay in seed precipitation, reduction of forest cover, degradation of natural resources, and changes in the distribution of disease putting more people at risk from incidences of malaria and dengue fever.

IPCC (2007) noted that an increase in average temperature will adversely affect crop production, especially in lowland areas where heat has become a limiting factor. This in turn increases evapo-transpiration rate of plants, and increases chances for severe drought. Hence, agriculture in Ethiopia is mostly described by

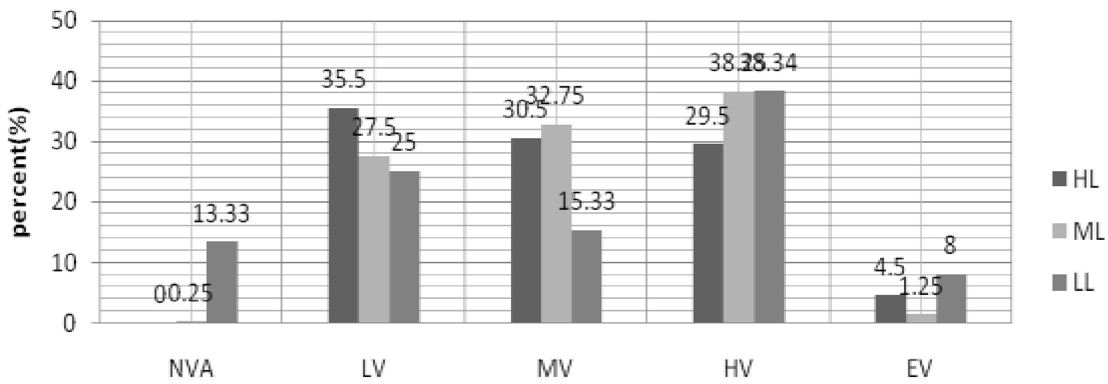



**Figure 5.** Farmers view of extent of vulnerability levels to climate variability and change in the surveyed agro ecologies (percent). NVA, Not Vulnerable at All; LV, less vulnerable; MV, more vulnerable; HV, highly vulnerable; EV, extremely vulnerable.

extreme dependence on rain systems (Woldeamlak, 2009) and most of the farmers pay more attention to recent climate information, noticed by Maddison (2007) and Gbetibouo (2009).

In his study on the central highlands of Ethiopia, Woldeamlak (2009) reports increasing incidences of agricultural pests and diseases as one of the manifestation of climate change. Besides, Abate (2009) indicated in his study on climate change impact on farmers' livelihood and coping mechanism in the west Arsi zone, that drought and delay in the onset of rain leads to poor grass regeneration and forage deficit, water shortages and heat stress on livestock.

To this end, farming activities are highly vulnerable to climate variability and change. This is aggravated due to low economic capability to adapt at a household level and their high dependence on rain fed agriculture for their sustenance. Therefore, it is clear from the analysis that a particular type of farming system and productivity depends upon the climate variability and change already happening, adaptation capacity and willingness to adapt to changing climate situations.

## CONCLUSION AND RECOMMENDATION

It is apparent that climate change is a reality that the world is facing. This study proved that climate change and variability is a reality at the micro (agro ecological) level. This study compared observational climate data analysis with local farmers' knowledge in the Didessa sub-basin. Farmers' perception can help to leverage knowledge from the observational data (metrological evidences).

The study showed that majority of the farmers in the Didessa sub-basin perceived shifts in the timing of seasons, increases in temperature, and decreases in rainfall. Farmers perceived changes in the rainy season more than other seasons. They expressed shortening of the rainy season and increased variability in terms of intensity and distribution. The analysis of the climate data also revealed that temperature is increasing while rainfall is decreasing across agro ecological zones. This observed temperature variability is compatible with the farmers' local perceptions.

However, for long-term periods it was difficult to relate perceptions precipitation changes over time because there are disparities among farmers in the study area and statistical trends detected in climate records. Farmers understand the *agronomic distinctiveness* but not *meteorological drought.* The study found that the most common causes of climate change were supernatural forces, deforestation, and pollution according to farmers' perceptions. This result in perceived impacts on the lives and livelihoods of farmers in Didessa sub-basin with variation across agro ecological zones

Complementing observational data analysis with local knowledge is a new way of deepening scientific understanding, especially where observational records are rare and uncertain. Therefore, we recommend that farmers perceptions of climate variability and change needs to be taken seriously by scientists and policymakers in dealing with ways of utilizing the metrological information to develop and implement useful options that will rescue the farming households from the impacts of climate change and variability. In addition, scientists and policymakers have to integrate the metrological data into farmers' perceptible way of climate variability for future adaptation and mitigation mechanisms.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## APPENDIX

**Box: 4.1. Farmers' explanations of climate variability and change (Household survey, 2015)**

A farmer in his late 50s explained from Arjo (Dega agro ecology) that: I used to cultivate ''daguja and Maize (long duration sorghum variety and maize) from year to year and the crop harvests were much higher (about 80 ''qunna" which is equivalent to 4 tons per hectare). In recent years, however, I am not able to plant ''daguja" and "maize" because the rain starts too late. In previous years, *when I was younger, effective rain (belg rain) used to start early in the month of February,* and continued until the end of October but in recent times it starts later (usually mid of July) and ends earlier (early September or sometimes mid of August). Furthermore, in the past, the amount of rainfall per rainy day was not too much or too little but recently, it looks as if ''it gets people angry", that is, higher rainfall intensity over shorter periods. In the past, the planting dates were on time and almost consistent from year to year, but recently we have followed the approach of ''Robnaan Facaasi" which means sow whenever it rains because*, rains are now unpredictable*. As a result, crops face severe moisture stress starting from the early development stages and yield is usually low. Besides, livestock are extremely affected by shortage of food.

**Box: 4.2. Farmers' perception and explanations of climate variability and change (Household survey, 2015)**

Another, 69 years old farmer at Agaro mentioned his perception of climate change: "I have been living here in Agaro (Woyina-Dega) agro ecology. However, in recent years, it is too hot to live. Our local community (where they live and sustain their livelihoods) is becoming hotter and hotter. I am in fear that it is going to be as hot as areas where people are talking about (e.g. afar, and other lowland areas of Ethiopia). In the past, the rainy season begins on time, and once the rainy season has started it extends into October. In recent years, the rainy season starts late and is short. Previously, Agaro was a midland zone well known for coffee production that dominated in terms of production, although recently changes in climate (coffee bear disease), coffee production and productivity reduced. For example, I had produced a lot per hectare of coffee but now I have only produced five quintal. Crop yields have reduced. We have not experienced any food insecurities so far, but, I anticipate such things in the future if it continues like this.

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# Identification and mapping QTLs of bolting time in purple cai-tai (*Brassica rapa* L. var. purpurea)

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**Bolting time is a crucial agronomic trait for yield and quality in purple cai-tai (*Brassica. rapa* L. var. purpurea), but the genetic mechanism controlling the procedure remains unknown. In the present study, a double haploid (DH) population derived from two inbred lines of purple cai-tai 4-1 and 040-3 was constructed to identify the quantitative trait loci (QTLs) of bolting time. Genetic linkage map was performed by JoinMap version 3.0 using SSR, SRAP and ESTP molecular markers. A total of one hundred and thirty-eight molecular markers were integrated into ten linkage groups (LGs), which were anchored to the corresponding chromosome of the *B. rapa* reference genome. The genetic linkage map covers 1253.1 cM, with an average distance of 9.08 cM between two adjacent markers. Five quantitative trait loci (QTLs) were identified to control bolting time and explaining variations from 17.7 to 44.2%. The genetic results of bolting time will be useful for future breeding of late bolting in purple cai-tai.**

**Key words:** Purple cai-tai (*Brassica rapa* L. var. *purpurea*), bolting time, quantitative trait loci (QTL).

## INTRODUCTION

*Brassica rapa* is consisted of various vegetables such as Chinese cabbage, non-heading Chinese cabbage, and turnip. Non-heading Chinese cabbage includes economically important vegetable taxa with a wide range of morphologies, such as pakchoi (*Brassica campestris* ssp. *chinensis* Makino var. communis Tsen et Lee), purple cai-tai (*B. rapa* L. var. *purpurea*, Canjie et al., 2019), rosette bok choy (*B. campestris* ssp. *chinensis* Makino var. rosularis Tsen et Lee), and taicai (*B. campestris* ssp. *chinensis* Makino var. tai-tsai Hort). Purple cai-tai is a natural early bolting mutant which bolting earlier without vernalization, and it is an important vegetable in the middle and lower reaches of Yangtze river.

In *B. rapa*, one of the most important agronomic traits is bolting because premature bolting significantly affects the quality and yield of the economic products (Kitamoto et al., 2014). Bolting times are regulated by multiple genes. In *Arabidopsis thaliana*, over 300 regulatory genes for bolting and flowering time have been isolated (Bouché et al., 2016). Many QTLs of bolting and flowing have been charactered in *B. rapa*. In the past two decades (Nishioka et al., 2005; Lou et al., 2007, 2011; Li et al., 2009; Kakizaki et al., 2011; Li et al., 2015).

During the elucidation of the genomes of crop species, it is crucial to assign molecular markers to the linkage

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groups (LGs) and construct genetic maps. A number of genetic linkage maps have been produced for *B. rapa* based on diverse marker types including Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD), Simple sequence Repeats (SSR), Amplified Fragment Length Polymorphisms (AFLPs), and Sequence-related amplified polymorphism (SRAP) (Kim et al., 2006; Suwabe et al., 2006; Soengas et al., 2007; Yan et al., 2009; Honghao et al., 2014; Haidong et al., 2016).

SRAP has some advantages, such as simple, a apposite throughput rate, targeting open-reading frames (ORFs), and so on (Uzun et al., 2009). SSR markers are useful to construct high-density maps because of its high polymorphism levels, its co-dominant character, its abundance and wide distribution during the genome and the utility as convenient anchor points in the integration of intraspecific and interspecific consensus maps (Acher et al., 2004). Expressed sequence tag polymorphism (ESTP) markers are transferable between species and between genera (Brown et al., 2001).

Although many QTLs about bolting have been isolated and characterized with molecular markers, the report of QTL and the markers based on sequence-tagged Polymerase chain reaction (PCR) mapped in purple cai-tai is limited (Canjie et al., 2019), especially those which may provide anchors to the genome of *B. rapa* and are readily transferable to other populations. Thus, the objective of this research was to identify QTLs controlling bolting in two years. Our results should be useful to understand the genetic mechanism about the bolting in purple cai-tai, and contribute to breeders for designing effective strategies for better cultivar.

## MATERIALS AND METHODS

### Plant materials and DNA isolation

Double haploid (DH) population consists of 140 individual DH lines was employed for trait assay and genetic mapping. The population was developed from microspore culture of F1 buds of the cross between 040-3, a cultivar with early bolting which was derived from 040 and 4-1, a high inbred line with late bolting, which was obtained by seven generations of self-pollination of cultivar Daguzi. The plants of parents, F1 and 140 individual DH lines were cultivated in an open field at the Institute of Economic Crops of Hubei Academy of Agricultural Science, Wuhan, China (30.57°N, 114.3°E) from September of 2012 to April of 2013, and September of 2013 to April of 2014. The bolting time (that is, days after sowing to appearance of macroscopic floral bud) was judged by the observation recorded every third day (Wang et al., 2018) in 2013, 2014 spring.

### Detection of DNA polymorphism

DNA was isolated from fresh and young leaves of the parental and 140 DH lines according to the protocol published by Guillemaut and Laurence (1992). 106 SSR markers, and 4 ESTP markers and 652 SRAP markers were used to filtrate the polymorphism of the two parents and $F_1$. The experiment of SRAP was carried out following the procedure reported by Li and Quiros (2001), with minor modifications. SSR and ESTP markers were obtained as described by Choi et al. (2007) and HyeRan et al. (2009) (Supplementary Table 1). PCR was performed in a 10 μl reaction mixture containing 2 μl DNA template (40 ng), 1 μl 10 × PCR buffer ($MgCl_2$), 0.2 μl forward primer (10 μM), 0.2 μl reverse primer (10 μM), 0.8 μL dNTPs (10 mM), 0.2 μl TaqDNA polymerase (2.0 U/μl), and 5.6 μL $ddH_2O$ (Biomed Tec Co., Beijing, China). PCR conditions were as the follows:an initial denaturation step at 94°C for 4 min, followed by 35 cycles of DNA amplification(94°C for 30 s, 60°C for 30 s, and 72°C for 60 s), with a final 7 min extension at 72°C (Mastercycler nexus, Eppendorf, German). The PCR products were separated by electrophoresis on 9% polyacrylamide gels (acrylamide/ bisacrylamide = 29:1) and screened with silver staining (Choi et al. 2007).

### Linkage analysis, map construction and QTL analysis

A scoring system was applied for the reproducibly polymorphic makers among the parent lines in the DH population. Linkage assay and the construction of maps were carried out by JoinMap Version3.0 (Stam, 1993; Van Ooijen and Voorrips, 2001). SSR and ESTP markers previously mapped (Yan et al., 2011) were utilized for the identification of LGs in the LOD groups with a threshold range of 3.0–8.0. The annotation of LGs was identical with the second generation of referenced LGs in *B. rapa* (A1–A10). A composite interval mapping (CIM) reported by Zeng (1994) was employed for the analysis of QTLs for bolting time by a QTL Cartographer (version 2.5) (Basten et al., 2002). In order to estimate the appropriate significance threshold of a logarithm of odds (LOD), a test of 1,000-permutation was carried out via the QTL Cartographer.

## RESULTS

### Polymorphism screening of primers between parents

In order to construct the genetic linkage map, the two parents and $F_1$ were filtrated for polymorphism with 652 SRAP markers and 106 SSRmarkers, and 4 ESTP markers. In total, 183 (24.02%) out of 762 primers (or primer combinations, abbreviated as PCs), including128 SRAP PCs, 42 SSR PCs, and 3 ESTP, produced polymorphic loci. A total of 129 polymorphic loci were selected with the help of 128 polymorphic SRAP primer combinations. Meanwhile, 42 SSR and 3 ESTP polymorphic loci were obtained. All these obtained polymorphic markers were employed for the assay of DH population (Supplementary Table 2).

### Construction of genetic linkage map

A total of 140 DH individuals from $F_1$ progenies of two purple cai-tai “4-1” and “040-3” were used for genotyping and linkage analysis. There were 25.0% of 184 polymorphic markers not assigned. As shown in Figure 1, a total of 138 markers were anchored to 10 LGs which spanned 1253.1 cM of map distance with an average distance of 9.08 cM. The location of 10 LGs on their corresponding chromosomes (A1-A10) was confirmed via 32 SSR and 2 ESTP markers of which the map positions were already known on the reference maps of *B. rapa*.

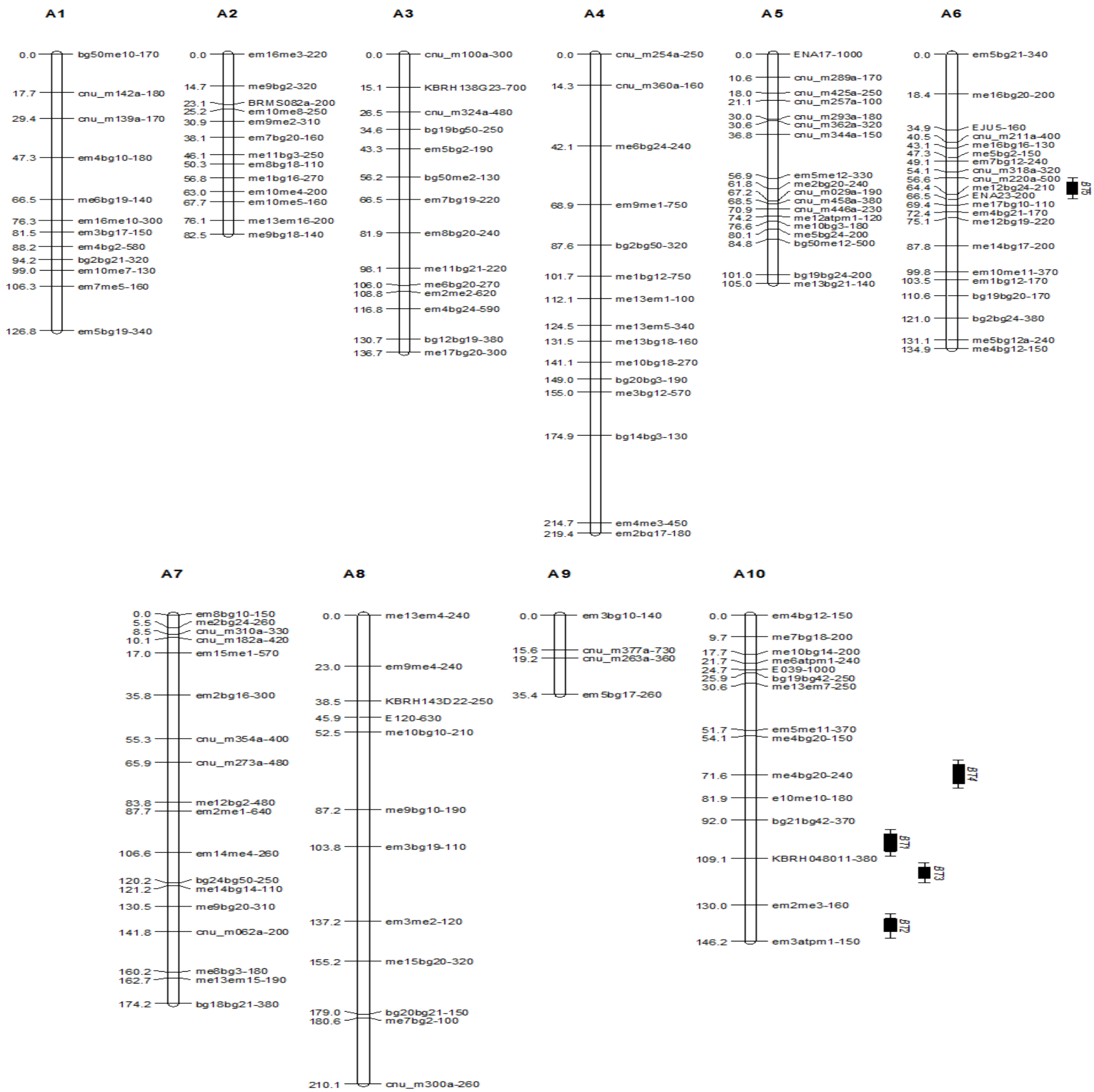


**Figure 1.** Genetic linkage map and localization of QTLs of bolting time traits on a population of 140 DH lines of purple cai-tai.

The length range of individual LGs varied from 35.4 cM (A9) to 219.4 cM (A4).

## QTL analysis for bolting time

As shown in Table 1, the parental lines, 040-3 and 4-1, revealed significant difference of the bolting time. All seven plants of 040-3 exhibited stably bolting time at 40 DAS in 2013 spring and 41 DAS in 2014 spring, respectively. For 4-1 of late bolting parent line, bolting time was detected between narrow ranges from 127 to 129 DAS in 2013 spring, and 128 to 132 DAS in 2014 spring, respectively. These results indicated that the genetic background of these two purple cai-tai lines is nearly homozygous with little environmental effect. Further, the average bolting time of the $F_1$ showed 96±2.2, 97±3.2 DAS which is slightly larger than that of the mid-parent (84±1 DAS, 85.5±1.4 DAS) in 2013 and 2014 spring, respectively. The bolting time of 137 out of the 140 $F_2$ DH progenies were checked that revealed a continuous distribution from 57 to 141 DAS in 2013

**Table 1.** Variation in bolting and flowering time of parents and F2 population.

| Environment | Generations | No. of plants | Bolting time (days) |
|---|---|---|---|
| | 040-3 | 7 | 40 |
| | 4-1 | 10 | 128±1 |
| Spring-2013 | F1 | 10 | 96±2.4 |
| | Mean of F2 population | 137 | 94.7±3.5 |
| | Range of F2 population | 137 | 57-141 |
| | 040-3 | 10 | 41 |
| | 4-1 | 10 | 130±1.4 |
| Spring-2014 | F1 | 10 | 97±3.2 |
| | Mean of F2 population | 137 | 93.1±2.8 |
| | Range of F2 population | 137 | 55-140 |

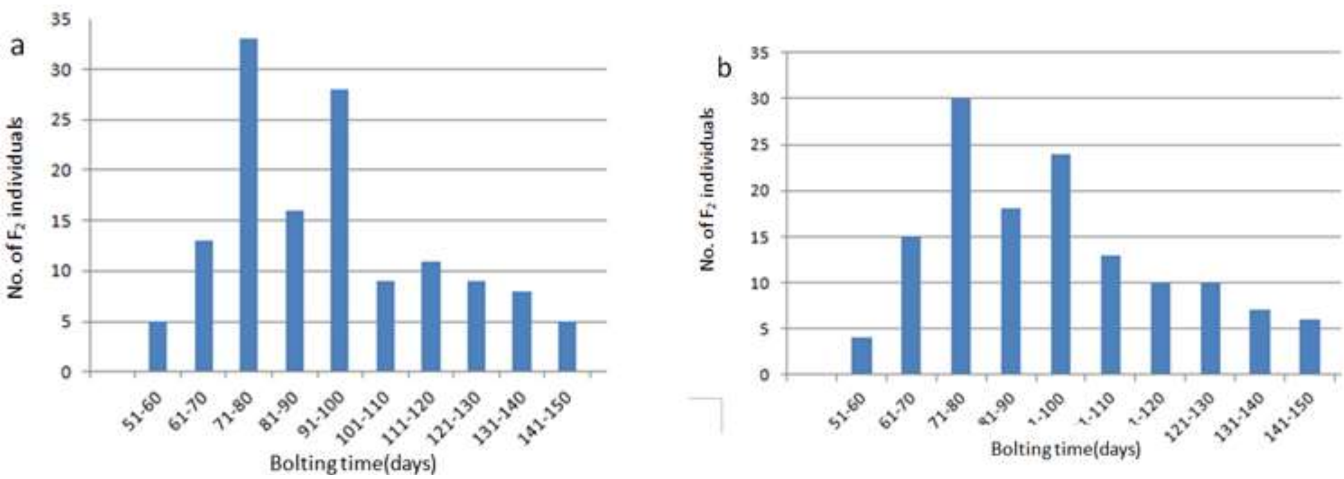


**Figure 2.** Frequency distribution of bolting time in the F2 population. (a) 2013 spring, (b) 2014 spring.

spring, and from 55 to 140 DAS in 2014 spring. The other 3 of the 140 $F_2$ DH progenies died before bolting. These results suggested that the bolting time in purple cai-tai probably be controlled by quantitative trait locus.

The frequency distributions of bolting time in the $F_2$ populations revealed continuous distribution, also showing that bolting time are quantitative traits controlled by polygenes (Figure 2). QTL analysis was performed individually for each of the 2013 and 2014 tests. Five QTLs for bolting were detected in A6 and A10 (four regions). The largest QTL effect (LOD of 11.73) on bolting time, named as BT1, was detected between the loci KBRH048O11-380 and bg21bg42-370 on A10, which explained approximately 44.2% phenotypic variation. Other four QTLs, named as BT2, BT3, BT4 and BT5, were mapped in A10 and A6 chromosome explaining 42.7, 41.6, 34.2, and 17.7% phenotypic variation, respectively. Remarkably, BT1, BT2, BT4 were detected twice in 2013 and 2014, but BT3 only in 2013 and BT5 only in 2014 (Table 2).

## DISCUSSION

A genetic linkage map was constructed via a segregating population of 140 purple cai-tai DH lines. This linkage map contains 104 SRAP, 32 SSR, and 2 ESTP markers which were grouped on 10 LGs, and each LG was anchored to the corresponding chromosome of the *B. rapa* reference map based on the common SSR and ESTP (Yan et al., 2011; HyeRan et al., 2009; Su Ryun Choi et al., 2007). It indicates that this map can be integreted into orther genetic linkage map of *B. rapa* and be useful for other researchers. Covered with a total genetic distance of 1253.1 cM, the linkage map in the present study is comparable to the published sequenced BAC anchored reference genetic map which is 1,123.3 cM (HyeRan et al., 2009) and the sequence-based genetic linkage map which is 1234.2 cM illustrated by Yan et al. (2011). The genetic map lengths differences among various reports are attributed to the scoring errors for the most parts. In addition, the differences have also

**Table 2.** QTL detected for the bolting time traits based on CIM (composite interval mapping) analysis.

| QTLs | Years | Marker interval | Group | QTL position | Peak LOD[a] | Addition effect |
|---|---|---|---|---|---|---|
| BT1 | 2013 | bg21bg42-370-- KBRH048011-380 | A10 | 102.049 | 11.73 | 15.42 |
| BT1 | 2014 | bg21bg42-370-- KBRH048011-380 | A10 | 102.049 | 11.24 | 14.53 |
| BT2 | 2013 | em2me3-160--em3atpm1-150 | A10 | 138.992 | 10.77 | 15.69 |
| BT2 | 2014 | em2me3-160--em3atpm1-150 | A10 | 138.992 | 10.54 | 15.23 |
| BT3 | 2013 | KBRH048011-380--em2me3-160 | A10 | 116.12 | 11.06 | 15.24 |
| BT4 | 2013 | me4bg20-150--e10me10-180 | A10 | 72.605 | 10.64 | 13.82 |
| BT4 | 2014 | me4bg20-150--e10me10-180 | A10 | 72.605 | 9.93 | 12.51 |
| BT5 | 2014 | cnu_m220a-500--ENA23-200 | A6 | 61.628 | 5.0 | 10.12 |

been reported to be caused by the type of markers, number of individuals, number of markers, recombination frequency, LOD values, and the software employed (Gosselin et al., 2002). The density of marks in the linkage map in the present study is more lower than the maps of Xiaowu et al. (2011) and Lei et al. (2018), so it needs to add marks to this linkage map for further research.

In total, five QTL affecting bolting time were identified in this study. The QTL BT5 near the marker cnu_m220a in A6 is similar with the qFT6.1 in *B. rapa* L. (Yating et al., 2016), it is a new QTL or the same QTL, need further verification. There is no similarity of the other four QTLs BT1, BT2, BT3 and BT4 with the previous studies (Jonathan et al., 1995; Hidetoshi et al., 2001; Yating et al., 2016) ,they may be new QTLs and subject to be further verify. The number of loci influencing bolting is different with previous genetic analyses (Jonathan H et al., 1995; Hidetoshi et al., 2001), that mostly attributable to different population, number of individuals, number of markers, and so on. In the future, a common linkage map will be employed to comparatively assay these QTLs for the elucidation of the genetics of bolting in *Brassica* crops. Moreover, it might make a contribution to the breeding of novel cultivars with controlled bolting.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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**Supplementary Table 1.** Primers for SSR, ESTP and SRAP marker assays.

| Marker assay name | Assay type | Forward primer | Reverse primer |
|---|---|---|---|
| KBRH138G23 | SSR | TTTGACATCGTGCAATGCTA | TTGGGCTGGTCCTGAAGATA |
| KBRH139B23 | SSR | ATCTCATGGTTGGTTCACCG | ATTTCCAAAACACACACGCA |
| KBRH143D22 | SSR | GATGTGATACTTTGGCGACGG | TGAAGGATAATATGGTCTTGGCC |
| KBRH143F19 | SSR | GCATGCAAGCTTGGAACTGAT | CAGTCACGCTTTCTGACGAAAA |
| KBRH143H15 | SSR | TCTGCATCAAAATGCTAAAATGA | TGATCTTTTAGAAACAAAGATCGAG |
| KBRH143K20 | SSR | CAAATGTCTCAAGACACATAAACCA | CTAAAGCAGCAATTGGGTGTTC |
| KBRH048O11 | SSR | GCCTCTACCTGGCTTCAGCA | TCATTTGGCGCATACTTCCA |
| EJU3 | SSR | CCTCTTTTAATTCAAACAAGAAATCA | TTCGGACAATGGCAGTGATA |
| EJU5 | SSR | GGCACGTACATGGAGGATTC | TGTTGGTCGAGCTGTTTCAG |
| ENA17 | SSR | CAGTTATTTCGCCTCGTCT | TATTTGTGGTCTGTTATTGGA |
| ENA18 | SSR | TTAAAATGAAACCCACCCGA | TGTTGGGCAACATCCATTTA |
| ENA23 | SSR | GCTGTGCCAGTTCCTCTTTC | TCATTCCAAATGGCCTTACC |
| ENA28 | SSR | GGAGTCCGAGCGTTATGAAT | CTTCATCGACCCACCTTGTT |
| ENA4 | SSR | ACTTCTCTTTATTCACTTCCCA | GAGGGTGGTTGGTTCATT |
| ENA6 | SSR | CTCGTCTTCTTCACCTACAAC | CTGACATCTTTCTCACCCAC |
| cnu_m008a | SSR | GTTGCTGGGCTTGCAGTTAT | GAGCGTACCAGCAACCTCTC |
| cnu_m016a | SSR | GGTGAATGGAATCTTGTCTTGA | CCCAACAATCCCAGAAACAC |
| cnu_m020a | SSR | GGCTCTCCTCATCGTCAAAA | AATTCCGATTGCGACAAAAC |
| cnu_m029a | SSR | TACCCATTGGTGTCCTCCAG | TCGTTCTCGAATGTGAATTGTC |
| cnu_m030a | SSR | GAAACAAATTATTTAAAAATCAGACCA | TGGAACAATCCGTAAAACTATGC |
| cnu_m034a | SSR | TCACCGCCATAATTTGATCC | CCCTCTCAACAAGGTATGCAA |
| cnu_m037a | SSR | CCTAGTTCCTTGCACTCATGC | TTGTCTTTCAGATTGAAAACTTCG |
| cnu_m038a | SSR | GGCATGTGTCAATGAGTTGG | CTCCCACTCCTCCATTCAAC |
| cnu_m044a | SSR | TGTTTTGATCTTTACTGTTTTTGGA | AATGTTTTTATATCACTATTGCCAAAT |
| cnu_m046a | SSR | GCTAAAGGTTTAGTCCAAATAGGATTC | GCAAAATGATGCCCCATAAA |
| cnu_m050a | SSR | AGCCCAAGCTCGTATTCCTT | AAAATCGGGACAACCACCTA |
| cnu_m052a | SSR | GGAATCCTACGGAAGAGCAA | AAGGTAACGGTGGCAGTGAG |
| cnu_m062a | SSR | ATCGGCGCTGGTTATGTCA | CTAGGCTGCCCTTTCCGATT |
| cnu_m068a | SSR | CCATATGACTAACTTGACACTTTTGAA | TTCCCGGAAAGTCTTCTTGG |
| cnu_m073a | SSR | TGGCATTGACAGAGCTAGTA | TTTATTTAGTTTCATACCCT |
| cnu_m090a | SSR | GCAAAGATCGGCGAAGAAGA | TGCAGACACATTCGAACAAACA |
| cnu_m098a | SSR | TGCGACCCAAGTAGGTGAAAC | TGTCTCTCGCTCATTCATCCAA |
| cnu_m100a | SSR | AAAGTTCACACAAATGATTTTGATATT | TTTTCTAGGAATGGTCCAAACTT |
| cnu_m114a | SSR | AGTCGGAGGAAACGCGAAATTA | CGAAATAAAGACAGACAGAGACATCCA |
| cnu_m119a | SSR | ACACCTACTTGTTTCCATCCAAAT | CGGGTATTTGCGTTGTTTCC |
| cnu_m132a | SSR | CCATGGCCTCTCGTATTGCT | CCAACGGAGTGTTCCCAATC |
| cnu_m139a | SSR | TCAAGCGCAACAAACATTGG | TGGTGTTAGGGTTTAAGGTTGTGG |
| cnu_m142a | SSR | GACCTTCGGTTCAGGGTATGG | CTGAACGGTCAATTTGTTTGG |
| cnu_m146a | SSR | TCATACCAACGTGCTTTGAAGA | GTGTGGCCGGATCTGATCTA |
| cnu_m148a | SSR | CACAAGCATTCTACCATAGCAAAGTC | TGCACATATGGCATGTTGTTTG |
| cnu_m149a | SSR | GGAAGCCTCTGTGCGAAAAA | TGCCGACGATTTGATAGAGGA |
| cnu_m157a | SSR | CCGCAGTTGATCCATTAGCC | ACGCTGCATCCACATGAAAC |
| cnu_m172a | SSR | GGAATGGAACACCGGATTAGC | TCGGATCTGATTTGTCGGATTT |
| cnu_m173a | SSR | TGTATTCCATTATTTCCGACTAACCT | CCGCATTTTAAAAACGTGAGAAA |
| cnu_m179a | SSR | TGGTTACACCTAGTTCCTTGCACTC | GGCCTTTGCCCGTTTAGTTTTA |
| cnu_m182a | SSR | TTCATCACCGTCTTATGTTGTGC | GGCAGGTGGAATATGTGGAAAT |
| cnu_m207a | SSR | GGACCCGGAATACCTCAAAAGA | CATCAATAGCTCCGACACAATCC |
| cnu_m211a | SSR | TGTAAAGTTGTGCAAGGATTGTG | TGGGTTTGTGAAAATATGGTGAAA |
| cnu_m215a | SSR | CCAACCATTTCGTTAGTCAACC | TTACGCATGTACCTGCACTAAAAA |
| cnu_m220a | SSR | ATCAGAACCGAATCCGACCA | CAATGGTTGCAATGTTATTTGGA |

**Supplementary Table 1.** Contd.

| | | | |
|---|---|---|---|
| cnu_m225a | SSR | TTGCGTTTTCTCGTCGTCAA | CCCCGAGATAAATGGCACAC |
| cnu_m241a | SSR | AATGCTGTGTCCATGACCAA | CGGGCATCCACCTAATTTGT |
| cnu_m246a | SSR | AAAGCCATCCATCCATCAAGC | GATGCAACATTTGACTGTGTTAGAGC |
| cnu_m252a | SSR | TGAAAATCAACACGAACACACAGA | CTCGTGGGGGAATGAGTGAG |
| cnu_m254a | SSR | AAGCTTGAGCTTCCAGCCTTC | ATCAGTGCCGGCCTTGAATA |
| cnu_m256a | SSR | TTGAAATACATGATACCCCAACCA | CCGTTTTCAGGGCACAGTTT |
| cnu_m257a | SSR | TGCATGATGTTCATGTCTTGTAAA | TCCTTCTGTAAACCGGTTGTAATTT |
| cnu_m263a | SSR | GAGGAAGTACGGCAAGAAACCA | AGGACACATGTCCACATGAAAA |
| cnu_m268a | SSR | TCATTGGTGAAGAACCCACAAA | GCGACCATAAAAAGAGAGTGAGAA |
| cnu_m273a | SSR | ATAAGGGCATCGCCTCAACA | TGCACGCATCCACATAAACA |
| cnu_m277a | SSR | GCCATGAGCATTCGGTTAGG | TGAACTCTGGTTGGATTGACGA |
| cnu_m280a | SSR | TGTTACCACAGGAACCGTTCAA | CTTGGGCACACCATCATCTG |
| cnu_m284a | SSR | TCGGTTAAATCGAGTAACGATG | TTTCAGGACCTAGACGTTACCAA |
| cnu_m286a | SSR | AGTTGCCCCTATTCATGCAC | AATGCGTTCATGTGGGGATA |
| cnu_m288a | SSR | GCGTTTCGTCCTCTTCTCAC | TTACCCACCTTGGCTTCATC |
| cnu_m289a | SSR | CCCCTGGACTCCGTTTATCT | GATCTACGACGATCGGATGC |
| cnu_m293a | SSR | AAAAAGAAATGGATATTGTGTGAAA | CCTGGATCAAGACCACGAAG |
| cnu_m295a | SSR | GCTGCCTAATAGGGTGCTTG | AGAGCGCATTCAAGTCTGGT |
| cnu_m296a | SSR | TCTCGTCGCTCTGAATTGTG | TTGTGAAATCAAAGCAAAAAGG |
| cnu_m300a | SSR | AATTAGCGCGATAACATAAATAAAAA | AAATTGCTTAACTATTAAAACTGCAAA |
| cnu_m308a | SSR | GTTTGGGCCATCATGAAAAA | TGGTTGCAAAATGTCACAGAA |
| cnu_m310a | SSR | GGCAGGTGGAATATGTGGAA | GCACTATCATCATCAAACAGAACA |
| cnu_m316a | SSR | TCAAGCATGTCCTTAAAACTCTGA | GCGTTCACGTTTCCCATATC |
| cnu_m318a | SSR | TTATCAACATATTTTCAATCATTCCA | GCTTTGGACTATGCTTCTAAAGTACG |
| cnu_m320a | SSR | TTTTTCCTTTGGCTTAAACTGA | GCCAAAGCCACAAGATAACAT |
| cnu_m321a | SSR | TTGAATAATGACCCCAAATATCA | TCAATAGGTATTAACCAATTCTACCG |
| cnu_m324a | SSR | TTTTCAACTCCCACATGCAC | TGGGTATGTGCCAAATTGTTT |
| cnu_m327a | SSR | TTCTTGACCAAAAGAATCATGG | CTAACACGGGGAAAAGCAGA |
| cnu_m332a | SSR | TCGAACCGAAGTAAATAACGGACT | TTTCGCCCACTGACGCTATT |
| cnu_m338a | SSR | GCAACGATGAATCCCTAAACGA | AAATCCTCCCACTGTTTCCGAT |
| cnu_m344a | SSR | CCCAAATACGAAAACAAAGTTTGAC | AGGATCTCATCCGCTTTCCA |
| cnu_m354a | SSR | AAAGAAAACAAAGTGTCATTGTCTCA | TCTACCGGTTGAACCAGAGTTTTT |
| cnu_m356a | SSR | CGCATTTTCGCCGTCATTA | ACATCAGGCCGTCCCACTAA |
| cnu_m360a | SSR | ATCAGTGCCGGCCTTGAATA | AAGCTTGAGCTTCCAGCCTTC |
| cnu_m362a | SSR | CCTCTGCTGAAGGAGGCAAA | AGGTGGCTCTAGCGGAAGGT |
| cnu_m364a | SSR | ACCTGCCACCCTGTCAAAAC | GCACTAACCGTCCCTCTCCTC |
| cnu_m371a | SSR | TTTTTGGGTTTCTTCTCAAAATGC | ACTCCAGCGAATTTGGCTTT |
| cnu_m372a | SSR | CCAGTGGCCAATACGAAACC | TGATGGAGAGTGGGTTGTGC |
| cnu_m377a | SSR | TCAGTTGTCGGATCGTCTATG | CACTTATCTTTCCTTTGAAGTTGTTG |
| cnu_m379a | SSR | ACACCACTAAAACATTGCCATA | ACCGAAGGAGACTGCAAAGA |
| cnu_m384a | SSR | TGAAGGTGATGATGACGATGA | TCATGGTCTACAAAGACATACGG |
| cnu_m396a | SSR | TCATCATTAAAATGAGTTAAAATTCG | TTTTGGTGATCTTTTCTAAATTTTTC |
| cnu_m397a | SSR | TCTTCAAGTCAAAATACTCACATTCA | AAACGACAAATACATATGACAGTTTTA |
| cnu_m398a | SSR | TGACATTCGCATCAGATTTGT | TTGGGCTTCACGCATAAGAT |
| cnu_m400a | SSR | CGAGTTTTTGTGTGTACGTATAGTAAT | CCAAAGTGCGTAAAGGAAGG |
| cnu_m402a | SSR | GCCGACTCCTAGTGAGGAAA | TGTGTTTTGGGCTCAAAGGT |
| cnu_m409a | SSR | TTCCGGTCACTTCTAGCTTCA | TTTTGGTGGTTAGTATGTCGCTAT |
| cnu_m416a | SSR | TGGTGGGTCGTAACAGATGA | GCTCGCTTCCCAAATATGAA |
| cnu_m425a | SSR | TCGTTTGACCAACCGTACAA | CTTGCCAGCGTTGATACAGA |
| cnu_m439a | SSR | CCCTACGGACGGATGAGTAA | TCTGAGTGGCACCAGCATTA |
| cnu_m442a | SSR | CGATTTGGACAATGACTAGTGG | AACGCCATGGAAACAGAAAC |
| cnu_m446a | SSR | CACGTACGTCTTGGATGAATAAA | ATCTCACGTGGAGCACCATT |

**Supplementary Table 1.** Contd.

| | | | |
|---|---|---|---|
| cnu_m457a | SSR | CTGCTCCTTCACGTTTTCATCA | ACGGACAGCAACAACAACAAGA |
| cnu_m458a | SSR | GGGGTGAATCTTGGATGAGG | CTGACGGATTCCCAACGAAT |
| cnu_m459a | SSR | CAAAGCCGGATTTCTTTTAGCA | TTTAAAAGTATTCTAACAAATCCGTTG |
| E039 | ESTP | CTTGAGTGCTCAGGTCAAAGC | GAACCCTTACCCCCAAGACTAC |
| E120 | ESTP | ATCATAACCCTCAGGTTTGACATC | ACATCAAGCTCCTCTCTGGGTA |
| E129 | ESTP | AGATGGTAAAAGAGCACAAGCC | TTCAAGCTACCGATCCAACTG |
| E138 | ESTP | TGCTATCACAGTAGGGATTGCTT | CACTCCCACTCCTCCTAGTCC |
| atpm1 | SRAP | CTCTTGGTGATTTCAGCCAC | |
| bg10 | SRAP | CGTTTCTTCTCGCATTTCTC | |
| bg12 | SRAP | TCTAAGACCTCCACAGTAAG | |
| bg14 | SRAP | GCGTGGAAGCTGGAAGTCAAC | |
| bg16 | SRAP | TGATACCACTTGCGATACCA | |
| bg17 | SRAP | TGGTATCGCAAGTGGTATCA | |
| bg18 | SRAP | GCAAGTCTCTCAGGTTATTC | |
| bg19 | SRAP | GCTCTTCATCAGTTCTTGGT | |
| bg2 | SRAP | GACCAAATATAAAACACTAACTA | |
| bg20 | SRAP | TCCTCTCCACTTTTGTCTTC | |
| bg21 | SRAP | AACTCGCTTGCTTAGATATG | |
| bg24 | SRAP | CACCTTTTCCTACTCCTATC | |
| bg3 | SRAP | GGAACACTTAATGGTACGGT | |
| bg42 | SRAP | ACACATAATCTTCTACAAATAC | |
| bg50 | SRAP | AAGTCGTTGTAGTATAGTGG | |
| me1 | SRAP | TGAGTCCAAACCGGATA | |
| me2 | SRAP | TGAGTCCAAACCGGAGC | |
| me3 | SRAP | TGAGTCCAAACCGGAAT | |
| me4 | SRAP | TGAGTCCAAACCGGACC | |
| me5 | SRAP | TGAGTCCAAACCGGAAG | |
| me6 | SRAP | TGAGTCCAAACCGGACA | |
| me7 | SRAP | TGAGTCCTTTCCGGTAA | |
| me8 | SRAP | TGAGTCCAAACCGGACG | |
| me9 | SRAP | TGAGTCCTTTCCGGTCC | |
| me10 | SRAP | TGAGTCCAAACCGGACT | |
| me11 | SRAP | TGAGTCCTTTCCGGTGC | |
| me12 | SRAP | TGAGTCCAAACCGGTAG | |
| me13 | SRAP | TGAGTCCAAACCGGTCA | |
| me14 | SRAP | TGAGTCCAAACCGGTAA | |
| me15 | SRAP | TGAGTCCAAACCGGTGC | |
| me16 | SRAP | TGAGTCCAAACCGGAAC | |
| me17 | SRAP | TGAGTCCAAACCGGCAT | |
| em1 | SRAP | GACTGCGTACGAATTAAT | |
| em2 | SRAP | GACTGCGTACGAATTTGC | |
| em3 | SRAP | GACTGCGTACGAATTGAC | |
| em4 | SRAP | GACTGCGTACGAATTTGA | |
| em5 | SRAP | GACTGCGTACGAATTAAC | |
| em6 | SRAP | GACTGCGTACGAATTGCA | |
| em7 | SRAP | GACTGCGTACGAATTCAA | |
| em8 | SRAP | GACTGCGTACGAATTCAC | |
| em9 | SRAP | GACTGCGTACGAATTACG | |
| em10 | SRAP | GACTGCGTACGAATTGAT | |
| em11 | SRAP | GACTGCGTACGAATTATG | |
| em12 | SRAP | GACTGCGTACGAATTCGA | |
| em13 | SRAP | GACTGCGTACGAATTTAG | |

**Supplementary Table 1.** Contd.

| | | |
|---|---|---|
| em14 | SRAP | GACTGCGTACGAATTTCG |
| em15 | SRAP | GACTGCGTACGAATTGTC |
| em16 | SRAP | GACTGCGTACGAATTGGT |

**Supplementary Table 2.** Detail of the primers for detection of DNA polymorphism and assigned linkage group.

| Marker assay name | Assay type | Polymorphism | Assigned linkage group |
|---|---|---|---|
| KBRH138G23-700 | SSR | O | A3 |
| KBRH139B23 | SSR | × | × |
| KBRH143D22 | SSR | O | × |
| KBRH143F19 | SSR | × | × |
| KBRH143H15 | SSR | O | × |
| KBRH143K20-320 | SSR | O | A7 |
| KBRH048O11 | SSR | O | × |
| EJU3 | SSR | × | × |
| EJU5-130 | SSR | O | A6 |
| ENA17 | SSR | O | × |
| ENA18 | SSR | × | × |
| ENA23-200 | SSR | O | A6 |
| ENA28 | SSR | × | × |
| ENA4 | SSR | O | × |
| ENA6 | SSR | × | × |
| cnu_m008a | SSR | × | × |
| cnu_m016a | SSR | × | × |
| cnu_m020a | SSR | O | × |
| cnu_m029a-190 | SSR | O | A5 |
| cnu_m030a | SSR | × | × |
| cnu_m034a | SSR | × | × |
| cnu_m037a | SSR | × | × |
| cnu_m038a | SSR | × | × |
| cnu_m044a | SSR | × | × |
| cnu_m046a | SSR | × | × |
| cnu_m050a | SSR | × | × |
| cnu_m052a | SSR | × | × |
| cnu_m062a-200 | SSR | O | A7 |
| cnu_m068a | SSR | × | × |
| cnu_m073a | SSR | O | × |
| cnu_m090a | SSR | O | × |
| cnu_m098a | SSR | × | × |
| cnu_m100a-300 | SSR | O | A3 |
| cnu_m114a | SSR | × | × |
| cnu_m119a | SSR | × | × |
| cnu_m132a | SSR | × | × |
| cnu_m139a | SSR | O | × |
| cnu_m142a-180 | SSR | O | A1 |
| cnu_m146a | SSR | O | × |
| cnu_m148a | SSR | × | × |
| cnu_m149a | SSR | × | × |
| cnu_m157a | SSR | × | × |
| cnu_m172a | SSR | × | × |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| cnu_m173a | SSR | × | × |
| cnu_m179a | SSR | × | × |
| cnu_m182a-420 | SSR | O | A7 |
| cnu_m207a | SSR | × | × |
| cnu_m211a | SSR | O | × |
| cnu_m215a | SSR | × | × |
| cnu_m220a-500 | SSR | O | A6 |
| cnu_m225a | SSR | × | × |
| cnu_m241a | SSR | × | × |
| cnu_m246a | SSR | × | × |
| cnu_m252a | SSR | × | × |
| cnu_m254a | SSR | O | × |
| cnu_m256a | SSR | × | × |
| cnu_m257a-100 | SSR | O | A5 |
| cnu_m263a-360 | SSR | O | A9 |
| cnu_m268a | SSR | × | × |
| cnu_m273a | SSR | O | × |
| cnu_m277a | SSR | × | × |
| cnu_m280a | SSR | × | × |
| cnu_m284a | SSR | × | × |
| cnu_m286a | SSR | × | × |
| cnu_m288a | SSR | × | × |
| cnu_m289a-170 | SSR | O | A5 |
| cnu_m293a-180 | SSR | O | A5 |
| cnu_m295a | SSR | × | × |
| cnu_m296a | SSR | × | × |
| cnu_m300a-260 | SSR | O | A8 |
| cnu_m308a | SSR | × | × |
| cnu_m310a | SSR | O | × |
| cnu_m316a | SSR | × | × |
| cnu_m318a-320 | SSR | O | A6 |
| cnu_m320a | SSR | × | × |
| cnu_m321a | SSR | × | × |
| cnu_m324a | SSR | O | × |
| cnu_m327a | SSR | O | × |
| cnu_m332a | SSR | × | × |
| cnu_m338a | SSR | × | × |
| cnu_m344a-150 | SSR | O | A5 |
| cnu_m354a-400 | SSR | O | A7 |
| cnu_m356a | SSR | × | × |
| cnu_m360a | SSR | O | × |
| cnu_m362a-320 | SSR | O | A5 |
| cnu_m364a | SSR | × | × |
| cnu_m371a | SSR | × | × |
| cnu_m372a | SSR | × | × |
| cnu_m377a-730 | SSR | O | A9 |
| cnu_m379a | SSR | × | × |
| cnu_m384a | SSR | O | × |
| cnu_m396a | SSR | × | × |
| cnu_m397a | SSR | × | × |
| cnu_m398a | SSR | × | × |
| cnu_m400a | SSR | × | × |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| cnu_m402a | SSR | × | × |
| cnu_m409a | SSR | × | × |
| cnu_m416a | SSR | O | × |
| cnu_m425a-250 | SSR | O | A5 |
| cnu_m439a | SSR | × | × |
| cnu_m442a | SSR | × | × |
| cnu_m446a-230 | SSR | O | A5 |
| cnu_m457a | SSR | × | × |
| cnu_m458a-380 | SSR | O | A5 |
| cnu_m459a | SSR | × | × |
| hri_mBRMS082a-200 | SSR | O | A2 |
| E039-1000 | ESTP | O | A10 |
| E120-630 | ESTP | O | A8 |
| E129 | ESTP | × | × |
| E138 | ESTP | O | × |
| bg50me10-170 | SRAP | O | A1 |
| em4bg10-180 | SRAP | O | A1 |
| me6bg19-140 | SRAP | O | A1 |
| em16me10-300 | SRAP | O | A1 |
| em3bg17-150 | SRAP | O | A1 |
| em4bg2-580 | SRAP | O | A1 |
| bg2bg21-320 | SRAP | O | A1 |
| em10me7-130 | SRAP | O | A1 |
| em7me5-160 | SRAP | O | A1 |
| em5bg19-340 | SRAP | O | A1 |
| em16me3-220 | SRAP | O | A2 |
| me9bg2-320 | SRAP | O | A2 |
| em10me8-250 | SRAP | O | A2 |
| em9me2-310 | SRAP | O | A2 |
| em7bg20-160 | SRAP | O | A2 |
| me11bg3-250 | SRAP | O | A2 |
| em8bg18-110 | SRAP | O | A2 |
| me1bg16-270 | SRAP | O | A2 |
| em10me4-200 | SRAP | O | A2 |
| em10me5-160 | SRAP | O | A2 |
| me13em16-200 | SRAP | O | A2 |
| me9bg18-140 | SRAP | O | A2 |
| bg19bg50-250 | SRAP | O | A3 |
| em5bg2-190 | SRAP | O | A3 |
| bg50me2-130 | SRAP | O | A3 |
| em7bg19-220 | SRAP | O | A3 |
| em8bg20-240 | SRAP | O | A3 |
| me11bg21-220 | SRAP | O | A3 |
| me6bg20-270 | SRAP | O | A3 |
| em2me2-620 | SRAP | O | A3 |
| em4bg24-590 | SRAP | O | A3 |
| bg12bg19-380 | SRAP | O | A3 |
| me17bg20-300 | SRAP | O | A3 |
| me6bg24-240 | SRAP | O | A4 |
| em9me1-750 | SRAP | O | A4 |
| bg2bg50-320 | SRAP | O | A4 |
| me1bg12-750 | SRAP | O | A4 |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| me13em1-100 | SRAP | O | A4 |
| me13em5-340 | SRAP | O | A4 |
| me13bg18-160 | SRAP | O | A4 |
| me10bg18-270 | SRAP | O | A4 |
| bg20bg3-190 | SRAP | O | A4 |
| me3bg12-570 | SRAP | O | A4 |
| bg14bg3-130 | SRAP | O | A4 |
| em4me3-450 | SRAP | O | A4 |
| em2bg17-180 | SRAP | O | A4 |
| em5me12-330 | SRAP | O | A5 |
| me2bg20-240 | SRAP | O | A5 |
| me12atpm1-120 | SRAP | O | A5 |
| me10bg3-180 | SRAP | O | A5 |
| me5bg24-200 | SRAP | O | A5 |
| bg50me12-500 | SRAP | O | A5 |
| bg19bg24-200 | SRAP | O | A5 |
| me13bg21-140 | SRAP | O | A5 |
| em5bg21-340 | SRAP | O | A6 |
| me16bg20-200 | SRAP | O | A6 |
| me16bg16-130 | SRAP | O | A6 |
| me5bg2-150 | SRAP | O | A6 |
| em7bg12-240 | SRAP | O | A6 |
| me12bg24-210 | SRAP | O | A6 |
| me17bg10-110 | SRAP | O | A6 |
| em4bg21-170 | SRAP | O | A6 |
| me12bg19-220 | SRAP | O | A6 |
| me14bg17-200 | SRAP | O | A6 |
| em10me11-370 | SRAP | O | A6 |
| em1bg12-170 | SRAP | O | A6 |
| bg19bg20-170 | SRAP | O | A6 |
| bg2bg24-380 | SRAP | O | A6 |
| me5bg12-240 | SRAP | O | A6 |
| me4bg12-150 | SRAP | O | A6 |
| em8bg10-150 | SRAP | O | A7 |
| me2bg24-260 | SRAP | O | A7 |
| em15me1-570 | SRAP | O | A7 |
| em2bg16-300 | SRAP | O | A7 |
| me12bg2-480 | SRAP | O | A7 |
| em2me1-640 | SRAP | O | A7 |
| em14me4-260 | SRAP | O | A7 |
| bg24bg50-250 | SRAP | O | A7 |
| me14bg14-110 | SRAP | O | A7 |
| me9bg20-310 | SRAP | O | A7 |
| me8bg3-180 | SRAP | O | A7 |
| me13em15-190 | SRAP | O | A7 |
| bg18bg21-380 | SRAP | O | A7 |
| me13em4-240 | SRAP | O | A8 |
| em9me4-240 | SRAP | O | A8 |
| me10bg10-210 | SRAP | O | A8 |
| me9bg10-190 | SRAP | O | A8 |
| em3bg19-110 | SRAP | O | A8 |
| em3me2-120 | SRAP | O | A8 |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| me15bg20-320 | SRAP | O | A8 |
| bg20bg21-150 | SRAP | O | A8 |
| me7bg2-100 | SRAP | O | A8 |
| em3bg10-140 | SRAP | O | A9 |
| em5bg17-260 | SRAP | O | A9 |
| em4bg12-150 | SRAP | O | A10 |
| me7bg18-200 | SRAP | O | A10 |
| me10bg14-200 | SRAP | O | A10 |
| me6atpm1-240 | SRAP | O | A10 |
| bg19bg42-250 | SRAP | O | A10 |
| me13em7-250 | SRAP | O | A10 |
| em5me11-370 | SRAP | O | A10 |
| me4bg20-150 | SRAP | O | A10 |
| me4bg20-240 | SRAP | O | A10 |
| e10me10-180 | SRAP | O | A10 |
| bg21bg42-370 | SRAP | O | A10 |
| em2me3-160 | SRAP | O | A10 |
| em3atpm1-150 | SRAP | O | A10 |
| em8bg19-130 | SRAP | O | × |
| me12bg10-240 | SRAP | O | × |
| em1bg10-260 | SRAP | O | × |
| bg12bg50-370 | SRAP | O | × |
| me14bg10-240 | SRAP | O | × |
| me17bg24-330 | SRAP | O | × |
| me5bg12-270 | SRAP | O | × |
| bg18bg20-120 | SRAP | O | × |
| me3bg18-140 | SRAP | O | × |
| me14bg21-190 | SRAP | O | × |
| bg2bg42-270 | SRAP | O | × |
| me1atpm1-160 | SRAP | O | × |
| me11bg18-330 | SRAP | O | × |

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# Sensory evaluation of coffee cultivars in the Campo das Vertentes Mesoregion, Minas Gerais

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**The objective of this study was to evaluate the sensory characteristics of coffees from *Coffea arabica* L. groups, in municipalities located in the Campo das Vertentes mesoregion, in the State of Minas Gerais. Natural and parchment coffees, after drying, consisted of 100% mature fruits. The experiment was carried out on 14 coffee farms located in the municipalities of Carmo da Mata, Oliveira, Santo Antônio do Amparo and Bom Sucesso, in the 2016/17 agricultural crop. In order to conduct the study, coffee fruit collections of nine groups of commercial cultivars were submitted to two types of post-harvest processing, known as "terrarium nut" and "parchment", combinations that resulted in 250 samples. Sensory analysis was performed according to the protocol of the Specialty Coffee Association - SCA, with panelists accredited by the Coffee Quality Institute - CQI. The natural post-harvest processing (terrarium nut) showed higher scores for most groups of cultivars when compared to parchment processing. Cultivars Topázio, Bourbon Amarelo, Catucaí Amarelo, Icatu Amarelo and Icatu Vermelho stood out with the highest averages for all sensory attributes.**

**Key words:** *Coffee arabica* L., sensory analysis, Specialty Coffee Association (SCA), quality.

## INTRODUCTION

Understanding, making viable and improving evaluations is some of the technical requirements used in coffee growing that can aid producers in trade competitiveness. Producing economically viable coffees, coupled with preservation of sensory quality and physical aspects can be an indispensable tool in improving batch pricing. Producers have increasingly sought to know the quality of their coffees, contributing to crop management, pre-harvest, harvest, post-harvest and especially commercialization decisions.

Considering studies on climate change, it is suggested that higher altitudes may become one of the prime factors for the production of quality coffees, as mild temperatures allow slower fruit maturation and, consequently, the accumulation of flavor precursors in the beverage (Worku et al., 2018). This statement may depend on the expression of the sensory qualities of coffee cultivars, according to the planting implantation altitude (Ribeiro et al., 2019).

Increasing efforts by coffee-consuming countries to

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**Table 1.** Edaphoclimatic characteristics of the municipalities of Santo Antônio do Amparo, Bom Sucesso, Carmo da Mata and Oliveira, in the State of Minas Gerais-Brazil.

| Municipality | Climate (Köppen-Geiger classification: Cwa) | Average altitude (m) | Coordinate |
|---|---|---|---|
| Santo Antônio do Amparo | Humid subtropical | 935 | Latitude: -20.9552, Longitude: -44.9167 20° 57' 19" South, 44° 55' 0" West |
| Bom Sucesso | Humid subtropical | 930 | Latitude: -21.0336, Longitude: -44.7526 21° 2' 1" South, 44° 45' 9" West |
| Carmo da Mata | Humid subtropical | 800 | Latitude: -20.5559, Longitude: -44.8625 20° 33' 21" South, 44° 51' 45" West |
| Oliveira | Humid subtropical | 988 | Latitude: -20.6971, Longitude: -44.8278 20° 41' 50" South, 44° 49' 40" West |

Source: http://pt.db-city.com/2018.

understand the "sensory identity", production traceability and collaboration of the coffee producing region on coffee quality, have moved producing countries to produce more single-source batches of very high quality, corroborating transparency in commercial transactions and, consequently, value addition and regional recognition (Smrke et al., 2015).

The quality of the coffee from the Campo das Vertentes - MG Mesoregion, located in the Southeast region of Brazil, has been a national and international highlight among buyers, consumers and connoisseurs. Understanding the coffee quality potential of cultivars from this region can bring many benefits, contributing to geographical indication processes, enabling post-harvest techniques, and improving communication mechanisms. One of the ways to evaluate coffee quality is by the SCA (Specialty Coffee Association) sensory analysis, which allows a quantitative and qualitative description of the beverage. It is one of the most respected analyses in the world conducted by CQI (Coffee Quality Institute) trained and accredited panelists, who delineate trade guidelines between specialty coffees or commodities, sensory particularities of batches, as well as an open and descriptive communication about their potential.

Thus, this study aimed to evaluate the sensory attributes of nine arabica coffee groups, implanted in municipalities located in the Campo das Vertentes Mesoregion in the State of Minas Gerais and to characterize the qualitative contributions of different post-harvest processing methods, wet and dry. The sensory profiles were described by accredited panelists for use of the Specialty Coffee Association - SCA protocol.

## MATERIALS AND METHODS

The study was carried out in the State of Minas Gerais - Brazil, in the region called Campo das Vertentes, comprising 14 coffee farms distributed in the municipalities of Santo Antônio do Amparo, Bom Sucesso, Carmo da Mata and Oliveira (Table 1).

First, the boundary space of each crop was delimited, respecting three initial rows after the "carrier" and three plants in the row to start the collection; the quadrant for the collection was a maximum of 5 ha crop. The ripe fruits were harvested manually and selectively in "zig and zag" in the middle and upper third of the plants, totaling approximately 25% of the harvested crop area, for the composition of each sample. After the fruits were collected, the samples were reviewed and the remaining green fruits were removed. It is noteworthy that the collections were planned according to the natural maturation of the crops, without the application of ripeners. Each sample resulted in 20 L of ripe fruits, emphasizing that, on farms that adopt the wet processing 10 L of coffee were peeled to comprise the wet sampling and the other 10 L for the dry route. For farms that have only dry processing, 10 L of ripe fruits were collected.

Commercial cultivars of the species *Coffea arabica* L. belonging to nine groups were evaluated; these are genetic materials widely found in the producing regions of Brazil. The cultivar groups under study are: Acaiá, Bourbon Amarelo, Catuaí Vermelho, Catuaí Amarelo, Catucaí Amarelo, Icatu Amarelo, Icatu Vermelho, Mundo Novo and Topázio.

For sample drying, wooden frames with 0.80 x 0.80 cm shading screens were used, and the entire procedure was performed on the properties that participated in the project. The drying layer for natural coffees was "fruit to fruit" in the early days, until the husk darkened. For parchment coffees, the procedures of 3 cm thick endocarp bean layer ("parchment coffee") were adopted at the beginning of drying, with 4 daily revolves until the remaining mucilage on the parchment was dry. Subsequently, the drying layer was thickened to 6 cm, the samples were rolled 8 times a day and the cover was received.

All samples were covered at about 3.30 p.m. to take advantage of the heat retained in the fruit mass, during the day, at night and discovered at 8 a.m., to avoid the absorption of local moisture by the fruits, consisting of raffia bag (for retention of condensed water by the fruit mass) and canvas (thermal insulation), both clean.

These procedures were followed until the coffee samples reached a humidity of 11 to 12% (b.u), measured by a G939 STD Gehaka equipment. The samples carefully benefited from the Coffee Post Harvest Technology Pole – Universidade Federal de Lavras - UFLA.

For the sensory analyses, only the beans retained in the 16/64-inch sieve were used. All extrinsic and intrinsic defects were removed, in addition to the mocha beans, for a better sample roasting uniformity.

The sensory analyses were performed in the 2016/17 agricultural season, by two accredited expert panelists, to evaluate specialty coffees (Q-Graders), using the methodology proposed by the Specialty Coffee Association - SCA (Lingle, 2011). The analyzed samples were roasted within 24 h in a Probat TP2-Leogap roaster prior to tasting for $CO_2$ release, with a color profile ranging from #58 for roasted beans to #63 for roasted and ground beans, verified by the Mbasic Agtron equipment.

Roasting time was conducted from 8 to 12 min, with an average roasting time between samples of 9 min and 15 s. Importantly, after the roasting process, the samples were immediately cooled exclusively by air and stored in airtight containers for 24 h until tasting. The beans called “quakers” were removed from the roasted sample due to their depreciating characteristics to the sensory attributes.

In addition to the roasting profile, the SCA sensory evaluation form enabled the verification of 10 important attributes with scales and intensities: fragrance, flavor, finish, acidity, body, balance, uniformity, clean cup, sweetness and overall impression.

According to the sensory evaluation form of the SCA, the accredited panelist determined the different sensory characteristics among the different samples, besides describing the scores of the identified flavors. This becomes a valuable tool for targeting the different quality standards of coffees from around the world. The evaluation form consists of the attributes fragrance/aroma, taste, finish, acidity, body, balance and overall impression, with ¼ point (0.25) between the numerical values “6” and “10”, and “0” to “10” for uniformity, clean cup and sweetness; both units represent the construction of coffee quality levels.

The following sensory attributes were evaluated: Aroma/ Fragrance, Flavor, Finish, Body, Acidity, Balance, Uniformity between cups, Absence of defects, Sweetness and Overall Impression. The sum of the scores obtained in the attributes resulted in the final scores.

Initially, descriptive statistics of the sample scores were obtained, as well as the detailed description of the data. In addition, a histogram of the total sample scores was obtained to demonstrate the frequency of the final scores, besides the survey of the minimum and total maximum scores for all cultivar groups.

Variance analyses were performed for each of the evaluated attributes. The effects of panelist, municipality, property, cultivar group and post-harvest processing were considered. In these analyses, the panelist effect was considered as random and the others as fixed.

After analysis of variance, if the F test for the cultivar groups were significant, the Tukey test, considering 5% significance, was applied to identify the cultivar groups that presented higher average scores. The processes that guaranteed higher average scores of each cultivar group were also identified. However, in the latter case, the F test is conclusive, and it is not necessary to use another means test, such as Tukey, since there are only two different processing forms. Statistical analyses were performed using the R software (R Core Team, 2017).

Principal component analysis - PCA was performed using the PAST3 software, with the grouping performed by the k-mean analysis using the Euclidean distance (Hammer et al., 2001).

## RESULTS AND DISCUSSION

The scores given for the characteristics uniformity between cups, absence of defects and sweetness were identical and corresponded to the total score (10) for all samples, as the panelists did not find any differences between the coffees and/or depreciation. Thus, the use statistical analyses would not be justified in this case. This finding highlights the seriousness of proper management regarding the collection and processing of coffee samples.

Table 2 presents the sensory behavior of coffee from the analyzed cultivars regarding the attributes fragrance, finish and flavor.

Topázio, Bourbon Amarelo and Catucaí Amarelo cultivars got the highest scores for fragrance, highlighting the cultivar groups Icatu Amarelo, Icatu Vermelho and Catuaí Vermelho (Table 2).

According to Bhumiratana et al. (2011), coffee aromas are probably one of the most important factors in differentiating quality. Cultivars Acaiá, Catuaí Amarelo and Mundo Novo had the lowest averages. The volatile compounds present in coffee represent a signature of the biochemical composition of coffee beans and their development in the plant (Bertrand et al., 2012).

For flavor, only ‘Mundo Novo’ differed statistically by the Tukey test (Table 2). Flavor characterizes what coffee has in its potential and is one of the attributes of greater weight in judging by verifying basic to complex flavors, their intensity and quality.

Cheng et al. (2016) emphasized that, although many studies have been conducted on the influence of genotypes and environments on coffee quality, this is a highly complex process. Through the analysis of averages among the post-harvest processing methods, the statistical difference for ‘Mundo Novo’ is possibly attributed to lower scores obtained by the parchment processing since, for the natural processing (Table 3), coffees had higher scores. It is observed that the sensory expression of coffees can change between harvests, due to several edaphoclimatic factors affecting crops.

Salla (2009) reports that there is a strong interference of genetic constitution in flavor determination. The author evaluated the effects of post-harvest processing methods, genotypes and production environments on coffee quality. The sensory quality of coffee cultivars varied as a function of processing and cultivation environment. However, some cultivars showed high genetic stability for beverage quality in all cultivation sites and different post-harvest processing methods.

For finish and acidity, cultivars Icatu Amarelo, Topázio, Icatu Vermelho, Bourbon Amarelo, Catucaí Amarelo and Acaiá had the highest averages, differing statistically from the other cultivars (Table 2). Fruit coloration, when ripe, may be possibly related to the sensory quality of the coffee. Studies under different environmental conditions have found better sensory quality in yellow fruit cultivars (Ribeiro et al., 2019).

Ribeiro (2013) evaluated the effect of genotype, altitude, slope and processing methods of cultivars Bourbon Amarelo and Acaiá on the relationship between

**Table 2.** Mean Fragrance and Flavor of coffee beverages as a function of cultivar groups evaluated in the Campo das Vertentes Mesoregion, in the state of Minas Gerais.

| Cultivar | Attribute | | | | | |
|---|---|---|---|---|---|---|
| | Fragrance | Flavor | Finish | Acidity | Overall score | Total score |
| Topázio | $7.77^{A}$ | $7.40^{A}$ | $7.24^{A}$ | $7.33^{A}$ | $7.45^{A}$ | $81.68^{A}$ |
| Bourbon Amarelo | $7.73^{A}$ | $7.38^{A}$ | $7.22^{A}$ | $7.25^{A}$ | $7.42^{A}$ | $81.69^{A}$ |
| Catucaí Amarelo | $7.68^{A}$ | $7.35^{A}$ | $7.21^{A}$ | $7.21^{A}$ | $7.38^{AB}$ | $81.50^{AB}$ |
| Icatu Amarelo | $7.56^{AB}$ | $7.39^{A}$ | $7.20^{A}$ | $7.28^{A}$ | $7.25^{AB}$ | $81.26^{ABC}$ |
| Icatu Vermelho | $7.52^{AB}$ | $7.41^{A}$ | $7.17^{A}$ | $7.27^{A}$ | $7.18^{ABC}$ | $81.28^{ABC}$ |
| Catuaí Vermelho | $7.52^{AB}$ | $7.18^{A}$ | $7.16^{A}$ | $7.24^{A}$ | $7.16^{ABC}$ | $80.28^{BCD}$ |
| Acaiá | $7.46^{B}$ | $7.20^{A}$ | $7.08^{AB}$ | $7.15^{AB}$ | $7.14^{BC}$ | $80.46^{BC}$ |
| Catuaí Amarelo | $7.44^{B}$ | $7.28^{A}$ | $6.95^{BC}$ | $7.11^{B}$ | $7.13^{BC}$ | $80.07^{CD}$ |
| Mundo Novo | $7.43^{B}$ | $6.96^{B}$ | $6.78^{C}$ | $7.04^{B}$ | $7.01^{C}$ | $79.35^{D}$ |
| Coefficient of variation (%) | 4.58 | 5.55 | 5.67 | 4.76 | 6.41 | 2.82 |

Means followed by the same letter in the column do not differ by the Tukey test ($p<0.05$).

**Table 3.** Mean fragrance, flavor, body, balance, overall and overall score of coffee beverages as a function of cultivar groups and post-harvest processing, parchment (CD) and natural, evaluated in the Campo das Vertentes Mesoregion, in the state of Minas Gerais.

| Cultivar | Attribute | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fragrance | | Flavor | | Body | | Balance | | Overall | | Overall score | |
| | Processing | | | | | | | | | | | |
| | CD | Natural | CD | Natural | CD | Natural | CD | Natural | CD | Natural | CD | Natural |
| Acaiá | $7.38^{A}$ | $7.55^{A}$ | $7.03^{B}$ | $7.35^{A}$ | $7.20^{B}$ | $7.42^{A}$ | $6.92^{B}$ | $7.19^{A}$ | $6.98^{B}$ | $7.32^{A}$ | $79.57^{B}$ | $81.36^{A}$ |
| Bourbon Amarelo | $7.53^{B}$ | $7.93^{A}$ | $6.99^{B}$ | $7.75^{A}$ | $7.17^{B}$ | $7.58^{A}$ | $7.08^{B}$ | $7.49^{A}$ | $7.17^{B}$ | $7.74^{A}$ | $79.74^{B}$ | $83.42^{A}$ |
| Catuaí Vermelho | $7.56^{A}$ | $7.68^{A}$ | $6.98^{B}$ | $7.38^{A}$ | $6.97^{B}$ | $7.42^{A}$ | $6.78^{B}$ | $7.23^{A}$ | $6.99^{A}$ | $7.28^{A}$ | $78.98^{B}$ | $81.35^{A}$ |
| Catuaí Amarelo | $7.22^{B}$ | $7.55^{A}$ | $7.11^{A}$ | $7.43^{A}$ | $7.04^{B}$ | $7.43^{A}$ | $6.96^{A}$ | $7.14^{A}$ | $7.08^{A}$ | $7.24^{A}$ | $79.74^{A}$ | $80.92^{A}$ |
| Catucaí Amarelo | $7.59^{B}$ | $7.78^{A}$ | $7.22^{B}$ | $7.48^{A}$ | $7.29^{B}$ | $7.46^{A}$ | $7.12^{B}$ | $7.25^{A}$ | $7.15^{B}$ | $7.37^{A}$ | $80.74^{B}$ | $81.79^{A}$ |
| Icatu Amarelo | $7.36^{B}$ | $7.78^{A}$ | $7.05^{B}$ | $7.72^{A}$ | $7.10^{B}$ | $7.58^{A}$ | $7.06^{B}$ | $7.49^{A}$ | $7.21^{A}$ | $7.57^{A}$ | $79.85^{B}$ | $83.16^{A}$ |
| Icatu Vermelho | $7.51^{A}$ | $7.53^{A}$ | $7.53^{A}$ | $7.28^{A}$ | $7.06^{B}$ | $7.56^{A}$ | $7.36^{A}$ | $7.21^{A}$ | $7.12^{A}$ | $7.23^{A}$ | $81.42^{A}$ | $81.06^{A}$ |
| Mundo Novo | $7.33^{A}$ | $7.54^{A}$ | $6.75^{B}$ | $7.16^{A}$ | $7.05^{B}$ | $7.28^{A}$ | $6.99^{A}$ | $7.02^{A}$ | $6.97^{A}$ | $7.06^{A}$ | $78.66^{A}$ | $80.05^{A}$ |
| Topázio | $7.61^{B}$ | $7.93^{A}$ | $7.30^{B}$ | $7.49^{A}$ | $7.25^{B}$ | $7.56^{A}$ | $7.17^{A}$ | $7.38^{A}$ | $7.35^{A}$ | $7.49^{A}$ | $81.03^{A}$ | $82.36^{A}$ |
| Coefficient of variation (%) | 4.58 | | 5.55 | | 4.32 | | 4.87 | | 6.16 | | 2.68 | |

Means for each variable followed by the same letter in the line do not differ by the F test.

the levels of chemical compounds analyzed with the sensory quality of specialty coffees. The author reports that 'Bourbon Amarelo' obtained more expressive sensory scores at the highest altitude, referring to the crop location area.

For the sensory attribute body, there was no statistical difference when comparing the nine cultivar groups studied (Table 2). Climatic diversity can cause variations in coffee characteristics (acidity, body and aroma), even in regions that favor the production of specialty coffees (Avelino et al., 2005).

The consistency of panelists regarding the overall score was that cultivars Bourbon Amarelo and Topázio had the highest averages, followed by Icatu Amarelo, Catucaí Amarelo, Icatu Vermelho and Catuaí Amarelo (Table 2). For the total score, cultivars Bourbon Amarelo and Topázio also showed the highest averages, with 81.68 and 81.69 respectively. It is worth mentioning the contribution of fragrance to the final score, since most cultivar groups that have the highest scores for this attribute, obtained the highest final scores. This can be of great relevance, demonstrating that not always are the quality of acidity and body the precursors with the highest importance in the final score.

With a commercial approach, coffees with scores above 80 points are classified as specialty coffees, enabling better pricing. Strategically for producers, it is essential to perform a sensory evaluation in the batches so that there is no mixing of coffees with different qualities. It is important to note that even coffees with scores below 80 points can achieve good commercial placing on the market, as long as they meet buyers' preferred qualities or even in the face of seasonal fluctuations in coffee supply and demand and their qualities.

Due to its high yield and adaptability, cultivar Mundo Novo is widely cultivated in Brazil (Carvalho et al., 2006). However, it presented limitations in the production of specialty coffees when compared to Bourbon Amarelo genotypes, indicating that the quality of its production is dependent on the conditions of the cultivation environment (Figueiredo et al., 2018).

Table 3 presents the averages given by the coffee panelists for the nine groups of coffee cultivars, regarding the post-harvest parchment method, compared to the natural processes. It can be seen from the table that cultivar groups Acaiá, Catuaí Vermelho, Icatu Vermelho and Mundo Novo did not differ statistically in the two post-harvest processing methods for fragrance. The lowest averages assigned by the panelists for fragrance were for cultivars Bourbon Amarelo, Catuaí Amarelo, Catucaí Amarelo, Icatu Amarelo and Topázio, when processed wet (Table 3).

For flavor, most cultivars have higher averages when processed naturally at post-harvest. Only cultivars Catuaí Amarelo and Icatu Vermelho did not differ statistically between the processes (Table 3).

For body, the highest averages were attributed to all groups of naturally processed coffee cultivars (Table 3). An analogy between beverage body and seed germination studies may possibly contribute to the explanation of the results.

Bytof et al. (2007) reported that when peeling coffee fruits for seed production, there is accelerated energy expenditure in the endosperm for embryo germination. Compared to coffee with the pericarp (with the husk), energy expenditure is slower. The length and timing of coffee seed germination processes differed significantly between wet and dry processed beans. The highest germination activity occurred 2 days after the onset of wet processing, while the maximum corresponding to dry processing appeared about one week after the start of post-harvest treatment. This energy results from the use of carbohydrates and lipids, components that constitute tactile perception, beverage viscosity after roasting.

Cultivars Catuaí Amarelo, Icatu Vermelho, Mundo Novo and Topázio did not present statistical differences between the post-harvest processing methods (natural and parchment) for balance (Table 3).

Cultivars Catuaí and Icatu (Table 3) did not differ statistically between parchment and natural processes for overall score. Cultivars Mundo Novo and Topázio are also included in these groups. Natural processing allowed a higher average for most cultivar groups (total scores) with scores above 80 by the SCA protocol.

It is observed that the cultivar groups Acaiá, Bourbon Amarelo, Catuaí Vermelho, Catucaí Amarelo and Icatu Amarelo differed by the means test between post-harvest processing methods. The highest average scores for natural and parchment processing methods were obtained for 'Icatu Vermelho' and 'Bourbon Amarelo', respectively.

The permanence of the husk gives the fruit a more resistant structure and the need for drying time to the ideal storage point, when compared to any other processing method. Besides, greater care and attention are required to avoid depreciation of dried husked coffees due to undesirable fermentation as a function of mucilage.

Analyzing the histogram with the total scores (Figure 1), regardless of coffee cultivar groups and the post-harvest processing methods, it is observed that the highest score frequency given by the panelists is between 82.50 and 85.00 points by the SCA protocol, reinforcing that the Vertentes Region has high quality coffees. This result may be provided by a combination of appropriate crop management techniques, the correct processing of coffee fruits and the genotypic adaptability of cultivar groups, in a favorable environment for the production of specialty coffees. Some coffee samples scored above 89 points, a quality that is highly valued in the specialty coffee market.

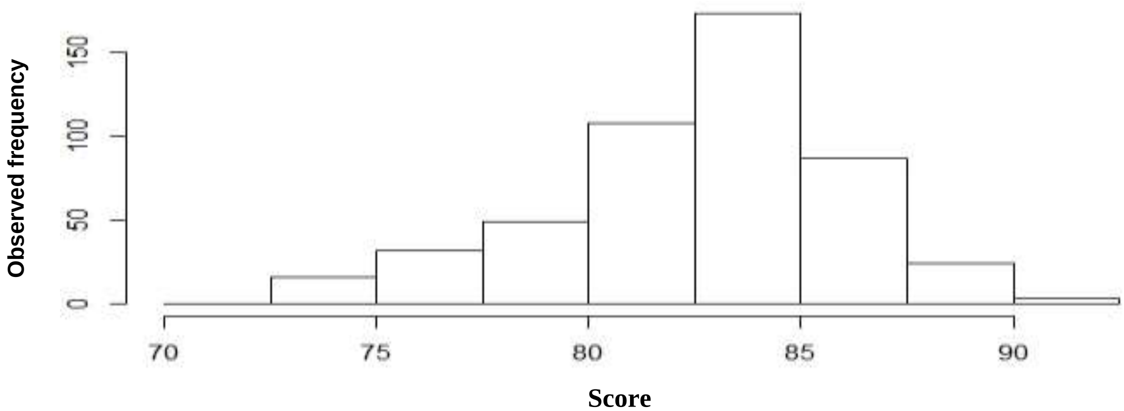


**Figure 1.** Observed frequency of the total coffee score, evaluated in 9 cultivar groups in the Campo das Vertentes Mesoregion, in the State of Minas Gerais.

**Table 4.** Minimum and maximum total scores of coffee beverages of cultivar groups evaluated in the Campo das Vertentes Mesoregion, in the state of Minas Gerais.

| Cultivar groups | Total score | |
|---|---|---|
| | Minimum | Maximum |
| Bourbon Amarelo | 77.00 | 90.25 |
| Topázio | 77.00 | 88.50 |
| Icatu Amarelo | 80.00 | 89.50 |
| Catucaí Amarelo | 73.00 | 90.75 |
| Icatu Vermelho | 74.25 | 87.50 |
| Catuaí Amarelo | 74.00 | 86.75 |
| Acaiá | 74.00 | 88.00 |
| Catuaí Vermelho | 75.00 | 88.50 |
| Mundo Novo | 73.50 | 89.25 |

It is noteworthy that all cultivar groups presented wide variation of total scores, and only the group 'Icatu Amarelo' had a minimum total score above 80 points (Table 4). In the item total maximum score, all cultivars had scores above 86 points, which allow the appreciation of coffees above the market price.

In this type of data analysis, it is possible to group the treatments that present similarity in their variables. The largest data variation is in principal component 1, with 87.38%, where the largest distribution of the treatments in the horizontal axis is observed (Figure 2).

In general, Bourbon Amarelo, Topázio and Icatu Amarelo cultivar groups presented the best results for the sensory variables. In addition, naturally processed coffees at post-harvest showed the best sensory responses, since there is a spatial separation of treatments between natural and parchment coffees, and the latter is inversely distributed in the analyzed sensory variables (Figure 2). For 'Mundo Novo', natural processing enables a better correlation with sensory attributes. The groups 'Catucaí Amarelo' and 'Icatu Vermelho' behaved in the same grouping, reflecting the stability in the sensory variables before the two post-harvest processing methods (Figure 2).

## Conclusion

(1) 'Topázio' and 'Bourbon Amarelo' groups, regardless of the post-harvest process used, present higher final and general average scores on the coffee beverage produced using them.

(2) 'Topázio', 'Bourbon Amarelo', 'Catucaí Amarelo', 'Icatu Amarelo' and 'Icatu Vermelho' showed higher averages for all sensory attributes considered by the Sensory Analysis Protocol of the Specialty Coffee Association - SCA.

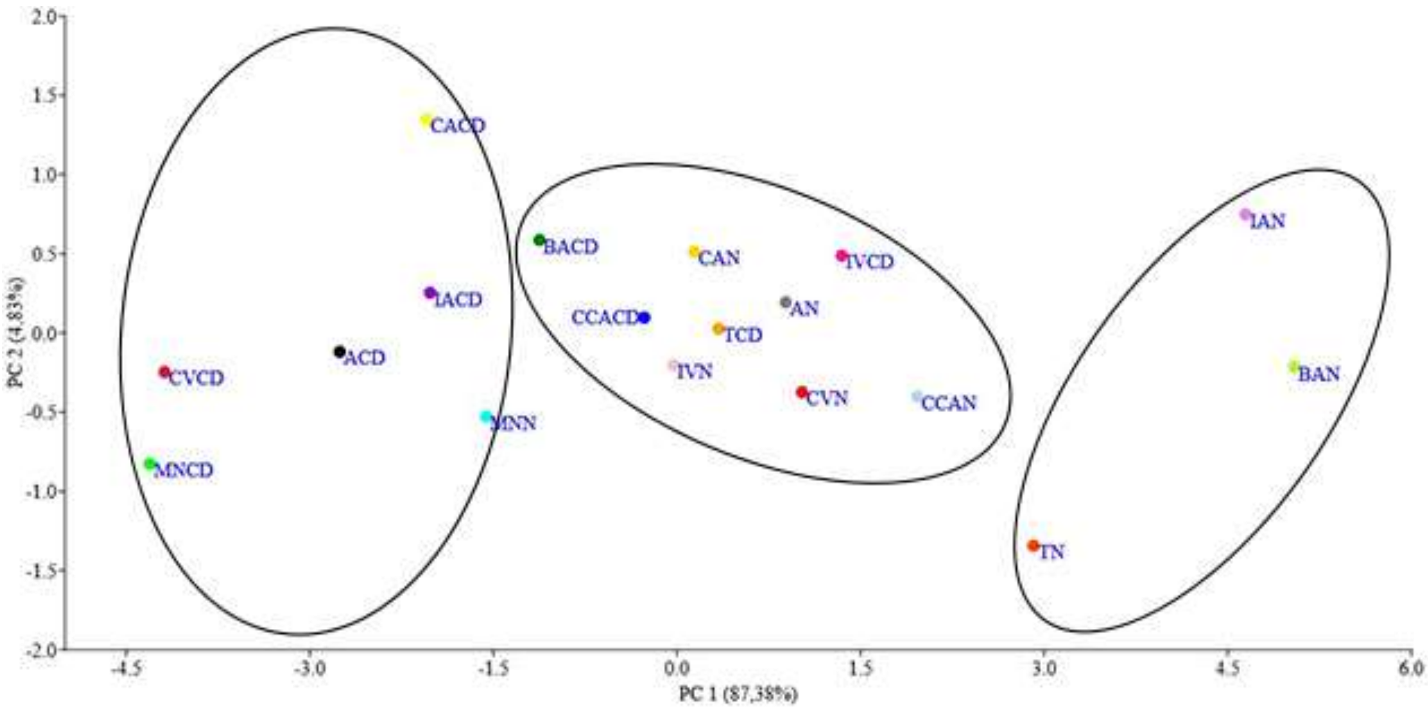


**Figure 2.** Relationship between the two principal components for cultivar groups and post-harvest processing methods, evaluated in the Campo das Vertentes Mesoregion, in the State of Minas Gerais. ACD=Acaiá Parchment, AN=Acaiá Natural, BACD= Bourbon Amarelo Cereja Parchment, BAN= Bourbon Amarelo Natural, CVCD Catuaí Vermelho Parchment, CVN= Catuaí Vermelho Natural, CACD= Catuaí Amarelo Parchment, CAN= Catuaí Amarelo Natural, CCACD= Catucaí Amarelo Parchment, CCAN= Catucaí Amarelo Natural, IACD= Icatu Amarelo Parchment, IAN= Icatu Amarelo Natural, IVCD= Icatu Vermelho Parchment, IVN= Icatu Vermelho Natural, MNCD= Mundo Novo Parchment, MNN= Mundo Novo Natural, TCD= Topázio Parchment, TN= Topázio Natural.

(3) All cultivar groups present higher average scores for the sensory attribute "body", when submitted to natural postharvest processing.
(4) Natural processing enables higher total scores.
(5) All cultivar groups have potential for the production of specialty coffees and can obtain maximum scores above 86 points.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Heterosis and combining ability for storage root, flesh color, virus disease resistance and vine weight in Sweet potato [*Ipomoea batatas* (L.) Lam.]

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**This study was done to determine the mid-parent heterosis, the general (GCA) and specific (SCA) combining abilities of storage root yield, sweet potato virus resistance (SPVD), flesh color and vine weight of candidate sweet potato clones. Sixteen selected genotypes from two gene pools were crossed in an 8B×8A cross having 64 families. Trials were conducted with 1,896 offsprings and 16 parents during two seasons at the National Crops Resources Research Institute in Uganda (NaCRRI) using a Westcott design (only checks were replicated). Significant differences in performance were noted among families for all traits in both seasons (P ≤ 0.001). Magabali×NK259L and Resisto×Naspot 7 were the best crosses for improving total storage root while Naspot 5×Naspot 7 stood out as the best cross for flesh color. The ratio of general combining ability to specific combining ability (GCA/SCA) for storage root, flesh color and SPVD ranged from 0.55 to 0.79, implying that additive gene effects were more important than non-additive gene effects for these traits. For vine weight, non-additive gene effects tended to be predominant. A susceptible parent Magabali and a moderately susceptible parent Naspot 1 had the most resistant progenies. This suggests that SPVD resistant alleles could be homozygous recessive, which may be confirmed in further studies. Correlation studies between traits were almost all significant except for flesh color and storage root yield. There was a positive and significant correlation (*P ≤ 0.001*) between flesh color and SPVD resistance, with orange roots being the most resistant to SPVD. This important finding can help breeders to come up with orange-fleshed sweet potatoes that are highly resistant to virus diseases.**

**Key words:** Sweet potato combining ability, heterosis, genetic effect, storage root, sweet potato virus resistance (SVDP), flesh color, vine weight.

## INTRODUCTION

Undernourishment and severe food insecurity continue to be major concerns in most parts of the world especially in Africa. New statistics indicate a rise in world hunger in recent years after a prolonged decline. An estimated 821 million people, approximately one out of every nine people in the world are undernourished. Therefore, considerable efforts need to be done to achieve the objective of a world without hunger (WFP, 2018). In order to meet that goal, crops that can be resilient under a wide range of environmental conditions need to be promoted worldwide. In that regard, root and tuber crops can play a significant role as solutions. Among tuber and root crops, sweet potato [*Ipomoea batatas* (L.) Lam] plays an important role in human and animal nutrition and is known to have the potential to be resilient to climate change thanks to its plasticity to adapt to different environments and agro-systems, its productivity and short growth cycle (Glato et al., 2017).

Sweet potato is grown for food and nutritional security with the white- and cream-fleshed storage roots usually consumed as raw, boiled or fried as calorie sources, while the orange-fleshed sweet potato (OFSP) having sufficient beta-carotene levels to alleviate vitamin A deficiency (Gurmu et al., 2014). Additionally, sweet potato leaves are used as vegetable and aboveground biomass is widely used as animal feed (David et al., 2018). Bearing in mind the importance of this crop, efficient sweet potato breeding programs are vital to increase food security and improve health worldwide (in sub-Sahara Africa specifically). Two keystones of any breeding program being the characterization of the crossing parents and evaluation of their performances, bringing about two concepts core to any crop improvement program: combining ability and heterosis. The former is defined as a cultivar's ability to transmit desirable genes or characters to their progenies in a very efficient way (Fasahat et al., 2016). Combining ability analysis helps breeders in identifying the potential parents and also informs on the genetic action governing the expression of a given trait (Rukundo et al., 2017). When crossing a genotype to several others we can calculate its mean performance in all crosses: this is referred to as the general combining ability (GCA) (Fasahat et al., 2016). GCA is directly related to the breeding value of a parent and is associated with additive genetic effects, while specific combining ability (SCA) is the relative performance of a cross and is mostly associated with non-additive gene actions, such as dominance, over dominance, epistasis (Rukundo et al., 2017). Therefore, both GCA and SCA effects are important in a breeding program (Rukundo et al., 2017).

On the other hand, heterosis, also referred to as hybrid vigor, is of sound interest to breeding programs especially for cross-pollinated crops like sweet potato (Singh, 2015). It is defined as the superiority of F1 hybrids over both parents in terms of yield or some other characters (Chaurasia, 2012). The exploitation of heterosis in crop plants is considered to be one of the milestones in modern agriculture and is of huge economic importance (Hochholdinger and Baldauf, 2018). The utilization of heterosis has become a major practice for increasing productivity of plants, which has contributed to the great increase of agricultural products worldwide in the last several decades. However, studies on yield components, qualitative traits and heterosis in sweet potato are limited and very few as compared to other crops like corn and potato. Several hypotheses have been proposed to explain heterosis such as: masking of deleterious mutations by heterozygosity; overdominance of additive loci between genotypes; protein metabolism, dosage-sensitive genes; and possible epigenetic effects (McKeown et al., 2013). In other major crops such as maize (Matin et al., 2017) and cotton (Khokhar et al., 2013), combining ability and heterosis have been heavily studied. Genetic studies in sweet potato are limited due to several barriers, namely: it is self and cross-incompatibility, high level of polyploidy and limited flowering ability and seed setting (Mwanga et al., 2017). Keeping in view the economic importance of yield contributing traits (storage root weight, vine weight) in sweet potato and also the importance of flesh color as well as resistance to SPVD, the objectives of the present study were: (1) to evaluate the general combining ability effects (GCA) of parents, (2) to estimate the specific combining ability (SCA) effects of different cross combinations with regard to the aforementioned traits, (3) to calculate the extent of the mid-parent heterosis for all progenies, and (4) to infer the correlations between the measured traits.

## MATERIALS AND METHODS

### Origins of parental lines

The parental lines originated from different countries in Africa, United States of America and Peru as shown in the Table 1. They are part of the parental materials of the International Potato Center's East and Central Africa sweet potato breeding support platform based in Uganda. The list of the parents is shown in Table 1.

### Plant materials and crosses

The population used in this study (Mwanga Diversity Panel) is an 8B×8A cross comprising 16 parents without reciprocals and 1,896 progenies. The 16 parental accessions were chosen from a pool of other 80 and 50 sweet potato accessions based on two heterotic

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**Table 1.** Background information of the parents crossed in this study.

| Parent code | Name of cultivar | SPVD status | Country of origin |
|---|---|---|---|
| A1 | Ejumula | S | Uganda |
| A2 | NASPOT 1 | MR | Uganda |
| A3 | Dimbuka-Bukulula | S | Uganda |
| A4 | NASPOT 5/58 | S? | Uganda |
| A5 | NASPOT 7 | MR | Uganda |
| A6 | SPK004 | MR | Kenya |
| A7 | NASPOT 10 O | MR | Uganda |
| A8 | NK259L | MR | Uganda |
| B1 | Resisto | S | USA |
| B2 | Magabali | S | Uganda |
| B3 | NASPOT 5 | MR | Uganda |
| B4 | Wagabolige | MR | Uganda |
| B5 | Mugande | MR? | Uganda |
| B6 | NASPOT 11 | MR | Uganda |
| B7 | New Kawogo | MR | Uganda |
| B8 | Huarmeyano | S? | CIP/Peru |

SPVD: Sweet potato virus disease, S: sensitive, MR: Moderately resistant, S? and MR? status not yet established but close to be sensitive or moderately resistant, respectively.

groups separated by 18 SSR markers (David et al., 2018). 64 crosses (B×A: B being the female parent and A being the male parent) were done to generate seeds that were established *in vitro* (at BecA) and later transferred to Uganda for phenotypic evaluation. Crosses and field trials were conducted at the National Crops Resources Research Institute (00°31′30″N, 32°36′54″E), Namulonge, Uganda. Experiments were conducted for two seasons in 2018 (season A running from March to July and season B running from August to December). 10 plants were established for each progeny at the beginning of the trials. Among the traits that were looked at are sweet potato virus disease (SPVD), flesh color, vine weight and total storage root.

#### Traits measurements

Two quantitative traits and two qualitative traits were measured in this study. Data were recorded for storage root and vine weights (in kg per plot and each plot was 3 $m^2$), for flesh color (ranked from 1 to 9 where 1 represents a white flesh and 9 deep orange-fleshed root) and for SPVD status (also ranked from 1 to 9 where 1 represents minimum signs of infection and 9 very high level of infection).

#### Phenotypic data analysis

Data were obtained from a population of 1896 progenies and 16 parents (including 2 checks: Naspot 11 and Ejumula). BLUPs (Best Linear Unbiased Prediction) were generated for each residual across all traits for the two seasons (separately) with the following model using ASREML software implemented in R (Version. 3. 6):

$$Y_{ijk} = \mu + g_i + r_j + c_k + e_{ijk}$$

where $Y_{ijk}$ is the phenotypic performance of the ith genotype in the jth row and kth column, μ is the oval mean, $g_i$ is the effect of the genotype, $r_i$, is the effect of the ith row, $c_k$ is the effect of the kth column, and $e_{ijk}$ is the experimental error. It was assumed that row and column effects were random. Co-analysis of the two seasons together did not allow the combined mixed model to converge.

Descriptive statistical analyses were then done on BLUPs of checks, parents, all progenies and 10% of the best performing progenies for every trait in each season. Mid-parent heterosis was calculated for the overall population and per family using the following formula:

$$\text{Mid-parent Heterosis (\%)} = [(\text{F1} - \text{MP})/\text{MP}] \times 100$$

where F1 and MP represent the mean performance of the progenies and the two parents, respectively. Correlation of BLUPs was evaluated between seasons and between pairs of traits. All analyses were conducted using R software (Version 3.6.1). The combining ability of parental lines was calculated as the deviation of the mean performance of the line in all its crosses from the mean of all crosses, while the specific combining abilities of crosses were computed as the deviation of the mean for each cross from the sum of general combining abilities of its two parental lines as defined in Griffin's method (Olfati et al., 2012a). The relative importance of GCA and SCA was estimated using the general predicted ratio (GPR) for the traits observed as follows (Baker, 1978):

$$\text{GCA/SCA} = (2\text{MSGCA}) / (2\text{MSGCA} + \text{MSSCA})$$

## RESULTS AND DISCUSSION

### Descriptive statistics on the overall population

Total storage root weight was better for the season A

**Table 1.** Descriptive statistics for BLUPs of the full population, checks and the best 10% clones for every trait in each season.

| Statistics | TSRW (Kg/plot) | | Flesh color (1-9) | | SPVDR (1-9) | | Vine weight (kg/plot) | |
|---|---|---|---|---|---|---|---|---|
| | SA | SB | SA | SB | SA | SB | SA | SB |
| Overall mean | 2.39 | 0.83 | 2.15 | 4.18 | 2.79 | 2.84 | 3.99 | 3.23 |
| Overall Min | 1.68 | 0.69 | 0.7 | 1.2 | 2.42 | 1.83 | 1.83 | 1.84 |
| Overall Max | 6.87 | 2.26 | 7.76 | 8.51 | 3.88 | 4.57 | 10.67 | 8.82 |
| Mean Checks | 3.92 | 1.16 | 3.17 | 3.84 | 3.21 | 3.30 | 4.72 | 4.18 |
| Mean best 10% | 2.95 | 0.92 | 6.55 | 6.85 | 2.57 | 2.04 | 6.06 | 5.10 |

TSRW: Total storage root weight, SPVDR: sweet potato virus disease resistance, SA: season A, SB: season B.

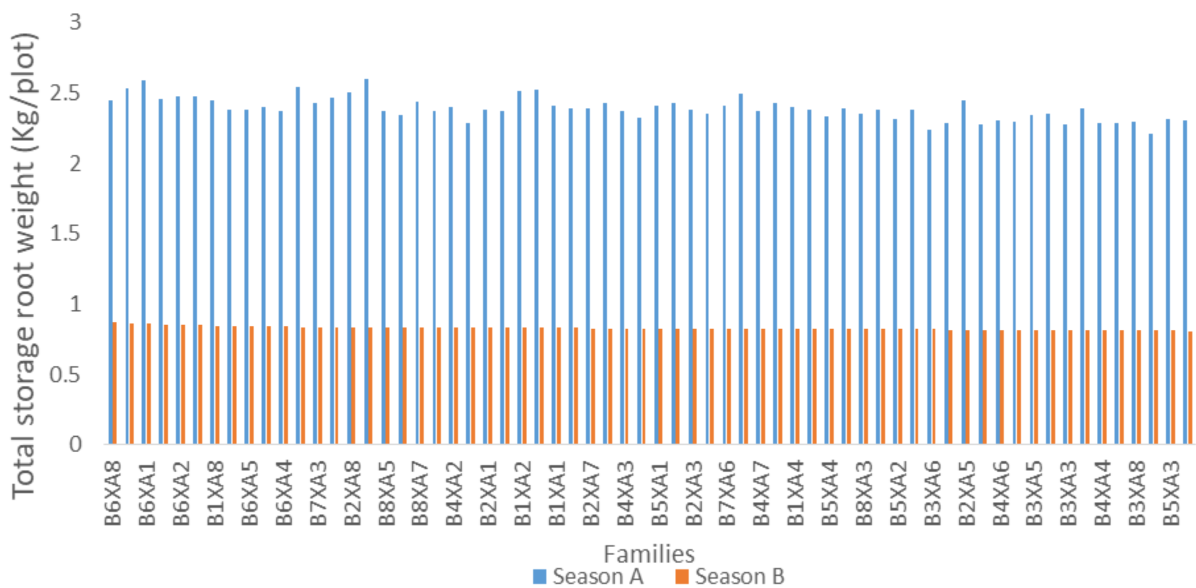


**Figure 1.** Mean performances of families using BLUPs for storage root weight in seasons A and B.

than for the season B. In season A, 50% of the genotypes had 2.36 kg/plot and above whilst the same percentage performed far less (0.82 kg/plot) in season B. At the same time the highest storage root weight yield was 6.87 and 2.26 kg/plot in seasons A and season B, respectively. The flesh color performance was better in season B than season A. 50% of the genotypes had a flesh color ranked at least 2 and the best genotypes for this trait scored 7 in season A, while half of the genotypes had a flesh color ranked at least at 4 and the best performance nearly reached 9 (8.51). The genotypes showed slightly better SPVD resistance status in season A than in season B. Less signs of disease were noticed in the first season where the most affected genotypes had a score of 3.88 on average as compared to the second season where the most affected genotypes had a score of 4.57 on average. The vine weight had also a better record for season A. Half of the genotypes had at least 3.92 and 3.07 kg/plot for seasons A and B, respectively. The highest performance was 10.67 kg/plot in the first season and 8.82 kg/plot in the second (Table 2).

### Analysis of variance

The mean performances for the measured traits were all significantly different between families as well as within families (Figures 1 to 4). Progenies form the families B7×A1, B6×A1, B7×A5, B6×A3 and B7×A7 had the highest storage root yield with 2.6, 2.58, 2.54, 2.53 and 2.52 kg/plot, respectively (Figure 1). Genotypes belonging to the families B1×A1, B3×A1 and B1×A7 ranked the highest for flesh color with 5.6, 5.0 and 4.5, respectively (Figure 2). For SPVD resistance, progenies from B3×A6, B3×A2 and B5×A2 showed less signs of virus disease with a rank of 2.7 in average (Figure 3).

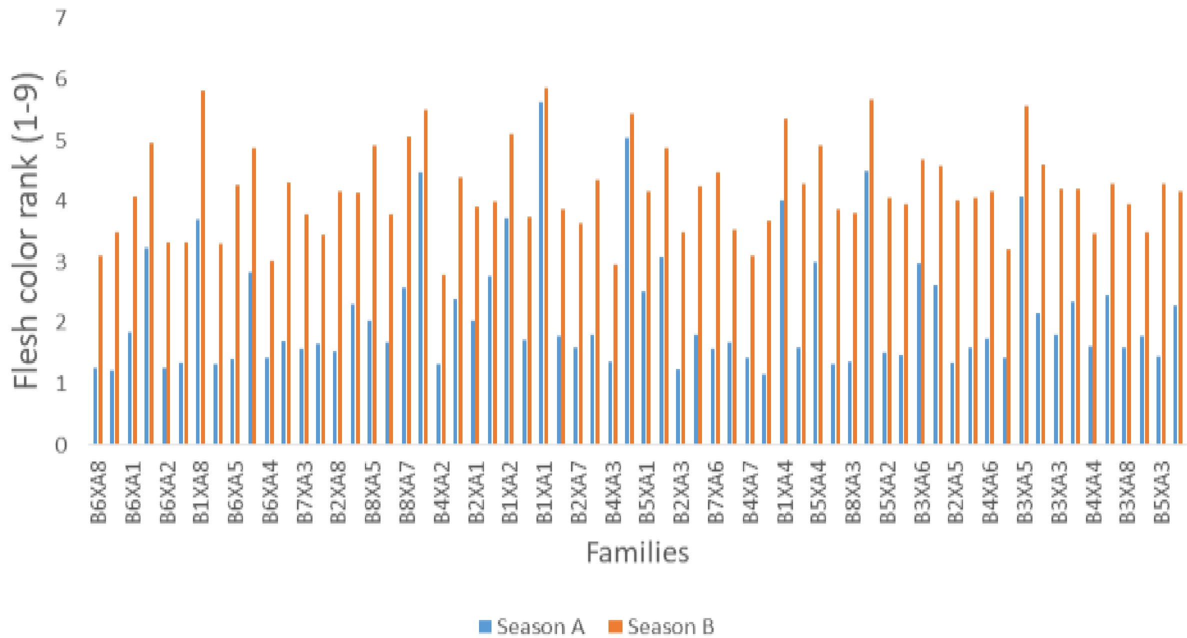


**Figure 2.** Mean performances of families using BLUPs for flesh color in seasons A and B.

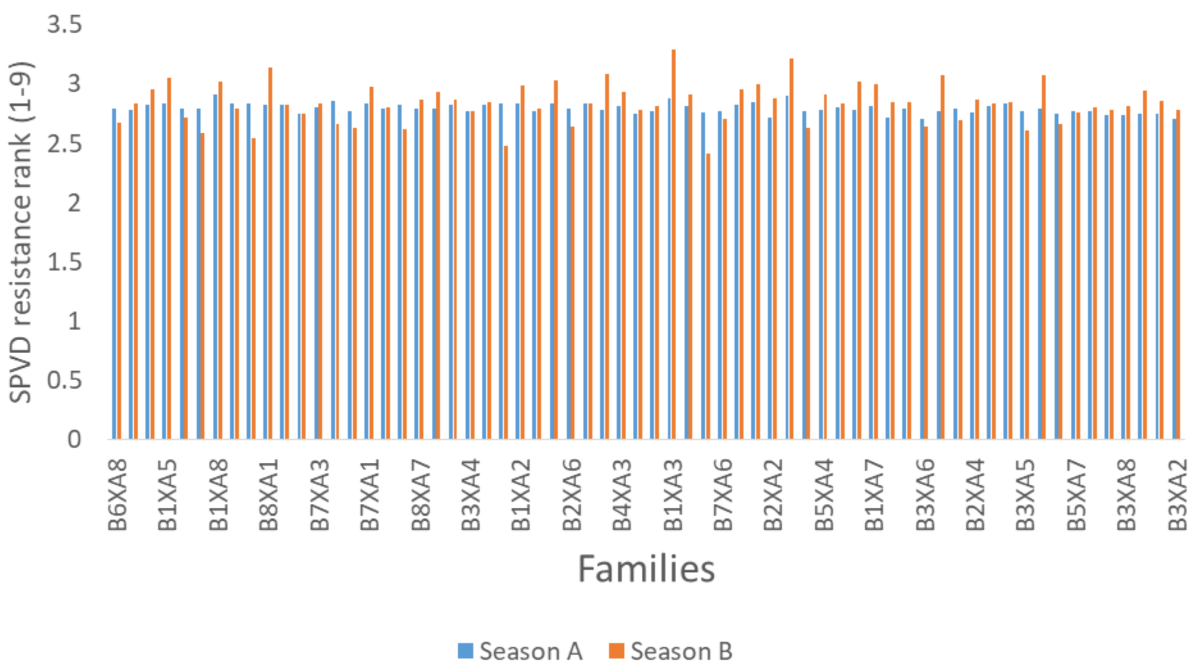


**Figure 3.** Mean performances of families using BLUPs for SPVD resistance in seasons A and B.

Genotypes from B2×A5, B5×A1, B8×A7 and B2×A8 had the highest vine weight (4.64-4.48 kg/plot) (Figure 4 and Table 3).

The parent’s storage root yield ranged between 2.31 and 4.63 kg/plot of which the highest were Naspot 11 and Ejumula (4.63 and 3.21 kg/plot, respectively), and the lowest was Magabali. Parent A1 (Ejumula) had the highest flesh color rank followed by parent B7 (New Kawogo) and parent B6 (Naspot 11) had the lowest score. Parent B3 (Naspot 5) was the most resistant to

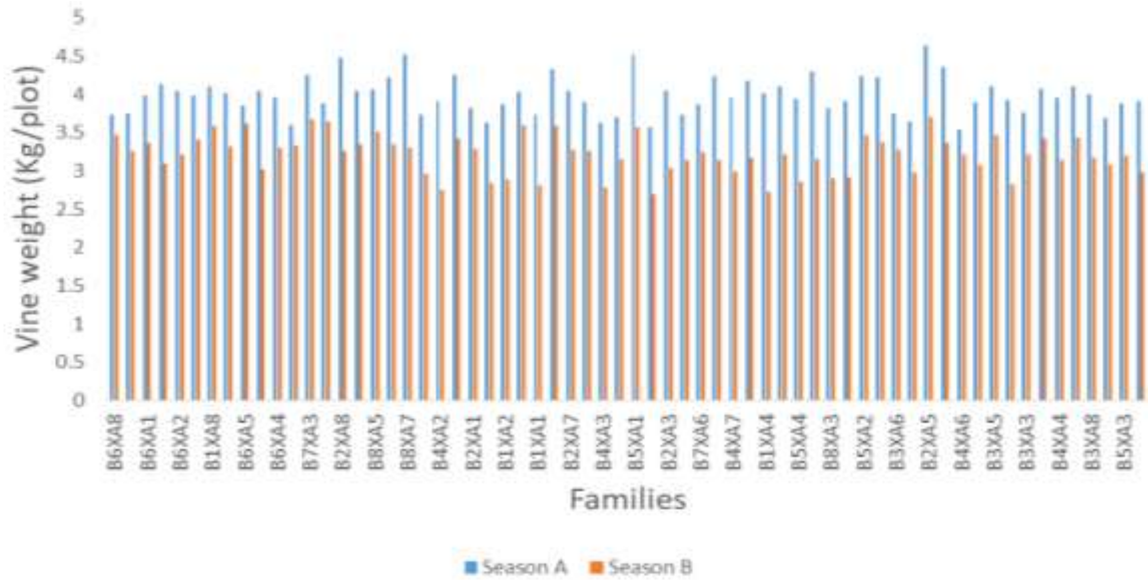


**Figure 4.** Mean performances of families using BLUPs for vine weight in seasons A and B.

**Table 3.** Combined analysis of variance using Kruskal-Wallis test for four agronomic traits of the F1 progenies in season A.

| Source | Df | Chi square | | | |
|---|---|---|---|---|---|
| | | TSRW | Flesh color | SPVDR | Vine weight |
| Family | 63 | 185.68*** | 352.41*** | 138.17*** | 122.48*** |

Df: Degrees of freedom, ***: data significant at P ≤ 0.001

**Table 4.** Combined analysis of variance using Kruskal-Wallis test for four agronomic traits of the F1 progenies in season B.

| Source | Df | Chi square | | | |
|---|---|---|---|---|---|
| | | TSRW | Flesh color | SPVDR | Vine weight |
| Family | 63 | 182.56*** | 518.66*** | 206.15*** | 200.35*** |

Df: Degrees of freedom, ***: data significant at P ≤ 0.001.

SPVD and parent A1 the most susceptible. Parent B6 had the highest vine yield with 5.57 kg/plot.

The mean performances for all the measured traits were significantly different and progenies performed better in season A than in season B. Progenies from B6×A8, B6×A3, B6×A1 and B1×A5 had the highest storage root production with, respectively 0.87 kg/plot for the for the first family and 0.86 kg/plot for the three last. Genotypes belonging to the family B1×A1 ranked the highest for flesh color followed by those from B1×A8, B1×A7 and B3×A5, respectively. Families B7×A8, B7×A4, B6×A5, and B6×A6 showed the best resistance status to SPVD while progenies from B2×A5, B7×A3 and B6×A7 had the best vine production (Table 4).

The parents' storage root yield ranged between 1.59 and 0.74 kg/plot with Naspot 11 having the highest yield followed by Naspot 10 O (0.85 kg/plot). Ejumula had the highest rank for flesh color with and Naspot 11 the lowest. Naspot 11 had the best resistance status along with New Kawogo while having also the highest vine production with Mugande and Magabali.

## Consistency of family performance across seasons

For storage root yield, families B6×A1 and B6×A3 were

**Table 5.** Mid-parent heterosis for the 30 best progenies based on the mean performances of the families on total storage root weight in both seasons.

| Season A TSRW | | | Season B TSRW | | |
|---|---|---|---|---|---|
| **Clone ID** | **Family** | **MPH (%)** | **Clone ID** | **Family** | **MPH (%)** |
| MDP139 | B1×A2 | 33.48 | MDP320 | B1×A8 | 24.28 |
| MDP131 | B1×A2 | 32.80 | MDP216 | B1×A5 | 21.24 |
| MDP110 | B1×A2 | 30.62 | MDP237 | B1×A5 | 21.10 |
| MDP525 | B2×A8 | 58.04 | MDP318 | B1×A8 | 17.66 |
| MDP524 | B2×A8 | 49.70 | MDP317 | B1×A8 | 17.64 |
| MDP523 | B2×A8 | 26.58 | MDP224 | B1×A5 | 17.50 |
| MDP513 | B2×A8 | 20.00 | MDP228 | B1×A5 | 16.85 |
| MDP507 | B2×A8 | 19.24 | MDP238 | B1×A5 | 15.42 |
| MDP1253 | B7×A1 | 29.24 | MDP311 | B1×A8 | 14.50 |
| MDP1247 | B7×A1 | 27.24 | MDP319 | B1×A8 | 14.13 |
| MDP1302 | B7×A5 | 58.90 | MDP321 | B1×A8 | 13.66 |
| MDP1297b | B7×A5 | 54.39 | MDP310 | B1×A8 | 13.36 |
| MDP1293c | B7×A5 | 37.48 | MDP239 | B1×A5 | 12.97 |
| MDP1303 | B7×A5 | 37.37 | MDP316 | B1×A8 | 12.81 |
| MDP1297 | B7×A5 | 34.11 | MDP328a | B1×A8 | 11.16 |
| MDP1294 | B7×A5 | 22.11 | MDP314 | B1×A8 | 10.14 |
| MDP1296 | B7×A5 | 18.11 | MDP221 | B1×A5 | 9.94 |
| MDP1303a | B7×A5 | 16.07 | MDP313 | B1×A8 | 9.62 |
| MDP1354 | B7×A7 | 34.09 | MDP329a | B1×A8 | 8.75 |
| MDP1354 | B7×A7 | 34.09 | MDP307 | B1×A8 | 7.71 |
| MDP1328 | B7×A7 | 25.15 | MDP330a | B1×A8 | 7.12 |
| MDP1328 | B7×A7 | 25.15 | MDP303 | B1×A8 | 6.83 |
| MDP1339 | B7×A7 | 22.56 | MDP220 | B1×A5 | 6.77 |
| MDP1339 | B7×A7 | 22.56 | MDP323a | B1×A8 | 6.21 |
| MDP1346 | B7×A7 | 19.55 | MDP315 | B1×A8 | 5.81 |
| MDP1346 | B7×A7 | 19.55 | MDP331a | B1×A8 | 5.46 |
| MDP1332 | B7×A7 | 17.14 | MDP322a | B1×A8 | 5.27 |
| MDP1332 | B7×A7 | 17.14 | MDP312 | B1×A8 | 4.98 |
| MDP1352 | B7×A7 | 16.41 | MDP226 | B1×A5 | 4.91 |
| MDP1352 | B7×A7 | 16.41 | MDP322 | B1×A8 | 4.85 |

TSRW: total storage root weight, MPH: mid-parent heterosis

consistently ranked among the best seven (~10%) in both seasons. Families B1×A1, B3×A5, B1×A6, B3×A1, B1×A4 and B1×A7 were consistent between the seasons for flesh color. No family was consistent between the seasons for SPVD while families B2×A5 and B2×A6 were the only consistent across the two seasons for vine weight.

## Heterosis

### *Heterosis for total storage root weight*

All the families were ranked based on their performances for the measured traits in each season and the seven best families (~10%) were chosen to calculate the mid-parent heterosis of the progenies. The progenies were thereafter ranked and the 30-best shown in Tables 5 to 8.

Clones MDP139, MDP131 and MDP110 (all belonging to B1×A2 family), showed the highest mid parent heterosis with 33.48, 32.80 and 30.62%, respectively on storage root weight in the season A. B1×A2 family had 3 progenies, while B2×A8, B7×A1, B7×A5 and B7×A7 had, respectively 5, 2, 8, and 12 progenies out of the 30 heterotic crosses. Clones MDP320, MDP216 and MDP237 had the best heterosis in season B with respectively 24.28, 21.24 and 21.10%. The 30 best progenies were shared only among two families namely

**Table 6.** Mid-parent heterosis for the 30 best progenies based the mean performances of the families on flesh color in both seasons.

| Season A Flesh color | | | Season B Flesh color | | |
|---|---|---|---|---|---|
| **Clone ID** | **Family** | **MPH (%)** | **Clone ID** | **Family** | **MPH (%)** |
| MDP126 | B1×A2 | 246.9869 | MDP317 | B1×A8 | 93.88 |
| MDP140 | B1×A2 | 246.6692 | MDP325a | B1×A8 | 93.78 |
| MDP122 | B1×A2 | 244.8855 | MDP308 | B1×A8 | 92.63 |
| MDP124 | B1×A2 | 244.2425 | MDP313 | B1×A8 | 92.62 |
| MDP134 | B1×A2 | 243.3149 | MDP319 | B1×A8 | 92.24 |
| MDP125 | B1×A2 | 243.0951 | MDP187 | B1×A4 | 75.94 |
| MDP123 | B1×A2 | 242.45 | MDP330a | B1×A8 | 72.40 |
| MDP128 | B1×A2 | 239.7813 | MDP315 | B1×A8 | 71.80 |
| MDP117 | B1×A2 | 235.5377 | MDP328a | B1×A8 | 71.07 |
| MDP183 | B1×A4 | 259.649 | MDP310 | B1×A8 | 70.98 |
| MDP197 | B1×A4 | 253.9032 | MDP322 | B1×A8 | 70.75 |
| MDP190 | B1×A4 | 240.4212 | MDP311 | B1×A8 | 70.55 |
| MDP199 | B1×A4 | 234.0935 | MDP305 | B1×A8 | 70.25 |
| MDP270 | B1×A6 | 248.9083 | MDP306 | B1×A8 | 70.04 |
| MDP271 | B1×A6 | 248.4737 | MDP323a | B1×A8 | 69.97 |
| MDP261 | B1×A6 | 246.7578 | MDP326a | B1×A8 | 69.04 |
| MDP262 | B1×A6 | 243.5808 | MDP318 | B1×A8 | 68.17 |
| MDP269 | B1×A6 | 241.2006 | MDP269 | B1×A6 | 65.28 |
| MDP298 | B1×A7 | 253.7948 | MDP636 | B3×A5 | 64.57 |
| MDP278 | B1×A7 | 248.2322 | MDP278 | B1×A7 | 58.60 |
| MDP280 | B1×A7 | 247.9483 | MDP289 | B1×A7 | 58.14 |
| MDP294 | B1×A7 | 240.583 | MDP288 | B1×A7 | 57.22 |
| MDP295 | B1×A7 | 238.3572 | MDP20 | B1×A1 | 56.93 |
| MDP281 | B1×A7 | 235.7962 | MDP3 | B1×A1 | 56.75 |
| MDP301 | B1×A7 | 235.3117 | MDP31 | B1×A1 | 56.71 |
| MDP626 | B3×A5 | 243.5736 | MDP280 | B1×A7 | 56.63 |
| MDP624 | B3×A5 | 241.9982 | MDP25 | B1×A1 | 56.59 |
| MDP636a | B3×A5 | 239.726 | MDP190 | B1×A4 | 56.42 |
| MDP647 | B3×A5 | 237.8907 | MDP35 | B1×A1 | 56.22 |
| MDP627 | B3×A5 | 237.5849 | MDP86 | B3×A1 | 56.10 |

MPH: Mid-parent heterosis.

B1×A8 with 21 progenies and B1×A5 with 9 progenies.

### *Heterosis for flesh color*

MDP183, MDP197 and MDP298 showed the highest heterosis for flesh color in season A with 259.64, 253.90, and 253.79%, respectively. Among the 30 best progenies, clones from B1×A2 family were 9, those from B1×A4 were 4, those from B1×A6 were 5, those from B1×A7 were 7 and those from B3×A5 were 5. In season B, the highest heterosis was recorded in B1×A8 family with clones MDP317, MDP325a and MDP308 having, respectively 93.88, 93.78 and 92.63%. Family B1×A8 had the highest number of progenies with high heterosis (16) followed by B1×A1 with 5 progenies.

### *Heterosis for SPVD resistance*

For this trait the negative heterosis is selected to indicate the level of resistance to sweet potato virus disease. In other words, most negative values display better heterosis for this trait. In that regard, clones MDP355 and MDP362 showed the best heterosis with -13.64 and -13.61% respectively, they both belong to B2×A2 (Magabali×Naspot 1) family. Progenies from B2×A2 family were 12 and those from B5×A2 were 5. During the

**Table 7.** Mid-parent heterosis for the 30 best progenies based the mean performances of the families on SPVD resistance in both seasons.

| Season A SPVDR | | | Season B SPVDR | | |
|---|---|---|---|---|---|
| **Clone ID** | **Family** | **MPH (%)** | **Clone ID** | **Family** | **MPH (%)** |
| MDP355 | B2×A2 | -13.64 | MDP1364b | B7×A8 | -56.90 |
| MDP362 | B2×A2 | -13.61 | MDP1366c | B7×A8 | -56.11 |
| MDP880 | B5×A2 | -10.98 | MDP1361 | B7×A8 | -54.84 |
| MDP366 | B2×A2 | -10.46 | MDP1356b | B7×A8 | -52.52 |
| MDP949 | B5×A6 | -10.11 | MDP1789 | B7×A4 | -44.72 |
| MDP370 | B2×A2 | -9.69 | MDP815 | B4×A8 | -35.55 |
| MDP893 | B5×A2 | -9.51 | MDP1362 | B7×A8 | -32.09 |
| MDP374 | B2×A2 | -9.40 | MDP1358b | B7×A8 | -30.53 |
| MDP369 | B2×A2 | -9.01 | MDP822 | B4×A8 | -30.38 |
| MDP609 | B3×A8 | -8.80 | MDP517 | B2×A8 | -29.91 |
| MDP585 | B3×A3 | -8.74 | MDP816 | B4×A8 | -27.97 |
| MDP575 | B3×A3 | -8.70 | MDP510 | B2×A8 | -27.88 |
| MDP874 | B5×A2 | -8.49 | MDP526 | B2×A8 | -27.43 |
| MDP367 | B2×A2 | -8.33 | MDP818 | B4×A8 | -26.14 |
| MDP538 | B3×A2 | -8.31 | MDP836i | B4×A8 | -25.88 |
| MDP360 | B2×A2 | -8.28 | MDP824 | B4×A8 | -23.54 |
| MDP357 | B2×A2 | -8.05 | MDP646f | B3×A5 | -23.28 |
| MDP359 | B2×A2 | -8.05 | MDP525 | B2×A8 | -23.22 |
| MDP363 | B2×A2 | -7.77 | MDP828 | B4×A8 | -22.34 |
| MDP361 | B2×A2 | -7.76 | MDP631 | B3×A5 | -22.13 |
| MDP533 | B3×A2 | -7.61 | MDP522 | B2×A8 | -21.91 |
| MDP557 | B3×A2 | -7.40 | MDP646 | B3×A5 | -21.86 |
| MDP672 | B3×A6 | -7.36 | MDP812 | B4×A8 | -21.83 |
| MDP872 | B5×A2 | -7.27 | MDP819 | B4×A8 | -21.52 |
| MDP868 | B5×A2 | -7.26 | MDP511 | B2×A8 | -21.39 |
| MDP551 | B3×A2 | -7.06 | MDP516 | B2×A8 | -20.68 |
| MDP553 | B3×A2 | -6.73 | MDP505 | B2×A8 | -20.66 |
| MDP881 | B5×A2 | -6.72 | MDP524 | B2×A8 | -20.54 |
| MDP375 | B2×A2 | -6.69 | MDP638 | B3×A5 | -20.14 |
| MDP354 | B2×A2 | -6.64 | MDP639 | B3×A5 | -19.75 |

SPVD: Sweet potato virus disease resistance, MPH: mid-parent heterosis.

season B, heterosis was better with the best performances shown by MDP1364b, MDP1366c and MDP1361 (-56.90, -56.11 and -54.84%, respectively), all belonging to B7×A8 family. NK259L was the most represented male parent, being involved in 24 out of the 30 heterotic crosses.

### *Heterosis for vine weight*

Clones MDP1546, MDP1588 and MDP842 had the highest heterosis for vine weight in season A with, respectively 166.91, 91.73 and 91.02%. Huarmeyano was involved in 10 crosses as female parent out of the 30 best. Progenies from B8×A8 and B8×A7 were five in each family. MDP1774, MDP1328 and MDP322a showed the best heterosis with 141.17, 130.20 and 106.84% in season B, respectively. B7×A3 and B7×A7 were the most represented with seven progenies each.

### Combining ability

The general combining ability for parents was calculated and presented in Tables 9 and 10. During season A, New Kawogo and Naspot 11 were the female parents that had the highest and positive GCA with 0.09 and 0.08, respectively, while Wagabolige and Naspot 5 showed the lowest GCA for storage root weight (-0.06 and -0.08),

**Table 8.** Mid-parent heterosis for the 30 best progenies based the mean performances of the families on vine weight in both seasons.

| Season A Vine weight | | | Season B Vine weight | | |
|---|---|---|---|---|---|
| **Clone ID** | **Family** | **MPH (%)** | **Clone ID** | **Family** | **MPH (%)** |
| MDP1546 | B8×A7 | 166.91 | MDP1774 | B7×A3 | 141.17 |
| MDP1588 | B8×A8 | 91.73 | MDP1328 | B7×A7 | 130.20 |
| MDP842 | B5×A1 | 91.02 | MDP322a | B1×A8 | 106.84 |
| MDP415 | B2×A5 | 87.71 | MDP1354 | B7×A7 | 102.74 |
| MDP1570 | B8×A8 | 81.67 | MDP1335 | B7×A7 | 99.24 |
| MDP1556 | B8×A7 | 80.58 | MDP321 | B1×A8 | 84.27 |
| MDP420 | B2×A5 | 78.67 | MDP1343 | B7×A7 | 81.84 |
| MDP54 | B2×A4 | 74.58 | MDP318 | B1×A8 | 78.51 |
| MDP523 | B2×A8 | 70.25 | MDP1192 | B6×A7 | 76.94 |
| MDP457 | B2×A6 | 68.84 | MDP1765 | B7×A3 | 70.82 |
| MDP435 | B2×A5 | 68.37 | MDP1195 | B6×A7 | 68.74 |
| MDP1584 | B8×A8 | 65.53 | MDP421 | B2×A5 | 68.44 |
| MDP857 | B5×A1 | 63.46 | MDP327a | B1×A8 | 68.07 |
| MDP514 | B2×A8 | 60.81 | MDP1759 | B7×A3 | 62.42 |
| MDP866 | B5×A1 | 60.21 | MDP1764 | B7×A3 | 56.82 |
| MDP1544 | B8×A7 | 57.63 | MDP313 | B1×A8 | 56.68 |
| MDP430 | B2×A5 | 55.28 | MDP1332 | B7×A7 | 55.84 |
| MDP1584a | B8×A8 | 47.74 | MDP1780 | B7×A3 | 51.12 |
| MDP423 | B2×A5 | 47.44 | MDP1183 | B6×A7 | 49.34 |
| MDP1547 | B8×A7 | 46.02 | MDP1784 | B7×A3 | 48.88 |
| MDP1589 | B8×A8 | 45.13 | MDP309 | B1×A8 | 48.05 |
| MDP840 | B5×A1 | 43.19 | MDP1769 | B7×A3 | 43.21 |
| MDP52 | B2×A4 | 43.05 | MDP1341 | B7×A7 | 42.00 |
| MDP434 | B2×A5 | 40.50 | MDP331a | B1×A8 | 40.18 |
| MDP521 | B2×A8 | 38.69 | MDP1347 | B7×A7 | 38.90 |
| MDP43 | B2×A4 | 37.78 | MDP438 | B2×A5 | 34.10 |
| MDP505 | B2×A8 | 36.50 | MDP412 | B2×A5 | 33.70 |
| MDP852 | B5×A1 | 35.82 | MDP1757 | B7×A3 | 31.61 |
| MDP68 | B2×A4 | 35.74 | MDP317 | B1×A8 | 31.16 |
| MDP1538 | B8×A7 | 35.11 | MDP1333 | B7×A7 | 30.29 |

MPH: Mid-parent heterosis.

respectively. Ejumula had the best GCA for the same trait as male parent with 0.05. Resisto showed the highest GCA for flesh color as female parent with 1.89 and Ejumula as male parent with 0.88. Naspot 5 had the lowest GCA as female parent (-0.04), while Naspot 1 along with SPK004 had the lowest GCA with -0.01 each on male parent's group for SPVD resistance. Magabali had the best performance for vine weight as a female parent with a GCA of 0.24, whilst Ejumula and Naspot 10 O were the best male parents with -1.18 each.

During the season B Naspot 11 stood out as best female parent with a GCA of 0.03 whilst all the male parents had a GCA of 0.01 except Naspot 5/58 which had a GCA of 0 for storage root weight. Resisto still had the best GCA as female parent (1.22), while Ejumula appeared to have the highest GCA (0.37) as male parent. Magabali along with SPK004 had the lowest GCA as female and male parents with -0.05 and -0.06, respectively for SPVD resistance. Naspot 11 had the highest GCA for vine weight with 0.19 and Ejumula the highest GCA among male parents with -0.29.

The seven best SCA were all positive for total storage root in seasons A and B. B2×A8 (Magabali×Huarmeyano) cross had the highest SCA in season A while B1×A5 was the best cross in season B with 0.09 and 0.02, respectively. For flesh color SCA ranged from 1.61 to 0.51 in season A and from 0.79 to 0.45 in season B. For both seasons, B3×A5 (Naspot 5 × Naspot 7) showed the

**Table 9.** GCA effects of parents for the measured traits in season A.

| Female parent | General combining ability Season A | | | | Observation |
|---|---|---|---|---|---|
| | **TSRW** | **Flesh color** | **SPVDR** | **Vine weight** | |
| New Kawogo | 0.09 | -0.35 | 0 | -0.03 | 218 |
| NASPOT 11 | 0.08 | -0.72 | 0.02 | -0.09 | 241 |
| Resisto | 0.04 | 1.89 | 0.06 | -0.1 | 240 |
| Magabali | 0.01 | -0.56 | 0 | 0.24 | 244 |
| Huarmeyano | -0.01 | -0.07 | 0 | 0.04 | 244 |
| Mugande | -0.04 | -0.13 | -0.02 | 0.13 | 242 |
| Wagabolige | -0.06 | -0.59 | 0.02 | -0.14 | 226 |
| NASPOT 5 | -0.08 | 0.51 | -0.04 | -0.02 | 240 |
| **Male parent** | | | | | |
| Ejumula | 0.05 | 0.88 | 0.02 | -1.18 | 224 |
| NASPOT 1 | 0.03 | -0.32 | -0.01 | -1.21 | 242 |
| NASPOT 10 O | 0.03 | 0 | 0.02 | -1.18 | 245 |
| NK259L | 0.01 | -0.3 | 0.01 | -1.19 | 240 |
| NASPOT 7 | 0 | -0.02 | 0.01 | -1.19 | 243 |
| Dimbuka-Bukulula | 0 | -0.5 | 0.01 | -1.19 | 241 |
| SPK004 | -0.03 | 0.15 | -0.01 | -1.21 | 243 |
| NASPOT5/58 | -0.07 | 0.22 | 0.01 | -1.19 | 218 |

TSRW: Total storage root weight, SPVD: sweet potato virus disease resistance.

**Table 10.** GCA effects of parents for the measured traits in season B.

| Female parent | General combining ability Season B | | | | Observation |
|---|---|---|---|---|---|
| | **TSRW** | **Flesh color** | **SPVD** | **Vine weight** | |
| NASPOT 11 | 0.03 | -0.67 | -0.11 | 0.19 | 241 |
| Resisto | 0.01 | 1.22 | 0.23 | -0.26 | 240 |
| New Kawogo | 0.01 | -0.16 | -0.07 | 0.13 | 218 |
| Huarmeyano | 0.01 | 0.32 | 0.15 | -0.1 | 244 |
| Magabali | 0.01 | -0.31 | -0.05 | 0.11 | 244 |
| Wagabolige | 0 | -0.79 | 0.02 | -0.17 | 226 |
| Mugande | 0 | 0 | -0.04 | 0.09 | 242 |
| NASPOT 5 | 0 | 0.35 | -0.11 | 0.04 | 240 |
| **Male parent** | | | | | |
| Ejumula | 0.01 | 0.37 | 0.09 | -0.29 | 224 |
| NASPOT 1 | 0.01 | -0.32 | 0.05 | -0.33 | 242 |
| NASPOT 10 O | 0.01 | -0.08 | 0 | -0.38 | 245 |
| NK259L | 0.01 | -0.02 | -0.12 | -0.5 | 240 |
| NASPOT 7 | 0.01 | 0.16 | -0.07 | -0.45 | 243 |
| Dimbuka-Bukulula | 0.01 | -0.31 | 0.08 | -0.3 | 241 |
| SPK004 | 0.01 | 0.18 | -0.06 | -0.44 | 243 |
| NASPOT5/58 | 0 | 0.06 | 0.06 | -0.32 | 218 |

TSRW: Total storage root weight, SPVD: sweet potato virus disease resistance.

highest SCA. B4×A5 and B7×A4 had the best (lowest) SCA for SPVD resistance, respectively for seasons A and B. The seven best SCA for vine weight ranged from 1.64 to 1.47 (in season A) and from 1.12 to 0.64 (in season) with B8×A7 being the best cross in season A and B1×A8 being the best cross in season B. The GCA/SCA ratios were calculated for all traits in each season separately and shown in Table 12. The ratios were all > 0.5 for all traits in both seasons except for vine weight where it was equal to 0.5 in season A and 0.46 in season B.

## Correlation tests between seasons and traits

Spearman rank tests were run to see the consistency of the performance of the progenies between the seasons.
All the correlations were significantly positive. The highest correlation was obtained for flesh color (R=0.41) followed by storage root weight (R=0.31). The lowest correlation was gotten between SPVD-SA and SPVD-SB (R=0.20).

Correlations between traits were computed using the mean performance of every clone in the two seasons for the measured traits. Storage root weight was significantly and positively correlated to other traits except for flesh color where the correlation was non-significant (Table 14). Flesh color performances were significantly correlated with SPVD performances, while a weak and significant negative correlation (-0.079) was found between flesh color and vine weight. Vine weight and SPVD status were also significantly and negatively correlated (-0.04).

# DISCUSSION

## Performance of F1's and parents in season A

This study was based on an unreplicated trial, which in many regards, is quite different from replicated designs. Replication along with randomization and blocking are the backbones of any experimental design (Girma and Machado, 2013), although during early breeding stages when the main focus is to rank genotypes based on their performances, it is more practical to conduct unreplicated designs. However, replication becomes mandatory in late stages of breeding programs as it increases the accuracy of estimates of cultivars differences as well as their respective performances, which is important for breeding value prediction immediately prior to commercial release (Kempton and Gleeson, 1997). In the early generations of a breeding like in our study, however, the benefits from replication are less clear because the main focus here lies on ranking genotypes rather than predicting their performances (Kempton and Gleeson, 1997). In our study yield on total storage root results can be analyzed from different angles. The population performed better in season A than season B with means of 1.68 and 0.69 Kg/plot respectively. Clearly, environmental conditions were more favorable in the first season. However, there was a consistency between seasons because the high yielding families did not differ that much across seasons. In both seasons, checks performed better than overall progenies and other parents, which is in agreement with the findings of (Mwanga et al. 2007, 2011) who recognized Naspot 11 and Ejumula as good performers for storage root yield. The frequency of the checks was maintained high to lower plot error considerably and to improve the efficiency of the ranking (Kempton and Gleeson, 1997). Because of field heterogeneity and the high number of progenies (1896), all the F1 individuals were ranked and the best 10% of the overall population was selected to infer the extend of the genetic gain of the crosses. This group had higher means than the overall population as well as the parents for all traits across seasons, indicating thus a genetic gain of some progenies over their respective parents. This not only shows that some crosses gave offsprings with added value, but also that some combinations (families) were more efficient than others. For flesh color the 10% best progenies had means superior to those of the checks in both seasons, showing a high genetic gain that was strongly statistically supported. Furthermore, Resisto was the female parent to almost all the 10% best progenies, possibly because it is an orange-flesh cultivar (Tumwegamire et al., 2014a, b) and also carries dominant alleles for this trait. The whole population showed a better resistance status to SPVD in season A than season B. This can be attributed to the buildup of viruses between season one and two of planting material. In fact, planting materials were taken from the same net-tunnels for both seasons. The best 10% offsprings performed however better in season B this could possibly be explained by their added genetic predisposition to resist to the virus. Vine weight performance was better in season A for the whole population as well as for the checks and the 10% best offsprings. This trait being genetically controlled; it appears that genotypes had a large variety of genetic makeups resulting in different vine weight yields. These present results are closely similar to the findings of Rahman et al. (2015).

Analysis of variance for family performance was done using Kruskal-Wallis test (a non-parametric test) because the data were not normally distributed. Significant Chi squares for families on total storage root demonstrated genetic variation between crosses. Significant differences ($p \leq 0.001$) were also noticed between families from the Kruskal-Wallis test for all the other traits across the two seasons. These results are consistent with the findings of Rukundo et al. (2017). That study concluded that there was a significant difference between means of sweetpotato families on storage root, flesh color, vine weight and biomass though the authors used ANOVA. Therefore, this suggests that crosses were actually consistently and significantly of different breeding value and that selection can be made for taking the better progenies to the next stage of breeding.

### Mid parent heterosis

Heterosis has been used in many crops to harness dominance variance through production of hybrids (Olfati et al., 2012b). The mid-parent heterosis, also relative heterosis for the seven best families (~10%) was calculated for all traits in every season. Specific parental backgrounds resulted in progeny with high MPH. In season A, for storage root weight, Resisto, Magabali and New Kawogo were the only female parent represented while Naspot 1, N259KL, Ejumula, Naspot 7 and Naspot 10 O were the male parents involved, showing that these parents can be candidates for having elite varieties for storage root. In season B on the other hand, Resisto was the only female parent for all the progenies, while the male parents were NK259L and Naspot 7. This further confirms the huge potential of using these cultivars to give high yielding progenies for storage root. In this study, the improved performances of hybrids relative to their parents can be explained by favorable allelic interactions at heterozygous loci that outperform either homozygous states or by the fact that deleterious and recessive alleles at different loci in the parental genomes are masked in the F1 hybrids thus producing a better phenotype.

For flesh color, Resisto was the best female parent in season A and B. In season B MDP317 clone, belonging to B1×A8 (Resisto×NK259L) had the highest MPH. This could be explained by the fact that dominant alleles brought by Resisto had masked the recessive alleles brought by NKL259L which is cream-flesh. This result could suggest that alleles for ‘orange-fleshed root’ are dominant against alleles for ‘cream-fleshed root’ in sweet potato. For disease resistance the best mid-parent heterosis is the negative ones because negative values show less signs of infections. In that regard MDP355 clone from B2×A2 (Magabali×Naspot 1) family showed the best heterosis in season A. Knowing that Magabali is very susceptible variety to SPVD (Mwanga et al., 2011), these findings may imply that genes responsible for SPVD resistance are homozygous and recessive in Magabali. Mid-parent heterosis for vine weight was better in seasons A than B. This can be attributed to the high buildup of virus in planting materials during season B. MDP1546 clone from B8×A7 family had the highest heterosis and Huarmeyano was the most represented female parent followed by Magabali and Mugande suggesting that these parents could be carriers of either recessive or dominant alleles for vine weight. MDP 1774 clone from B7×A3 family showed the highest heterosis in season B with New Kawogo being the most represented female parent and Naspot 10 O the most represented male parent. These findings are in agreement with previous results (Mwanga et al., 2011) where New Kawogo was described as being high biomass yielding cultivar.

### GCA and SCA effects

The GCA effects of parental lines were calculated and shown in Tables 9 and 10 for seasons A and B, respectively. Though significance levels were not calculated, the values obtained were statistically strong because each parent (male or female) was observed at least 218 times.

It appeared that for total storage root, New Kawogo and Naspot 11 were the best general combiners during the first season, while Naspot 11 only had the highest GCA effect among all parental lines in season B, an indication that these parents may be used for improving storage root yield. Combining ability studies have been conducted in many crops ranging from cereals, roots to legumes, indicating that it is a crucial tool in plant breeding (Fasahat et al., 2016). In sweet potato, though some more efforts need to be done, studies on combining ability estimation are readily available (Esan and Omilani, 2018; Musembi et al., 2015; Rukundo et al., 2017). Resisto and Ejumula had the GCA effects on flesh color suggesting that these parents must be included in a breeding scheme where the target is to develop cultivars with high beta-carotene content. Naspot 5 along with NKL259L, Naspot 7 and SPK004 were the best general combiners for sweet potato disease resistance, indicating that these parents, when included in a breeding program, will produce progenies with high resistance to SPVD. For vine weight, Mugande and Naspot 11 and Magabali were the best general combiners, these parents may be carriers of dominant alleles for high vine weight yields.

SCA effects of all crosses were calculated and ranked. The seven best SCA effects for every trait in each season were chosen and shown in Table 11. SCA effects for storage root were higher in season A (due to the virus buildup in season B) and the best cross being B2×A8 (Magabali×NK259L) could be explained by additive gene effects from both parents. For flesh color B3×A5 (Naspot 5×Naspot 7) stood out as the best cross in both seasons, indicating also additive genetic actions from both parents. Crosses B4×A5 (Wagabolige×Naspot 7) and B7×A4 (New Kawogo×Naspot 10 O) showed the best SCA for SPVD in seasons A and B, respectively. It appeared also that parent A6 (SPK004) was involved in 5 out of the seven best crosses in season B, provided that SPK004 is moderately resistant (Mwanga et al., 2007), this finding could suggest that this parent carries recessive alleles for SPVD resistance. For vine weight, the best SCA were all positive, this shows that non-additive genetic events were highly pronounced in the designated crosses. SCA effects were higher in season A with B8×A7

**Table 11.** SCA of the best crosses for the measured traits in seasons A and B.

| Season | TSRW | | Flesh color | | SPVDR | | Vine weight | |
|---|---|---|---|---|---|---|---|---|
| | Crosses | SCA | Crosses | SCA | Crosses | SCA | Crosses | SCA |
| | B2×A8 | 0.09 | B3×A5 | 1.61 | B4×A5 | -0.07 | B8×A7 | 1.67 |
| | B7×A1 | 0.07 | B3×A1 | 1.19 | B2×A2 | -0.06 | B2×A5 | 1.60 |
| | B6×A1 | 0.07 | B5×A4 | 0.76 | B1×A7 | -0.06 | B5×A1 | 1.59 |
| Season A | B7×A5 | 0.07 | B7×A4 | 0.75 | B5×A2 | -0.05 | B4×A8 | 1.57 |
| | B6×A3 | 0.06 | B1×A1 | 0.70 | B7×A5 | -0.04 | B7×A2 | 1.49 |
| | B5×A4 | 0.06 | B2×A8 | 0.56 | B4×A4 | -0.04 | B7×A3 | 1.48 |
| | B1×A2 | 0.06 | B8×A7 | 0.51 | B1×A6 | -0.04 | B3×A4 | 1.47 |
| | B1×A5 | 0.02 | B3×A5 | 0.79 | B7×A4 | -0.35 | B1×A8 | 1.12 |
| | B5×A5 | 0.02 | B5×A4 | 0.67 | B7×A8 | -0.24 | B2×A5 | 0.93 |
| | B6×A8 | 0.01 | B8×A7 | 0.64 | B6×A6 | -0.23 | B8×A5 | 0.84 |
| Season B | B3×A4 | 0.01 | B6×A5 | 0.59 | B1×A6 | -0.22 | B4×A8 | 0.81 |
| | B1×A8 | 0.01 | B4×A6 | 0.59 | B2×A6 | -0.17 | B6×A5 | 0.65 |
| | B4×A8 | 0.00 | B3×A1 | 0.48 | B3×A6 | -0.15 | B3×A5 | 0.65 |
| | B4×A2 | 0.00 | B1×A8 | 0.45 | B7×A6 | -0.14 | B7×A7 | 0.64 |

TSRW: Total storage root weight, SCA: specific combining ability, SPVDR: sweetpotato virus disease resistance.

**Table 12.** GCA/SCA ratios for the measured traits in seasons A and B.

| Parameter | TSRW SA | TSRW SB | Flesh color SA | Flesh color SB | SPVDR SA | SPVDR SB | Vine weight SA | Vine weight SB |
|---|---|---|---|---|---|---|---|---|
| GCA/SCA | 0.67 | 0.61 | 0.79 | 0.76 | 0.55 | 0.62 | 0.5 | 0.46 |

TSRW: Total storage root weight, SCA: specific combining ability, GCA: general combining ability, SPVDR: sweet potato virus disease resistance, SA: season A, SB: season B.

(Huarmeyano×Naspot 10 O) being the cross with the best SCA effect followed by B2×A5 (Magabali×Naspot 7). Magabali being a low yielding variety (Mwanga et al., 2007) and Naspot 7 being moderately yielding variety, this result agrees with the fact that non-additive genetic action is predominant for vine weight. Predominant genetic actions were looked at for each trait by calculating GCA/SCA ratios and the results are shown in Table 12. Ratios were greater than 0.5 for storage root, flesh color and SPVD resistance, implying predominance of additive over non-additive genetic effects. This ratio was however equal to 0.5 for vine weight in season A and to 0.46 in season B suggesting a significant role of non-additive genetic effect on this trait. These results are in strong agreement with the findings of (Rukundo et al., 2017). The same results were also obtained by Musembi et al. (2015) when studying the predominant genetic action for fresh root.

## Correlation tests between traits and seasons

Spearman rank correlation tests were conducted to see the consistency in the performances of each genotype from one season to the other. The results of the tests are assigned in Table 13. All the tests were significantly positive. However, the highest correlation coefficient did not reach 0.5, showing that the environmental factor between trials was quite significant. The strongest correlation was seen in flesh color followed by total storage root (0.31). The lowest correlation was between the virus resistance in seasons A and B and this is because planting material used in season B was already infested by SPVD.

Correlation tests were also conducted between traits. Storage root was positively correlated to all other traits but only significantly with SPVD resistance and vine weight at $P \leq 0.05$ and $P \leq 0.001$, respectively. These results can be explained by two facts: the slight positive correlation found between storage root yield and SPVD resistance is explained by the fact that estimation of virus symptoms was collected from vines rather than roots and genotypes with more vigorous vines tend to produce more storage roots. Part of these results is in agreement with the conclusion of Badu (2018) who found a positive and significant correlation between storage root yield and

**Table 13.** Spearman rank correlation tests between same traits across the two seasons.

| Correlation | TSRW-SB | Flesh color-SB | SPVDR-SB | Vine weight-SB |
|---|---|---|---|---|
| TSRW-SA | R=0.31*** | | | |
| Flesh color-SA | | R=0.41*** | | |
| SPVDR-SA | | | R=0.20*** | |
| Vine weight-SA | | | | R=0.26*** |

***: Signifiant P≤ 0.0001, R: Correlation coefficient, TSRW: total storage root weight, SCA: specific combining ability, GCA: general combining ability, SPVDR: sweet potato virus disease resistance, SA: season A, SB: season B.

**Table 14.** Pearson correlation tests of the mean performances of progenies for combined seasons.

| Correlation | TSRW | Flesh color | SPVDR | Vine weight |
|---|---|---|---|---|
| TSRW | | 0.0017$^{ns}$ | 0.06* | 0.16*** |
| Flesh color | | | 0.093*** | -0.079** |
| SPVDR | | | | -0.04* |
| Vine weight | | | | |

*,**,***, ns: Significant at P ≤ 0.05, P ≤ 0.01, P ≤ 0.001, non-significant, respectively. SPVDR: Swee tpotato virus disease resistance, TSRW: total storage root weight.

vine weight. This indicates the feasibility of improving sweet potato for better storage root yield as well as SPVD resistance. The same is also true for storage root and flesh color and vine weight. Flesh color was positively correlated with SPVD resistance at $P \leq 0.001$ and negatively correlated with vine weight at $P \leq 0.01$. And finally, there was a negative and significant correlation between SPVD resistance and vine weight at $P \leq 0.05$, probably because high virus infestations impeded establishment of vines. These findings will be insightful to sweet potato breeders as they allow them to predict in which way a given trait is going to vary if another improves or decreases.

## Conclusion

The different performances between the two seasons indicated that the environmental conditions as well the quality of the planting material between seasons had a true influence on the genotypes. Analyses of variance showed that some crosses performed significantly better than others. The GCA to SCA variance ratios indicated that additive gene action was more predominant than non-additive gene action in controlling all the traits observed except for vine weight. Magabali combined well with NK259L giving the highest root yielding progenies in season A while during season B the best cross was between Resisto and Naspot 7. Thus, these parents can be incorporated in breeding programs for improving storage root yield. Naspot 5 combined with Naspot 7 to give the best SCA for flesh color in both seasons. Therefore, progenies from this cross can be promoted to have varieties with high beta-carotene content. Combining SPK004 with New Kawogo or Naspot 5 will be a good strategy for improving resistance to SPVD. The combinations of Ejumula or Naspot 10 O with Huarmeyano are the best crosses for improving vine weight. Clones that had high heterosis for every trait in one season were not necessarily the same in the other season. This meant that they were unstable across environments and could be evaluated for use in further trials with more controlled conditions. One major finding is that it is possible to breed sweet potato varieties for having higher beta-carotene content as well as being resistant to SPVD. This result is more important when knowing that most of the current orange-fleshed sweet potato cultivars we have are susceptible to SPVD.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Spatial distribution and sampling size for monitoring of African maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) on maize (*Zea mays* L.) in Southern Ethiopia

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**The African maize stem borer (*Busseola fusca*) is one of the important biotic constraints for maize production in sub-Saharan African. This study determined the spatial distribution and sampling sizes for African maize stem borer in Southern Ethiopia. Twenty four maize farms were visited in 12 localities at three growth stage of maize. Data were collected on the number of infested and not-infested plants, and the number of larvae and pupae. There were variations in the levels of infestations and population density of *B. fusca* in the study areas and years. Percent infestation at mid-whorl stage of maize ranged from 13.6 to 25.9% and 19.5 to 41.4% in 2015 and 2016, respectively. Infestation increased through time and at harvesting stage reached ranges of 36.8 to 68.8% in 2015 and 65.5 to 80.7% in 2016. The optimal sample size for four fixed precision levels of 0.10, 0.15, 0.20 and 0.25 were estimated with Iwao's regression coefficients. The distribution pattern of B. fusca varied between maize growth stages, locations and years. At mid-whorl stage of maize, *B. fusca* infested plants were aggregated but in both at silking and harvesting stage uniformly distributed. At mid-whorl as well as silking stage of maize B. fusca larvae were aggregated. But, larvae at harvesting stage and pupae in both silking and harvesting stages of maize were randomly distributed. For 10% infestation, which is considered as action threshold level for stem borers management on maize, 22 sampling units (660 plants) at the precision of 20% are required.**

**Key words:** *Busseola fusca*, maize, spatial distribution, sample size, precision level.

## INTRODUCTION

Maize (*Zea mays* L.) is one of the main food and feed crops in Ethiopia and worldwide (FAO, 2018). In Africa, maize is among the most important field crop providing food, feed and fuel (Smale et al., 2011). Ethiopia is the

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fourth largest maize producing country in Africa, and first in the East African region (FAO, 2018). Maize is mainly grown in Oromia, Amhara, Southern Nations, Nationalities, and Peoples' Region (SNNPR), and Tigray (CSA, 2017/2018). In SNNPR, the average productivity of maize is 3.8 t/ha (CSA, 2017/18) which is slightly less than the national average yield of 3.9 t/ha (CSA, 2017/2018) but much lower than the world average of 5.8 t/ha (USDA, 2018). The low productivity of maize could be attributed to many abiotic and biotic factors (Getu et al*.,* 2001; Desalegn et al., 2012; Tilahun et al., 2012).

Among the biotic factors, insect pests, particularly stem borers are responsible for the low yield of maize crop (Getu et al., 2001; Wale et al*.,* 2006). Yield losses in Ethiopia due to stem borers vary with agroecology, but generally range from 15 to 100% depending on infestation by the pest species, crop and crop growth stage attacked (Wale et al., 2006). In East Africa, the noctuid *Busseola fusca* (Fuller) and the crambid *Chilo partellus* (Swinhoe) are the most important insect pests associated with maize (Mwalusepo et al., 2015). The two lepidopterous stem borers are economic pests of maize in Ethiopia (Wale and Ayalew, 1993; Wale al et., 2006). Gebre-Amlak (1985) reported that *C. partellus* was a predominant species at low elevation (less than 1700 m) and *B. fusca* was dominant at elevations between 1160-2600 m.a.s.l. and cooler areas of Ethiopia.

Spatial distribution is one of the characteristic properties of insect populations; in most cases, it allows us to define them, and is a typical trait in insect populations and is an important characteristic of ecological communities (Debouzie and Thioulouse, 1986). Understanding the distribution and phenology of insects in a different environment is important to plan management practices (Searle et al., 2013). No field sampling can be efficient without understanding the underlying spatial distribution (Taylor, 1984). Spatial distribution allows for the estimation of densities and in turn forms the basis for deciding on pest management programs (Khaing et al., 2002). The appropriate sampling pattern depends on the spatial distribution of the insect or disease (Lin et al., 1979). Insect populations may follow a random, uniform or aggregate distribution, but the degree of aggregation often varies among the population and species (Root and Cappuccino, 1992). The spatial distribution of stem borers varies among and within-host plants possibly due to their suitability for oviposition and larval development (Addo - Bediako and Thanguane, 2012).

Management method cannot be implemented effectively without accurate estimates of insect population and its effects on yield (Nabil, 2010). To estimate insect density, sampling time, sampling unit and sampling size are crucial (Southwood and Henderson, 2000). The number of samples size and units could be varied with insects being sampled, their distribution patterns (Southwood and Henderson, 2000); purpose of sampling, infestation pattern, severity and economic considerations (Frisbie and Whorter, 1986). Too few sample sizes will reduce the value of the estimate (Vlug and Paul, 1986) and too many will increase the cost of the program (Blackshaw and Hicks, 2013). Therefore, the present study was designed to determine the spatial distribution patterns and the sampling size for monitoring of *B. fusca* on maize.

## MATERIALS AND METHODS

### Study areas

The study was conducted in Wolaita, Sidama and Gurage zone (Table 1) which are found in southern Ethiopia during 2015 and 2016 main cropping seasons (May- October). The number of districts, localities, sampling farms, sampling plots and plants in each zone are described in Table 2. Districts and localities were selected based on road accessibility and intensity of maize production.

### Sampling procedures

In this study, twenty four maize farms in 12 localities having similar inputs and management practices were covered. The farms did not receive any insecticide treatment and grew the popular maize varieties BH540 and Shone. In each maize farm five sampling spots with a size of $9m^2$ each were measured in 'X' pattern at mid-whorl, silking and harvesting stages of maize. In each spot, the total number of plants (30 plants on the average) and those infested by stem borers (characterized by dead heart, scarified leaves, and larval entry and exit holes in stems, the presence of frass) were recorded and percent of infestation (%) calculated using the formula $IP\% = \frac{IP*100}{TP}$ Where, IP = infested plants, TP = total plants. When infestations were observed, ten plants were randomly selected from each spot and dissected to record the number of larvae and pupae. The same fields were used for samplings at different stages of maize.

### Spatial distribution pattern determination

The spatial distribution pattern of *B. fusca* was determined by using four indices, namely Taylor's power law, Iwao's mean crowding regression, Lloyd's mean crowding, and index of dispersion. Percent of infested plants and numbers of insects per spot were used.

### Taylor's power law (1984)

$\log(S^2) = a + b\log(\bar{X})$, where $S^2$ is the variance, $\bar{X}$ is sample mean, a is intercept and b is the slope. When b=1, b < 1 and b> 1 the distribution is random, uniform and aggregated, respectively.

### Iwao's regression (1968)

Iwao's regression method was used to quantify the relationship between mean crowding index (X*) and mean density (*m*) using by solving the following equation: $X^* = \alpha + \beta\bar{X}$ . Where α indicates the tendency to crowding (positive) or repulsion (negative) and β =1, β

**Table 1.** Description of the study areas in Southern Ethiopia.

| Zone | Altitude (m.a.s.l.) | Annual Average temperature ($^0$c) | | Annual Average rainfall (mm) | | Distance from Addis Ababa (km) |
|---|---|---|---|---|---|---|
| | | Minimum | Maximum | Minimum | Maximum | |
| Gurage | 1001- 3600 | 13.0 | 30.0 | 600 | 1600 | 158 |
| Sidama | 501-3000 | 10.0 | 25.0 | 801 | 1600 | 275 |
| Wolaita | 1200 - 2950 | 15.1 | 25.1 | 1200 | 1300 | 340 |

Source: SNNPRS Resource Potential and Investment Opportunities (2008).

**Table 2.** Study areas, number of sampling farms per locality, plots per farm and plants per plot.

| Study area | | | No of sampling farms/locality | No of sampling plots/farm | No of sampling plants / plot |
|---|---|---|---|---|---|
| Zone | Districts | Localities | | | |
| Gurage | Mareko | Dida Halibo | 2 | 5 | 10 |
| | | Dida Midore | 2 | 5 | 10 |
| | Meskane | Dida | 2 | 5 | 10 |
| | | Ochageneme | 2 | 5 | 10 |
| Hawassa | Wondo Genet | Aruma | 2 | 5 | 10 |
| | | Youwo | 2 | 5 | 10 |
| | Hawassa Zuria | Jara Kerara | 2 | 5 | 10 |
| | | Jara Demuwa | 2 | 5 | 10 |
| Wolaita | Damot Gale | Shasha Galea | 2 | 5 | 10 |
| | | Buge | 2 | 5 | 10 |
| | Boloso Sore | Wermuma | 2 | 5 | 10 |
| | | Dola | 2 | 5 | 10 |

< 1 and β > 1 the distribution is random, uniform and aggregated, respectively.

#### Index of dispersion ($S^2/\bar{X}$ and Z)

$S^2/\bar{X}$ variance ($S^2$) to mean ($\bar{X}$) ratio was calculated and values 1 random, < 1 uniform and > aggregated distribution. The index of dispersion (ID); ID = (n-1) $S^2/\bar{X}$, where n denotes the number of samples. The index was tested by Z value as follows:- $Z = \sqrt{2I_D} - \sqrt{(2v-1)}$, where v = n -1 if 1.96 >= Z > - 1.96 and the distribution is random but if Z < - 1.96 or Z > 1.96, it would be uniform and aggregated, respectively (Patil and Stiteler, 1978).

#### Lloyd's means crowding (1967)

$X^* = \bar{X} + S^2/\bar{X} - 1$, where $S^2$ is variance and $\bar{X}$ is the sample mean. To remove the effect of changing in density, the ratio of mean crowding to the mean was used and $X^*/\bar{X}$ =1 random, <1 uniform and >1 aggregated

#### Sample size determination

At mid-whorl stage of maize, the number of required sampling units per field were determined using proportion of infested plants and Kuno (1969) formula $n = (a+1/\bar{X} + \beta-1)/D^2$, Where n = number of sampling units; $\bar{X}$ means of infestation; a and β are coefficients obtained from Iwao's regression, D = precision level. The allowable precision levels (10, 15, 20 and 25 %) in ecological research (Southwood and Henderson 2000) were used.

#### Data analysis

*B. fusca* distribution indices were generated by using SPSS 21.0 software. Mean of *B. fusca* infestation, larvae and pupae in each zone, maize growth stage and year ware analysis using SPSS software. The count and percent data were transformed using square root and arcsine, respectively.

## RESULTS

### Levels of infestations and population density of *B. fusca* larvae and pupae

There were variations in the levels of infestations and population density of larvae and pupae in the different study locations and years (Table 3). Percent infestation by *B. fusca* at mid-whorl stage of maize ranged from 13.7

to 25.9% and 19.4 to 41.4% in 2015 and 2016, respectively. The level of infestation increased through time and at harvesting stage reached to ranges of 36.8 to 68.8% in 2015 and 65.5 to 80.7% in 2016. The numbers of larvae were higher at mid-whorl stage (4.14 to 5.8 / plant in 2015 and 4.05 to 7.7 /plant in 2016) than the subsequent stages of maize. Pupae were recovered starting the silking stage of maize and there were 0.5 to 1.14 and 0.7 to 1.55 pupae per plant in 2015 and 2016, respectively.

## Distribution of infested maize plants with *B. fusca*

In both years, at mid-whorl stage the index of dispersion and Lloyd's mean crowing ($S^2/\bar{X}$ and $X^*/\bar{X}$) *for* percent number of infested plants were greater than one and the coefficients of Taylor's power law (b) and Iwao's patchiness regression (β) were significantly greater than one (Table 4). Index of dispersion ($S^2/\bar{X}$) ranged from 0.99 to 1.81; Lloyd's mean crowing ($X^*/\bar{X}$) from 1.18 to 1.72; coefficients of Taylor's power law (b) from 1.10 to 1.91 and Iwao's regression (β) from 1.40 to 2.40; whereas at silking as well as maturity stage of maize, all the distribution indices were less than one. The study showed that at the mid-whorl stage of maize, the distribution pattern of *B. fusca* infestation was aggregated and uniform at both silking and harvesting stages of maize.

## Distribution of *B. fusca* larvae at mid-whorl stage of maize

During the study periods, *B. fusca* was the only stem borer species recorded in the three zones of the study areas. In both years, at the mid-whorl stage of maize, the index of dispersion ($S^2$/) for *B. fusca* larvae was greater than one (1.46 to 2.64); Z value was greater than 1.96 and Lloyd's mean crowding ($X^*/\bar{X}$) ranged from 1.10 to 1.51 (Table 5). The coefficients of Taylor's power law (b) and Iwao's (β) were significantly greater than one and ranged from 1.82 to 3.88 and 1.14 to 1.7, respectively. All the dispersion values indicated that at the mid-whorl stage of maize *B. fusca* larvae had an aggregated distribution.

## Distribution of *B. fusca* larvae and pupae at silking stage of maize

Similar to mid-whorl stage, at silking stage of maize, *B. fusca* larvae distribution indices ($S^2/\bar{X}$ and $X^*/\bar{X}$) were greater than one; Z values greater than 1.96; Taylor power and Iwao coefficients were significantly greater than one (Table 5). In both years, the index of dispersion ($S^2/\bar{X}$) ranged from 1.24 to 2.30; (Z) from 1.87 to 3.20; Lloyd's mean crowing ($X^*/\bar{X}$) ranged 1.08 to 1.52 and the Taylor power coefficients (b) ranged from 1.21 to 3.54. The slopes of the regression lines for Iwao's mean crowding regression were also numerically greater than one, ranged from 1.2 to 1.4. The dispersion values indicated that *B. fusca* larvae were aggregated at silking stage of maize. Index of dispersion ($S^2/\bar{X}$) of pupae ranged from 0.84 to 1.26; with (Z) values from 0.90 to 1.31; Lloyd's mean crowing ($X^*/\bar{X}$) ranged from 0.82 to 1.30 and coefficients of Taylor's power law *(*b) ranged from 0.94 to 1.34 (Table 6). The slopes of the regression of Iwao's were near to one and ranged from 0.89 to 1.24. All the indices indicated that at silking stage of maize *B. fusca* pupae had a random distribution.

## Distribution of *B. fusca* larvae and pupae at harvesting stage of maize

Unlike mid-whorl and silking stages, at harvesting stage of maize *B. fusca* larvae distribution indices ($S^2/\bar{X}$ and $X^*/\bar{X}$) were near to one; Z values less than 1.96; Coefficients Taylor power (b) and Iwao values (β) were not significantly different from one (Table 5 and 6). The larvae index of dispersion ($S^2/\bar{X}$) ranged from 0.97 to 1.26; Z values from 1.08 to 1.43; Lloyd's mean crowding ($X^*/\bar{X}$) from 0.99 to 1.38 and Taylor power coefficients (b) ranged from 1.0 to 1.36 (Table 5). Similarly, the slopes of the regression lines of Iwao's regression (β) were not significantly greater than one and ranged from 1.01 to 1.20. The index of dispersion ($S^2/\bar{X}$) for pupae ranged from 0.93 to 1.21 with (Z) values from 0.90 to1.53; Lloyd's mean crowding ($X^*/\bar{X}$) ranged from 0.86 to 1.15 and the coefficients of Taylor's power law (b) ranged from 0.68 to 1.32; Iwao's coefficients (β) from 0.82 to 1.09 (Table 6). These results indicate that in both years, the distribution of *B. fusca* larvae and pupae at harvesting stage of maize was random.

## Sampling size based on percent infestation of maize at mid-whorl stage

Sample size estimates were similar for the two years. The required sample units to estimate 5 to 30% mean infestation of maize by *B. fusca* ranged from,101 - 73, 45 - 32, 25-18 and 16 to 12 in 2015 and 104-76, 42-30, 26-19 and 17 to 12 in2016, for 10, 15, 20 and 25% precision, respectively (Figure 1). For 10% infestation, which is considered as action threshold level for stem bores management on maize, 85, 38, 22 and 14 sampling units were required for 10, 15, 20 and 25% precision, respectively.

# DISCUSSION

Infestation of maize with *B. fusca* was aggregated at

**Table 3.** Levels of infestations and population density of *B. fusca* larvae and pupae in 2015 and 2016.

| Location (Zone) | Stage of maize | 2015 | | | 2016 | | |
|---|---|---|---|---|---|---|---|
| | | Larvae/ plant | Pupae/ plant | Infestation (%) | Larvae/ plant | Pupae/ plant | Infestation (%) |
| Gurage | Mid-whorl | 5.8±0.24 | | 25.9±4.7 | 7.7±0.14 | | 41.4±1.2 |
| | Silking | 3.0±0.18 | 1.2±0.08 | 42.3±4.8 | 2.7±0.15 | 1.6±0.09 | 67.3±4.8 |
| | Harvesting | 1.2±0.08 | 0.6±0.06 | 68.8+5.2 | 1.3±0.11 | 0.7±0.08 | 80.7±5.4 |
| Sidama | Mid-whorl | 4.2±0.21 | | 13.6±2.7 | 4.1±0.21 | | 19.5±2.1 |
| | Silking | 2.5±0.17 | 1.1±0.06 | 21.3±2.1 | 2.3±0.14 | 1.4±0.12 | 33.0±3.9 |
| | Harvesting | 0.9±0.07 | 0.6±0.5 | 36.8±1.5 | 0.9±0.09 | 0.8±0.07 | 65.5±6.5 |
| Wolaita | Mid-whorl | 4.7±0.18 | | 21.5±2.5 | 6.0±0.17 | | 32.0±1.6 |
| | Silking | 3.0±0.19 | 1.3±0.07 | 45.0±1.2 | 2.3±0.08 | 1.6±0.14 | 60.8±2.3 |
| | Harvesting | 1.3±0.08 | 0.9±0.07 | 60.8±1.7 | 1.5±0.12 | 0.8±0.08 | 72.7±3.4 |

**Table 4.** Distribution of *B. fusca* infestation in maize field derived from two distribution indices and two regressions at three stages of maize crop.

| Zone | Maize growth stage | Year | Indices of dispersion | Lloyd's crowding | Taylor's power | | | Iwao's regression | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $S^2/\bar{X}$ | $X^*/\bar{X}$ | a | b | P-value | a | β | P-value |
| Gurage | Mid-whorl | 2015 | 1.67 | 1.59 | -0.71 | 1.40 | 0.04 | 0.71 | 2.40 | 0.01 |
| Sidama | | | 1.48 | 1.72 | -0.20 | 1.20 | 0.06 | -0.60 | 2.40 | 0.03 |
| Wolaita | | | 1.91 | 1.18 | -1.12 | 1.91 | 0.03 | 0.51 | 1.90 | 0.02 |
| Gurage | | 2016 | 0.99 | 1.31 | -0.40 | 1.70 | 0.03 | 0.24 | 1.90 | 0.02 |
| Sidama | | | 1.04 | 1.57 | -0.62 | 1.50 | 0.02 | 0.60 | 1.40 | 0.05 |
| Wolaita | | | 1.81 | 1.35 | -0.50 | 1.80 | 0.00 | 0.50 | 2.30 | 0.00 |
| Gurage | Silking | 2015 | 0.81 | 0.75 | 0.19 | 1.04 | 0.21 | -0.51 | 0.92 | 0.13 |
| Sidama | | | 0.70 | 0.88 | 0.77 | 0.72 | 0.15 | 0.23 | 0.75 | 0.22 |
| Wolaita | | | 0.82 | 1.12 | 0.35 | 0.59 | 0.11 | 0.31 | 0.83 | 0.10 |
| Gurage | | 2016 | 0.93 | 0.92 | -0.03 | 0.94 | 0.06 | -0.32 | 0.84 | 0.08 |
| Sidama | | | 0.87 | 0.97 | 0.08 | 0.87 | 0.07 | -0.11 | 0.97 | 0.21 |
| Wolaita | | | 0.76 | 0.83 | 0.07 | 0.66 | 0.09 | -0.21 | 0.86 | 0.09 |
| Gurage | Harvesting | 2015 | 0.99 | 0.55 | 0.03 | 0.78 | 0.12 | 0.03 | 0.85 | 0.19 |
| Sidama | | | 0.79 | 0.68 | 0.23 | 1.12 | 0.09 | -0.13 | 0.69 | 0.21 |
| Wolaita | | | 0.74 | 0.54 | 0.16 | 0.83 | 0.07 | -0.15 | 0.74 | 0.13 |
| Gurage | | 2016 | 0.88 | 0.57 | -0.02 | 0.65 | 0.73 | -0.10 | 0.91 | 0.62 |
| Sidama | | | 0.83 | 0.74 | -0.13 | 0.76 | 0.06 | -0.14 | 0.93 | 0.51 |
| Wolaita | | | 0.66 | 0.67 | -0.33 | 0.75 | 0.17 | -0.21 | 0.60 | 0.42 |

P -values test whether or not b and β values for Taylor's power law and Iwao's are significantly different from 1; "a" stands for the intercept ; "b and β" stands slope values for Taylor's power law and Iwao's regression, respectively.

mid-whorl stage but uniform at silking and maturity stage of maize. *B. fusca* larvae were aggregated at both mid-whorl and silking stage of maize. The aggregated distribution pattern of distribution pattern during season could be caused by changes in population density or movement of larvae. *Busseola fusca* females oviposit a highly variable number (from 100 up to 800) of round and flattened eggs in batches (Kruger et al., 2012); larvae migrate to neighboring plants throughout the larval stages (Van Rensburg et al., 1987; Calatayud et al., 2014). Sun and Du (1991) reported that rice stem borer (*Chilo suppressalis*) larvae have an aggregated distribution pattern the active seasons but the dispersal rate of larvae in changes with developmental stages. The

**Table 5.** Spatial distribution of *B. fusca* larvae derived from two distribution indices and two regressions at three growth stage of maize crop in 2015 and 2016.

| Zone | Maize growth stage | Year | Indices of dispersion | | Lloyd's crowding | Taylor's power law | | | Iwao's regression | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $S^2/\bar{X}$ | Z | $X^*/\bar{X}$ | a | b | p-value | $R^2$ | a | β | P-value |
| Gurage | Mid-whorl | 2015 | 1.87 | 2.67 | 1.27 | -2.41 | 3.88 | 0.01 | 0.86 | 1.40 | 1.7 | 0.01 |
| Sidama | | | 1.46 | 2.23 | 1.10 | -1.83 | 2.16 | 0.07 | 0.63 | 1.03 | 1.20 | 0.04 |
| Wolaita | | | 1.77 | 2.40 | 1.23 | -3.03 | 3.46 | 0.04 | 0.72 | 1.40 | 1.40 | 0.00 |
| Gurage | | 2016 | 2.64 | 2.82 | 1.25 | -0.88 | 2.37 | 0.01 | 0.79 | 2.30 | 1.45 | 0.03 |
| Sidama | | | 2.02 | 2.57 | 1.51 | -0.43 | 1.62 | 0.08 | 0.92 | 1.00 | 1.16 | 0.01 |
| Wolaita | | | 2.20 | 3.05 | 1.33 | -0.50 | 2.17 | 0.03 | 0.92 | 1.20 | 1.14 | 0.00 |
| Gurage | Silking | 2015 | 2.30 | 3.20 | 1.52 | -2.12 | 3.54 | 0.00 | 0.91 | 0.20 | 1.30 | 0.00 |
| Sidama | | | 1.24 | 1.87 | 1.21 | -0.87 | 1.97 | 0.03 | 0.48 | 0.95 | 1.13 | 0.06 |
| Wolaita | | | 1.93 | 2.65 | 1.38 | -2.28 | 2.37 | 0.02 | 0.76 | 0.52 | 1.26 | 0.01 |
| Gurage | | 2016 | 1.34 | 2.21 | 1.35 | 0.25 | 2.31 | 0.06 | 0.76 | 0.28 | 1.20 | 0.04 |
| Sidama | | | 1.30 | 1.98 | 1.08 | 0.37 | 1.21 | 0.03 | 0.94 | 0.38 | 1.17 | 0.07 |
| Wolaita | | | 1.74 | 2.35 | 1.53 | -1.20 | 1.50 | 0.04 | 0.91 | 1.14 | 1.12 | 0.02 |
| Gurage | Harvesting | 2015 | 1.09 | 1.19 | 1.21 | 0.21 | 1.18 | 0.56 | 0.87 | -0.59 | 1.01 | 0.12 |
| Sidama | | | 0.97 | 1.08 | 0.99 | 0.14 | 1.00 | 0.49 | 0.50 | -0.03 | 1.08 | 0.26 |
| Wolaita | | | 1.07 | 1.05 | 1.08 | -0.56 | 1.29 | 0.12 | 0.76 | -0.12 | 1.02 | 0.08 |
| Gurage | | 2016 | 1.18 | 1.43 | 1.38 | -0.01 | 1.36 | 0.08 | 0.65 | -0.12 | 1.10 | 0.06 |
| Sidama | | | 1.26 | 1.21 | 1.33 | 0.85 | 1.05 | 0.12 | 0.73 | -0.28 | 1.03 | 0.19 |
| Wolaita | | | 1.19 | 1.39 | 1.21 | -0.56 | 1.14 | 0.07 | 0.86 | -0.22 | 1.04 | 0.05 |

P - values test whether or not b and β values for Taylor's power law and Iwao's are significantly different from 1; "a" stands for the intercept ; "b and β" stands slope values for Taylor's power law and Iwao's regression, respectively.

**Table 6.** Spatial distribution of *B. fusca* pupae derived from two distribution indices and two regression at two growth stages of maize crop in 2015 and 2016.

| Zone | Maize growth stage | Year | Indices of dispersion | | Lloyd's crowding | Taylor's power | | | Iwao's regression | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $S^2/\bar{X}$ | Z | $X^*/\bar{X}$ | a | b | p-value | $R^2$ | a | β | P-value |
| Gurage | Silking | 2015 | 1.15 | 1.31 | 1.15 | -0.09 | 1.21 | 0.05 | 0.86 | 0.33 | 0.89 | 0.08 |
| Sidama | | | 0.84 | 1.15 | 0.82 | 0.22 | 0.94 | 0.12 | 0.63 | -0.21 | 1.24 | 0.04 |
| Wolaita | | | 1.08 | 1.21 | 1.09 | -0.44 | 1.34 | 0.20 | 0.71 | 0.50 | 1.16 | 0.07 |
| Gurage | | 2016 | 1.00 | 1.09 | 1.30 | 0.62 | 1.34 | 0.14 | 0.67 | 0.53 | 1.02 | 0.15 |
| Sidama | | | 0.94 | 0.90 | 1.00 | 0.83 | 1.00 | 0.06 | 0.70 | 0.83 | 0.94 | 0.15 |
| Wolaita | | | 1.26 | 1.01 | 1.09 | -0.21 | 1.03 | 0.11 | 0.88 | -0.43 | 1.06 | 0.06 |
| Gurage | Harvesting | 2015 | 1.21 | 1.53 | 1.04 | 0.04 | 1.42 | 0.33 | 0.61 | -0.06 | 1.09 | 0.63 |
| Sidama | | | 0.93 | 1.14 | 0.86 | 0.42 | 0.68 | 0.43 | 0.56 | 0.61 | 0.82 | 0.50 |

**Table 1. Contd.**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wolaita | | 1.02 | 1.22 | 1.07 | -0. 05 | 1.09 | 0.13 | 0.74 | 0.20 | 1.07 | 011 |
| Gurage | 2016 | 1.03 | 1.06 | 0.93 | -0.16 | 1.36 | 0.05 | 0.89 | 0.16 | 0.99 | 0.42 |
| Sidama | | 0.95 | 0.90 | 0.89 | 0.06 | 0.85 | 0.12 | 0.77 | -0.38 | 0.84 | 0.15 |
| Wolaita | | 1.12 | 1.15 | 1.15 | 0.21 | 1.14 | 0.09 | 0.82 | -0.21 | 1.03 | 0.12 |

P - values test whether or not b and β values for Taylor's power law and Iwao's are significantly different from 1; "a" stands for the intercept ; "b and β" stands slope values for Taylor's power law and Iwao's regression, respectively.

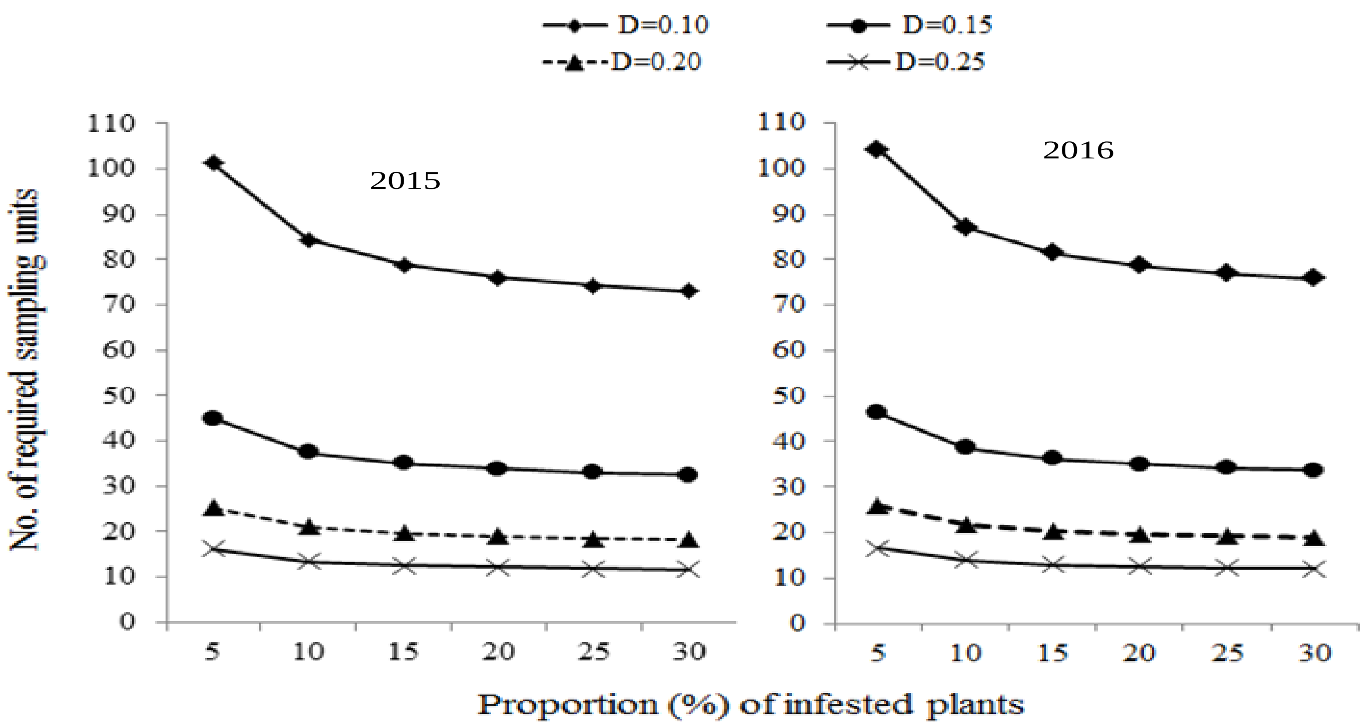


**Figure 1.** Relationship between number of required sampling units and proportion of infested maize plants by maize stem borer in 2015 and 2016 for four levels of precision (D = 0.10, D = 0.15, D = 0.20, D = 0.25 and D = 0.30) based on Kuno's (1969) method.

numbers of the required sampling units are dependent upon the insects being sampled, their distribution patterns, and other factors (Pedigo and Van Schaik, 1984). The required sampling size typically increased with higher precisions and for lower levels of infestations. An action threshold for *B. fusca* 10% infested plants has been recommended (Ong'amo et al., 2016; Van Rensburg et al., 1988). For 10% infestation 14 to

85 sampling units were required for precision ranges of 10 to 25%. A 25% level of precision is acceptable for scouting programs (Southwood, 1978). Taking a higher precision of 20%, for the 10% infestation 22 sampling units (660 plants) are required. In this study the required sample units were estimated with Kuno (1969)'s method which is based on Iwao's patchiness regression. Many studies used Taylor's power to estimate sample sizes. The Taylor's method reduces the necessary sample size when compared with Iwao's method (Darbemamieh et al., 2011; Ifoulis and Savopoulou-Soultani, 2006). Hence, the sample size recommended in this study could be considers as optimum.

Techniques of scouting which are easy to follow, save time and effort, and avoid needless insecticide applications are required. Although various studies have established the relationship between density of larvae of stem borers and yield loss in maize, count of larvae which employs destructive sampling is time consuming and not feasible to scout and decide on the management of stem borers. Van Rensburg and Pringle (1989) developed a sequential sampling method for egg surveys (based on the negative binomial distribution) and the method saved on time and effort required for sampling while allowing for more timely application of insecticides. We used presence and absence of infestation on maize by the stem borer which is easy to execute in the field.

This study concludes that, stem borer, B. *fusca* is a major constraint for the production of maize in southern Ethiopia. *B. fusca* has not one type of distribution pattern for all of its life stages. Infestation of maize with *B. fusca* is aggregated at mid-whorl stage but uniform at silking and maturity stage of maize. *B. fusca* larvae had an aggregated pattern at both mid-whorl and silking stage of maize and random at harvesting stage. At silking and harvesting stage of maize the distribution pattern of *B. fusca* pupa is random. The required sampling size typically increased with higher precisions and for lower levels of infestations. For 10% infestation, which is considered as action threshold level for stem borers management on maize, 22 sampling units (660 plants) at the precision of 20% are required.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Evaluation of the performance of coffee varieties under low moisture stressed areas of Southern Ethiopia

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**The experiment was conducted by using seven released coffee varieties at three locations in southern Ethiopia, Halaba, Loka Abaya and Dilla, to select coffee varieties with higher yield and tolerant to low soil moisture stress. It was arranged in RCBD having three replications. The result indicated that the varieties showed significant difference on main stem diameter, plant height at harvesting, plant height up to the first branch, number of primary branches, number of secondary branches, number of tertiary branches, number of main stems, fruiting nodes per branch (FNPB), number of beans per cherry (NBPC), canopy diameter, leaf area, number of leaves per branch, number of leaves per tree (NLPT), hundred bean weight (HBW), weight of fresh husk (WHF), weight of dried husk (WHD), weight of fresh husked bean (WHBF), and weight of dried husked bean (WHBD). Stand count at harvest (STCNT), leaf length (LL), leaf width (LW), bean thickness (BTH), bean length (BL), bean yield per tree (YPT), bean yield per plot (YPP), bean yield per hectare (YPHA) and weight of husked clean coffee (WHCC) were not statistically significant. Location specific significant variations were observed on some of the variables such as stand count, leaf length, and leaf width at Halaba; yield per tree, yield per plot, yield per hectare and weight of husked clean coffee were significant at all the three locations despite their non-significant value while combined. The coffee variety Catimor J-19 performed best at all location with respect to fresh bean yield and dried clean coffee followed by Angafa. Thus they can be promoted for larger commercial production at tested locations and locations with similar agro-ecological conditions.**

**Key words:** *Coffea arabica*, husked coffee, clean coffee, agroecology.

## INTRODUCTION

In Ethiopia, coffee cultivation plays a fundamental role both in the cultural and socioeconomic life of Ethiopians. It represents the major agricultural export crop, providing 20 to 25% of the foreign exchange earnings (ECFF, 2015). The coffee sector contributes about 4 to 5% to the country's Gross Domestic Product (GDP) and creates hundreds of thousands of local job opportunities (EBI, 2014).

A number of coffee varieties were developed through short and long term programs. The first 26 pure Arabica coffee varieties were developed from 1977 to 1981. Their performance varied with locations and management.

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From 1996 to 2006, 10 improved pure line varieties having a mean yield range of 15.4 to 25.5 and 9 to 21 q/ha on research and farmer managed field conditions, respectively were developed and released (Tesfaye et al., 2008). According to Minister of Agriculture (MoA, 2018), different high yielding, disease resistance, early bearing and high quality Arabica coffee varieties were identified and released for official production at major coffee growing areas of the country. But their performance varied with agro-climatic condition (Location) in addition to their genetic performance. Belete and Bayeta (2008) indicated that cultivar yield is affected by location, that is, those cultivars outperformed in one location performs differently in other location with different altitude and agro-climatic condition indicating the low stability of yield of coffee varieties across locations. Multi-location adaptation tests carried out in other countries also illustrated similar result that genotype-environment interaction is a common scenario in Arabica coffee genotypes like other crops (Agwanda et al., 1997).

The native home of coffee species is characterized by low-water-deficit conditions, which probably allowed evolution without the need to develop extensive mechanisms to cope with drought stress (Coste, 1992). Nevertheless, some coffee cultivars are known to differ in their responses to drought (Orozco and Jaramillo, 1978; Carr, 2001; DaMatta and Rena, 2001), suggesting that modern cultivars are not very close to their wild relatives in terms of drought tolerance. In fact, field observations have indicated that some cultivars may endure 6 to 7 months with no rain, even in sandy soils, but obviously at the expense of strong declines in crop yield (DaMatta and Ramalho, 2006).

The report by Chemura et al. (2014) indicated that C*offea arabica* varieties react differently to low soil moisture stress which otherwise had no statistically significant difference. They reported that no coffee variety was significantly superior in biomass before soil moisture deficit stress exposure. For most of the varieties, there was a reduction in fresh biomass and a slow buildup of dry biomass during period of soil moisture deficit stress. Some of coffee varieties showed positive changes in root biomass after 21 and 28 days of soil moisture. DaMatta (2004) pointed out that drought prone coffee farms are associated with low input systems and as such varieties that have better survival and yield stability under drought stress are of much greater value than those with greater yield potential under optimal conditions.

Despite the role of coffee in the national economy and in spite the country of origin of Arabica coffee, average national productivity has not exceeded six quintals (600 kg/ha) (Jefuka et al., 2012; Eshetu et al., 2000; Workafes and Kassu, 1999). This is very low in contrast to yield levels reported usually in some Latin American countries. The factors attributed to such low productivity include lack of resistant varieties to various diseases and insect pests and poor agronomic practices (Eshetu et al., 2000; Workafes and Kassu, 1999).

Lack of suitable varieties that exhibit stable yield performances across wide ranges of environments is the major factor among several production constraints contributing to low productivity of Arabica coffee in Ethiopia (Beksisa et al., 2018; Belete and Bayetta, 2008). Thus testing the adaptability of coffee varieties at range of environments and specific location is paramount important. Hence, this paper deals with adaptability of coffee varieties under low moisture stressed area of Southern Region of Ethiopia and recommend the best performing variety for further wider production.

## MATERIALS AND METHODS

### Location description

The trials were conducted at four different locations in southern region of Ethiopia: Dilla, Halaba and Loka Abaya. They represented wider range of altitude, 1500-1900 masl (Table 1).

### Research materials and source

Seven coffee varieties, Catimor J-19, Koti (85257), 74112, Odicha (97.4), Fayate (97.1), 1377 (Angafa), and one farmers' variety were tested at Dilla, Halaba and Loka Abaya. The first one was obtained from Tepi Agricultural Research Center while the other five were collected from Awadasub Center of Wondogenet Agricultural Research Center and the seventh was collected from farmers. The seedlings were developed by using 100 mm diameter and 250 mm length black polyethylene tube filled with 70% soil and 30% manure. Polyethylene tubes were covered with thick grass mulching until emergence after sowing. Mulch was removed just after emergence and the seedling were kept under shade structure. The developing seedlings were irrigated every three days by using watering can until 12 pair of leaves were developed which is the stage that the seedlings attain the final transplanting stage. Holes were dug and lest open for two months. The hole was filled with topsoil immediately before planting. The seedlings were field planted when they are approximately eight months old in randomized complete block design (RCBD) with three replications. The seedlings were planted 2 m between plants and 2 m between rows. They were mulched immediately after being planted so as to maintain soil moisture and protect the root zone from direct sun light. The plots received uniform application of NP fertilizer and other cultural practices throughout the growing period. The space left between plots was 4 m and the trees were arranged in a single raw. As it is a fast growing tree type, sesban (*Sesbania sesban* (L.) Merr.) was planted as temporary shading between every two coffee trees. The shade tree was planted at 4 m × 4 m spacing indicating that two coffee trees were shaded by one shade tree.

### Data collected and analysis

Information such as date of sowing, field management, date of seed emergence, date of transplanting, growth stage at transplanting and other relevant information have been reported to be recorded. Major yield and vegetative data such as bean length (mm), bean width (mm), hundred bean weight (g), yield per tree (kg), yield/plot (which will be converted to yield/hectare), disease types and their incidences such as coffee berry disease (%), bacterial blight, rust

**Table 1.** Coordinates, annual temperature and rain fall of Dilla, Loca Abaya and Halaba (Data collected from the nearby metrological station).

| Location | Altitude | Latitude | Longitude | Temperature (°C) | | Annual rainfall (mm) |
|---|---|---|---|---|---|---|
| | | | | Min. | Max. | |
| Dilla | 1,570 m | 6°24′38″N | 38°18′37″E | 13.1 | 28.2 | 1226.7 |
| Halaba | 1,726 m | 7°17'60.00" N | 38°06'60.00" E | 15.3 | 29.4 | 879.1 |
| Loka Abaya | 1690 | 6° 25' 59.99" N | 37° 52' 59.99" E | 13.9 | 27.9 | 937.6 |

incidence (%), height up to first primary branch (cm), number of primary branches (no), leaf length (cm), leaf width (cm), leaf area ($cm^2$), plant height (cm) and canopy diameter (cm) were collected. Yield and other agronomic data were analyzed by using SAS statistical software version 9.2 (SAS, 2008). Based on the analyzed data the best performing varieties were recommended for the areas and similar agro ecological locations for wider commercial production.

## RESULTS AND DISCUSSION

### Analysis of variance

The combined analysis of variances indicated that there was statistically significant difference among varieties for main stem diameter (MSTD in cm), plant height at harvesting stage (PLHT in cm), plant height up to the first branch (PLHTFB in cm), number of primary branches (NPB), number of secondary branches (NSB), number of tertiary branches (NTB), number of main stems (NMST), fruiting nodes per branch (FNPB), number of beans per cherry (NBPC), canopy diameter (CAND in cm), specific leaf area (ELA in $cm^2$), leaf area (LA in $m^2$), number of leaves per branch (NLPB), number of leaves per tree (NLPT), hundred bean weight (HBW in g), weight of fresh husk (WHF), weight of dried husk (WHD), weight of fresh husked bean (WHBF in g per plot) and weight of husked bean (WHBD in g/plot). But the other yield and yield components were found to be statistically non-significant. These included stand count at harvest (STCNT), leaf length (LL in cm), leaf width (LW in cm), bean thickness (BTH in cm), bean length (BL in cm), bean yield per tree (YPT in g), bean yield per plot (YPP in g), bean yield per hectare (YPHA in kg) and weight of husked clean coffee (WHCC in q/ha). Location specific significant variations were observed on some of the variables such as STCNT, LL and LW at Halaba; YPT, YPP, YPHA and WHAC at all the three locations despite their non-significant value while combined (Table 2). All the significant variables were considered for mean separation so as to come up with the recommendation with special focus to clean coffee yield per hectare. The significant difference detected among cultivars indicates the existence of genetic variability, and that allowed the selection of better cultivars in regard to coffee yield, so as to include them in the next value for cultivation and use (Teixeira et al., 2013).

### Performance of coffee varieties across locations

Main stem diameter (cm), PLHT, PLHTFB varied with varieties as the largest value was recorded from variety Angafa. But the highest result on NPB and NSB was obtained from variety Catimor J-19 while variety Koti showed the highest performance on number of tertiary branches (NTB) and number of main stem per plant, while the value obtained from Odicha was statically non-significant with Koti in case of NMST. Varieties Catimor J-19(5.6), Koti (5.0), 74112 (4.7) and Odicha (5.4) showed statistically non-significant result on fruiting nodes per branch (FNPB). Catimor J-19 also showed higher value for ELA, LA, NLPB and NLPT. As Shown in Table 3, varieties Catimor J-19 and Angafa outperformed all other varieties (Table 3). The least value was recorded from local farmer variety. This indicated that performance of coffee varied with varieties. The result is in line with the work of Tirunesh et al. (2015). They reported that three coffee genotypes: 8213, 8143 and 75187B exhibited superior performance consistently at all locations. Similarly, Gebreselassie et al. (2017a) indicated the existence of statistically significant differences among coffee hybrids for stem girth/diameter, plant height, number of main stem nodes and yield with the highest stem girth (7.13 cm), plant height (323.3 cm), and number of main stem nodes (58.06), while the lowest stem girth (6.36 cm), plant height (281.63 cm) and number of main stem nodes (45.06). On the contrary, Belete and Bayetta (2008), Demissie et al. (2011) and Belete et al. (2014) also confirmed that varieties exhibiting better adaptation at one location did not perform well at other locations.

All improved varieties showed statistically different performance compared with the local (farmers') variety for HBW, WHD, WPBF and WPBD. The highest value ofHBW was recorded by the variety Angafa despite the non-significant difference with the values obtained from Catimor J-19, Koti, Odicha, 74112, and Fayate (Table 4). The coffee variety Fayate showed the top performance with regard to WHD while variety Catimor J-19 outperformed all other varieties in case of WPBF and WPBD. However, the result obtained from Odicha and

**Table 2.** Analysis of variance indicating treatment, location, treatment by location interaction and error mean squares across locations.

| Variable | Mean square | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dilla | | Halaba | | Loca Abaya | | Combined | | |
| | Treatment (6) | EMS (12) | Treatment (6) | EMS (12) | Treatment (6) | EMS (12) | Treatment (6) | (TrtxLoc) | EMS (12) |
| STCNT | 0.2063$^{NS}$ | 0.4683 | 0.7619* | 0.76 | 0.2698$^{NS}$ | 0.27 | 0.471$^{NS}$ | 0.384$^{NS}$ | 0.4 |
| MSTD | 0.3411** | 0.0390 | 0.7848* | 0.23 | 5.8013** | 0.076 | 3.82** | 1.55** | 0.1 |
| PLHT | 730.28* | 206.60 | 2234.9** | 292 | 284.7$^{NS}$ | 171.1 | 2291** | 479.5* | 229.9 |
| PLHTFB | 75.805** | 7.7988 | 95.716* | 5.23 | 28.86** | 4.582 | 99.0** | 50* | 5.8 |
| NPB | 120.46* | 40.386 | 434.58** | 31.1 | 22.7* | 5.137 | 247.7** | 165.0** | 23.5 |
| NSB | 105.33** | 6.6281 | 36.998** | 2.03 | 31.0** | 0.533 | 111.6** | 30.9** | 3.8 |
| NTB | 5.5265** | 0.1048 | 0.0476$^{NS}$ | 0.05 | 0.9433** | 0.034 | 3.43** | 1.54** | 0.1 |
| NMST | 1.0494* | 0.2730 | 1.0299$^{NS}$ | 0.41 | 0.1515$^{NS}$ | 0.194 | 0.62* | 0.81** | 0.3 |
| FNPB | 11.691** | 1.0022 | 4.6835** | 0.39 | 0.0153$^{NS}$ | 0.723 | 7.9** | 4.2** | 0.9 |
| NBPC | 4.4643** | 0.507 | 5.7918* | 1.49 | 0.0030$^{NS}$ | 0.526 | 3.05** | 3.6** | 0.8 |
| CAND | 1651.8** | 84.38 | 2290.3** | 101 | 287.5$^{NS}$ | 117.4 | 3216.4** | 506.6** | 97.6 |
| LL | 2.0677$^{NS}$ | 2.436 | 13.214* | 3.24 | 1.4$^{NS}$ | 2.886 | 11.6** | 2.52$^{NS}$ | 2.6 |
| LW | 0.7805$^{NS}$ | 0.644 | 2.7793** | 5.97 | 0.2863$^{NS}$ | 0.694 | 2.56** | 0.64$^{NS}$ | 0.7 |
| ELA | 73.542* | 25.71 | 331.65** | 45.7 | 48.86* | 10.43 | 312.5** | 70.8* | 31.3 |
| LA | 7.454** | 0.0624 | 0.9833** | 0.02 | 0.2513** | 0.024 | 83.1** | 34.12** | 0.1 |
| NLPB | 313.54** | 3.03 | 1.53$^{NS}$ | 27.3 | 10.3030$^{NS}$ | 8.959 | 216** | 74.8** | 8.3 |
| NLPT | 66953** | 10404 | 64584** | 2867 | 12451** | 1883 | 354615** | 201432** | 4644.1 |
| BTH | 0.245$^{NS}$ | 0.3009 | 0.0137$^{NS}$ | 0.29 | 0.055$^{NS}$ | 0.175 | 0.05$^{NS}$ | 0.132$^{NS}$ | 0.3 |
| BL | 0.3469$^{NS}$ | 0.6327 | 0.0028$^{NS}$ | 0.30 | 0.1521$^{NS}$ | 0.215 | 0.062$^{NS}$ | 0.22$^{NS}$ | 17.6 |
| HSW | 200.83$^{NS}$ | 287.775 | 5214.6** | 444 | 594.3$^{NS}$ | 480.5 | 105* | 2479.3** | 450.9 |
| YPT | 920276* | 240455 | 968555* | 200000 | 952555* | 300000 | 2544276** | 148556$^{NS}$ | 319666.2 |
| YPP | 14491575** | 648408 | 6806556** | 800000 | 4636568* | 1000000 | 24083971** | 925364$^{NS}$ | 864302.5 |
| YPHA | 5660772** | 253284 | 2658811** | 300000 | 2000000* | 500000 | 9407801** | 361471$^{NS}$ | 337618.18 |
| WHF | 653915* | 226787 | 57688$^{NS}$ | 41500 | 40882$^{NS}$ | 100000 | 165922$^{NS}$ | 293281* | 121508.69 |
| WHD | 121963** | 8799.90 | 55646** | 1603 | 7043** | 365.9 | 81521** | 51566** | 3432.754 |
| WPBF | 1383855** | 275358 | 60185$^{NS}$ | 100000 | 336629* | 74264 | 1356315** | 296917* | 154112.06 |
| WPBD | 332189** | 66396.5 | 848218** | 10966 | 166902** | 10843 | 803725** | 27179** | 31249.76 |
| WHAC | 16.003* | 4.1812 | 16.8421* | 3.707 | 16.56* | 5.807 | 44.24** | 2.58$^{NS}$ | 5.5586443 |
| CBD | 1.2$^{NS}$ | 0.56 | 5.7** | 0.15 | 1.5* | 0.42 | 4.72** | 1.7** | 1.2 |
| CLS | 0.825* | 0.278 | 4.54** | 0.42 | 1.22* | 0.389 | 3.35** | 1.61** | 0.41 |

*Significant at p≤0.05, **significant at p≤0.01, the numbers in the parenthesis are degree of freedom, EMS=error mean square, NS=non-significant.

Angafa were statistically non-significant compared with Catimor J-19.

## Location specific performance of coffee varieties

The performance of coffee varieties varied with locations. At Halaba, stand count at harvest (STCNT), average leaf length (LL) and average leaf width (LW) showed specific and significant differences. Accordingly, higher value of stand count was recorded from varieties Catimor J-19, 74112, Odicha, Fayate, Angafa and local variety. The lowest was recorded from the variety Koti but not statistically significant from varieties Catimor J-19, 74112, Odicha, and local farmers' variety. In the same way coffee variety Fayate showed statistically higher average leaf length and width compared with 74112 and local variety. But it showed statistically no significant difference compared with Catimor J-19, Koti, Odicha, and Angafa. They are statistically at par (Table 5). It can be seen that the vegetative performances of improved varieties outweighed the local one in all aspects. Similarly, Abdulfeta (2018) reported very higher results of leaf width(8.77 m) and leaf length (18.4 m) coffee varieties.

Some yield traits such as YPT, YPP, YPHA and WHAC showed specific performance across all location despite their combined effect which was statistically non-significant. Accordingly, the highest values were obtained from Catimor J-19 irrespective of location differences following Angafa (Figure 1). Their performances were still

**Table 3.** Growth (agronomic) performance of coffee varieties combined over locations.

| Variable | Varieties | | | | | | | CV |
|---|---|---|---|---|---|---|---|---|
| | Catimor J-19 | Koti (85257) | 74112 | Odicha (97.4) | Fayate (97.1) | Angafa (1377) | Local | |
| MSTD | $1.8^{c}$ | $1.8^{c}$ | $1.3^{d}$ | $2.1^{c}$ | $2.5^{b}$ | $3^{a}$ | $1.1^{d}$ | 17.5 |
| PLHT | $77.1^{bc}$ | $89.6^{b}$ | $68.6^{c}$ | $84.2^{b}$ | $92.4^{b}$ | $111.3^{a}$ | $64.0^{c}$ | 18.1 |
| Plhtfb | $8.1^{d}$ | $13.6^{b}$ | $10.6^{c}$ | $12.0^{bc}$ | $11.9^{bc}$ | $18.6^{a}$ | $10.3^{cd}$ | 19.8 |
| NPB | $29.7^{a}$ | $21.8^{b}$ | $22.8^{b}$ | $30.8^{a}$ | $26.3^{ab}$ | $26.1^{ab}$ | $15.4^{c}$ | 19.6 |
| NSB | $12.7^{a}$ | $6.7^{b}$ | $4.4^{c}$ | $8.3^{b}$ | $8.0^{b}$ | $7.9^{b}$ | $1.4^{d}$ | 27.6 |
| NTB | $2.0^{b}$ | $2.5^{a}$ | $1^{d}$ | $1^{d}$ | $2.2^{b}$ | $1.5^{c}$ | $1^{d}$ | 15.4 |
| NMST | $1.8^{ab}$ | $2.3^{a}$ | $2.0^{ab}$ | $2.3^{a}$ | $2.1^{ab}$ | $2.1^{ab}$ | $1.6^{b}$ | 26.1 |
| FNPB | $5.6^{a}$ | $5.0^{ab}$ | $4.7^{ab}$ | $4.1^{b}$ | $4.0^{b}$ | $5.4^{a}$ | $2.9^{c}$ | 21.3 |
| NBPC | $4.7^{ab}$ | $4.0^{bc}$ | $3.9^{bc}$ | $4.1^{bc}$ | $4.3^{ab}$ | $5.2^{a}$ | $3.4^{c}$ | 21.5 |
| CanD | $83.8^{bc}$ | $76.9^{c}$ | $59.8^{d}$ | $77.9^{c}$ | $91.8^{ab}$ | $100.6^{a}$ | 45.0e | 12.9 |
| ELA | $33.3^{a}$ | $27.1^{b}$ | $21.7^{c}$ | $28.9^{ab}$ | $33.8^{a}$ | $32.6^{ab}$ | $18.8^{c}$ | 20 |
| LA | $2.7^{a}$ | $1.2^{b}$ | $0.6^{c}$ | $1.0^{b}$ | $1.2^{b}$ | $1.2^{b}$ | $0.5^{c}$ | 20.2 |
| NLPB | $25.8^{a}$ | $20.7^{b}$ | $12^{d}$ | $13.1^{d}$ | $14.8^{cd}$ | $16.5^{c}$ | $14.1^{cd}$ | 17.2 |
| NLPT | $810.4^{a}$ | $528.7^{b}$ | $257.3^{d}$ | $364.9^{c}$ | $353.2^{c}$ | $395.7^{c}$ | $226.1^{d}$ | 16.2 |

**Table 4.** Coffee bean yield and yield related treat combined over locations.

| Variety | HBW | WHD | WPBF | WPBD |
|---|---|---|---|---|
| Catimor J-19 | $98.0^{bc}$ | $288.5^{b}$ | $2296.2^{a}$ | $1426.9^{a}$ |
| Koti (85257) | $116.7^{ab}$ | $235.5^{bc}$ | $1850.3^{b}$ | $803.2^{b}$ |
| 74112 | $114.5^{ac}$ | $292.2^{b}$ | $1802^{b}$ | $907.7^{b}$ |
| Odicha (97.4) | $114.7^{ac}$ | $205.6^{c}$ | $2591.8^{a}$ | $1332.1^{a}$ |
| Fayate (97.1) | $106.6^{ac}$ | $445.8^{a}$ | $1747.1^{b}$ | $841.4^{b}$ |
| Angafa (1377) | $123.1^{a}$ | 138.5d | $2572.6^{a}$ | $1434.7^{a}$ |
| Local | $93.1^{c}$ | $271.7^{b}$ | $1744.9^{b}$ | $829.2^{b}$ |
| CV | 19.4 | 21.8 | 18.8 | 16.3 |

**Table 5.** Stand count (STCNT) in cm, leaf length (LL) in cm and leaf width performance of coffee varieties tested at Halaba.

| Variety | Stcnt | LL | LW |
|---|---|---|---|
| Catimor J-19 | $3^{ab}$ | $11.1^{ab}$ | $5.4^{ab}$ |
| Koti (85257) | $2.33^{b}$ | $10.5^{ab}$ | $5.4^{ab}$ |
| 74112 | $3^{ab}$ | $8.1^{bc}$ | $4.2^{bc}$ |
| Odicha (97.4) | $3.67^{a}$ | $11.3^{ab}$ | $5.4^{ab}$ |
| Fayate (97.1) | $3.67^{a}$ | $11.8^{a}$ | $5.9^{a}$ |
| Angafa (1377) | $3.67^{a}$ | $11.3^{ab}$ | $6^{a}$ |
| Local | $3^{ab}$ | $6.2^{c}$ | $3.3^{c}$ |
| CV | 16.04 | 17.91 | 13.87 |

in comparison with their initial yield which was recorded during their first release. In the same way the variety Catimor J-19 showed higher clean coffee per hectare in quintal across all locations followed by Angafa as shown in Figure 2. Accordingly, the highest clean coffee yield 11.96, 12.8 and 11.1 q/ha at Dilla, Halaba and Lokabaya, respectively were obtained from Catimor J-19 followed by Angafa (Figure 2). Such mean yield at the first two

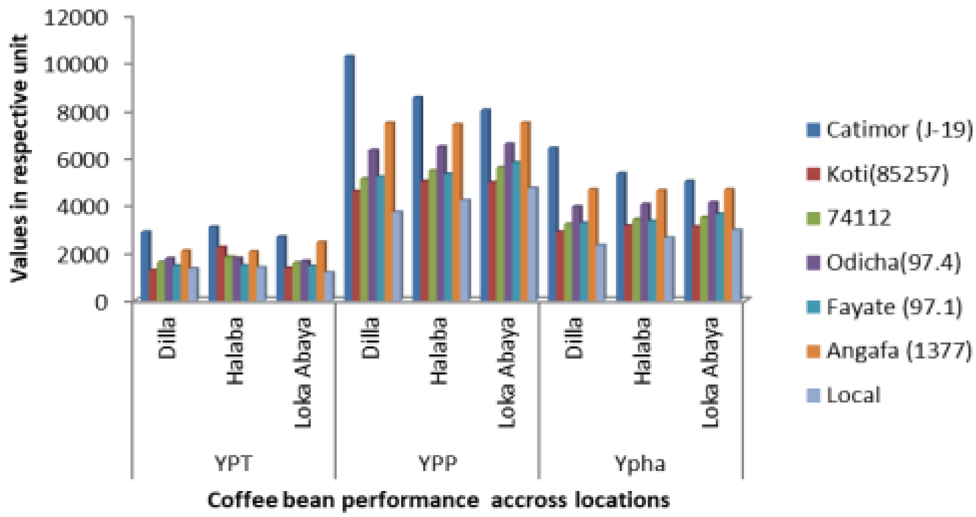


**Figure 1.** Coffee bean yield per tree, per plot and per hectare across locations.

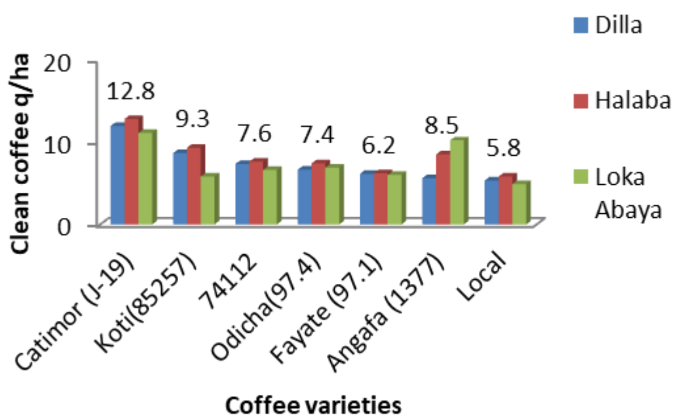


**Figure 2.** Clean cofee in quintal per hectare across locations.

bearing is very high as climax yield in Arabica coffee is attained starting from the fourth bearing stage (Wrigley, 1988). The overall performance of the varieties (Catimore (J-19) and Angafa was also higher at all environments. This is in line with the work of Agwanda et al. (1997) and Belete and Bayetta (2008) who reported the possibility of developing stable genotypes which can adapt across wide environments. The same result was obtained by Teixeira et al. (2013) as they reported that a significant difference was detected among coffee cultivars and the mean of the four harvests, green coffee yield was 14.7 q/ha with the range from 6.5 to 25.6 q/ha depending on cultivar. Gebreselassie et al. (2017b) also indicated that the existence of significant variation among coffee varieties for mean yield across locations with the highest five years mean yield 12.21 q/ha (2500 tree/ha) recorded at Awada followed by 5.342 q/ha at Komato and the lowest 4.34 q/ha recorded at Wonago growing condition.

## Characters association

Coffee bean yield and clean coffee yield were statistically significant and positively correlated with different growth parameters. Yield of clean coffee in quintals per hectare was found to be positively and significantly correlated with stand count at harvest, main stem diameter, number of secondary branches, number of fruiting nodes per branch, number of beans per cherry, canopy diameter, leaf length, leaf width, specific/single leaf area, leaf area per plant, number of leaves per branch, fresh bean yield per tree, bean yield per plot, bean yield per hectare,

**Table 6.** Coffee growth variables association with bean yield and clear coffee.

| Variable | HBW | YPT | YPP | Ypha | WHF | WHD | WPBF | WPBD | WHaC |
|---|---|---|---|---|---|---|---|---|---|
| Stcnt | 0.29* | (0.6)** | 0.07177 | 0.07177 | 0.17038 | -0.1518 | 0.11274 | 0.22925 | (0.59)** |
| MstD | -0.0397 | 0.26* | 0.29* | 0.29* | 0.11344 | -0.1717 | 0.34** | 0.36** | 0.26* |
| Plht | 0.27* | -0.0286 | 0.17006 | 0.17006 | 0.21083 | 0.02749 | 0.16726 | 0.13584 | -0.0286 |
| PlhtFb | 0.34** | -0.045 | -0.0434 | -0.0434 | 0.1208 | -0.1553 | 0.0873 | 0.00938 | -0.045 |
| NPB | 0.01394 | 0.03967 | 0.25* | 0.25* | 0.04485 | 0.35** | 0.1372 | 0.19001 | 0.03967 |
| NSB | 0.01581 | 0.39** | 0.60** | 0.60** | -0.1025 | 0.10637 | 0.39** | 0.26* | 0.39** |
| NTB | 0.06839 | -0.0077 | 0.06925 | 0.06925 | 0.30* | 0.14131 | -0.1487 | -0.0332 | -0.0077 |
| NMST | 0.23014 | -0.0765 | -0.0285 | -0.0285 | -0.0265 | 0.14436 | 0.07184 | -0.1578 | -0.0765 |
| FNPB | 0.34** | 0.31* | 0.50** | 0.50** | 0.32* | -0.1897 | 0.23364 | 0.16831 | 0.31* |
| NBPC | 0.02518 | 0.26* | 0.27* | 0.27* | 0.00646 | 0.09616 | 0.10788 | 0.0053 | 0.26* |
| CanD | 0.0209 | 0.31* | 0.42** | 0.42** | -0.0531 | 0.34** | 0.35** | 0.13385 | 0.31* |
| LL | 0.08599 | 0.34** | 0.32* | 0.32* | 0.01217 | -0.0497 | 0.13399 | 0.22136 | 0.34** |
| LW | 0.18414 | 0.30* | 0.36** | 0.36** | 0.21255 | -0.1301 | 0.31* | 0.29* | 0.30* |
| ELA | 0.10639 | 0.36** | 0.38** | 0.38** | 0.11394 | -0.1004 | 0.22367 | 0.28* | 0.36* |
| LA | -0.0079 | 0.34693 | 0.59** | 0.59** | 0.18772 | 0.00169 | 0.17897 | 0.32** | 0.35** |
| NLPB | 0.16743 | 0.26* | 0.47** | 0.46** | 0.30* | (0.31)* | 0.01397 | 0.28* | 0.26* |
| NLPT | 0.04402 | 0.22272 | 0.45** | 0.45** | 0.21957 | 0.04187 | 0.07906 | 0.22848 | 0.22272 |
| BTH | 0.20202 | 0.01814 | 0.10713 | 0.10713 | 0.30* | -0.1477 | 0.23747 | 0.19036 | 0.01814 |
| BL | 0.17693 | -0.1219 | 0.01808 | 0.01808 | 0.27* | -0.1193 | 0.13764 | 0.24* | -0.1219 |
| HSW | 1 | -0.1354 | 0.08513 | 0.08513 | 0.31* | (0.31)* | 0.02444 | 0.14073 | -0.1354 |
| YPT | | 1 | 0.72** | 0.72** | -0.0447 | -0.0598 | 0.28* | 0.30* | 1** |
| YPP | | | 1 | 1** | 0.13273 | -0.176 | 0.47** | 0.52** | 0.72** |
| Ypha | | | | 1 | 0.13273 | -0.176 | 0.47** | 0.52** | 0.72** |
| WHF | | | | | 1 | -0.1844 | -0.0806 | -0.0164 | -0.0447 |
| WHD | | | | | | 1 | -0.1643 | -0.2235 | -0.0598 |
| WPBF | | | | | | | 1 | 0.54** | 0.28* |
| WPBD | | | | | | | | 1 | 0.30* |

*Significant at p≤0.05, **=Significant at P≤0.01, numbers in the parenthesis are negative correlation coefficients.

weight of pulped bean per plot and weight of dried bean per plot (Table 6). This indicates that anything that contributed to improve the traits improves/bust clean coffee that could be harvested per hectare. The result is in line with the findings reported by Tirunesh et al. (2015). They reported that the growth characters such as canopy diameter with correlation coefficient of 0.57 and stem girth with the correlation coefficient of 0.59 exhibited strong positive correlation with yield indicating that these characters have strong tie to improve productivity per tree basis.

## Yield stability of coffee varieties across locations

The ASV parameter is used to quantify and classify the genotypes according to their stability performance. In this model, genotypes with least AMMI stability value (ASV) or have smallest distance from the origin are considered as the most stable, whereas those which have the highest ASV are considered as unstable (Mehari et al., 2014). The additive main effects and multiplicative interaction (AMMI) stability analysis of seven varieties on three environments indicated that the variability of performance of coffee varieties under different environmental condition. The clones 74112 followed by Catimor J-19 and Fayate had possessed wider adaptability as they found nearer to the origin. On the contrary, farmer varieties Angafa and Koti were found to be adapted to specific environmental conditions, Loca Abaya and Halaba, respectively which are far away from the origin of the plot (Figure 3). Similar research findings were reported by Beksisa et al. (2018) as they indicated that lack of suitable varieties that exhibit stable yield performances across wide ranges of environments is the major factor among several production constraints contributing to low productivity of Arabica coffee in Ethiopia. Two high yielding genotypes, namely (L52/2001) and (L55/2001), on average showed stable performance across environments. On the other hand, the study also illustrated the presence of location specific high yielding coffee genotype such as L56/2001. In this

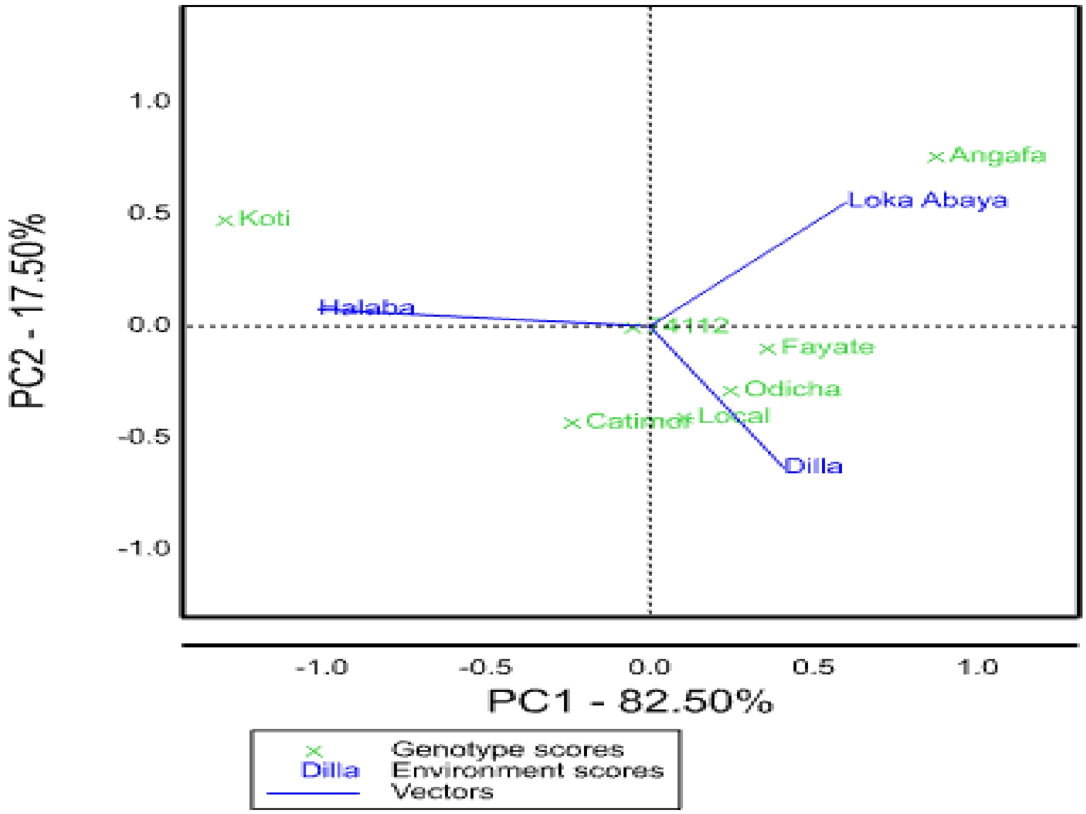


**Figure 3.** AMMI biplot (symmetric scaling indicating stability of coffee varieties across locations).

regard it can be seen that coffee by its very nature has a property to show stable and higher yield under different agro-ecological locations. Similarly, Tirunesh et al. (2015) confirmed that three coffee genotypes exhibited superior performance consistently at all locations irrespective of the interaction.

## Disease incidences

Coffee berry disease (CBD) is very sever and causes appreciable yield loss in areas and/or seasons where the weather is favorable. Temperature and rainfall (amount and duration), and relative humidity are decisively determining the occurrence, prevalence and severity of the diseases (Belachew and Demelash, 2015; Arega et al., 2008; Girma et al., 2008). Totally, more than 13 types of diseases were registered to affect coffee plant in Ethiopia. While major coffee diseases are coffee berry diseases (CBD) caused by *Colletotrichum kahawae,* coffee wilt disease (CWD) of *Gibberella Xylariales* and coffee leaf rust caused by *Hemileia vastatrix,* however, the rest diseases were considered to be minor (Eshetu et al., 2000; Belachew et al., 2015). Even if coffee is infected by different diseases, there was no severe disease attach on the tested varieties with the exception of coffee berry disease and coffee leaf spot. Varieties Catimor J-19, Koti, 74112, Odicha and Fayate showed resistant reaction to coffee berry disease compared with Ahgafa and the local farmers' variety. Among the three locations, the disease pressure at Dilla was very low compared to other location as there was no any statistically significant difference among the varieties on disease score (Table 7). Similarly, coffee leaf spot was prominent on some of the varieties while others showed less infestation level. Varieties Koti, Catimor J-19, 74112 and Angafa showed statistically non-significant but lower infestation level of coffee leaf spot. Odicha, Fayate and local farmers' varieties were highly infested by disease. The result obtained was in line with the findings of Arega et al. (2008), Bayetta (2001), Bayetta et al. (2000), and Mohammed and Jambo (2015). They indicated the existence of wider genetic variability among coffee cultivars for the reaction against coffee diseases.

## Conclusion

Despite the role of coffee in the national economy and in spite the country of origin of Arabica coffee, average national productivity has not exceeded six quintals (600 kg/ha). This is very low in contrast to yield levels reported

**Table 7.** Coffee berry and coffee leaf rust diseases incidence.

| Treatment | CBD | | | | CLS | | | |
|---|---|---|---|---|---|---|---|---|
| | Locations | | | Mean | Locations | | | Mean |
| | Dilla | Halaba | Loka Abaya | | Dilla | Halaba | Loka Abaya | |
| Catimor J-19 | $1^{a}$ | $1^{a}$ | $2^{a}$ | $1.3^{a}$ | $1.7^{a}$ | $2^{ac}$ | $2.7^{b}$ | $2.1^{ac}$ |
| Koti (85257) | $1.7^{a}$ | $2.3^{ac}$ | $2.3^{ac}$ | $2.1^{ab}$ | $1.7^{a}$ | $1^{a}$ | $2.7^{b}$ | $1.8^{a}$ |
| 74112 | $1.7^{a}$ | $2.7^{ad}$ | $2^{ac}$ | $2.1^{ab}$ | $2^{ab}$ | $2.3^{bc}$ | $2.7^{b}$ | $2.3^{ad}$ |
| Odicha (97.4) | $1.3^{a}$ | $1^{a}$ | $2^{ac}$ | $1.4^{a}$ | $1.3^{a}$ | $3^{c}$ | $3.3^{b}$ | $2.6^{bd}$ |
| Fayate (97.1) | $2.7^{a}$ | $2.3^{ac}$ | $2.3^{ac}$ | $2.4^{ac}$ | $2^{ab}$ | $4.7^{d}$ | $3^{b}$ | $3.2^{d}$ |
| Angafa (1377) | $2.7^{a}$ | $4^{c}$f | $2^{ac}$ | $2.9^{bc}$ | $2^{ab}$ | $1.3^{ab}$ | $1.3^{a}$ | $1.6^{a}$ |
| Local | $1.7^{a}$ | $4.7^{d}$f | $3.7^{b}$f | $3.3^{c}$ | $3^{b}$ | $3^{c}$ | $3^{b}$ | $3^{cd}$ |
| CV | 45.5 | 15.1 | 28.4 | 27.9 | 27 | 26.2 | 23.4 | 37.1 |

Values in the column followed by the same letter(s) are statistically non-significant; disease scoring was carried out by using 1-5 scale as 1 refers to zero while 5 refers to very high infestation

usually in some Latin American countries. The factors attributed to such low productivity include lack of resistant varieties to various diseases and insect pests, and poor agronomic practices.

Lack of suitable varieties that exhibit stable yield performances across wide ranges of environments is the major factor among several production constraints contributing to low productivity of Arabica coffee in Ethiopia which indicated that testing the adaptability of coffee varieties at range of environments and specific location is paramount important.

A number of coffee varieties developed through short term and long term programs. But their performance varies with agro-climatic condition (Location) in addition to their genetic potential. Coffee cultivars yields are affected by location, that is, those cultivars outperformed in one location might perform differently in other locations with different altitude and agro-climatic condition. Accordingly, variety Catimor J-19 performed best at all location with respect to fresh bean yield and dried clean coffee followed by Angafa indicating that the materials are well adapted to the edaphic and agro-climatic condition of Dilla, Halaba and Loka Abaya. In line with the great yield performance and stable yield across locations, coffee varieties tested were tolerant to common coffee diseases (CBD and CLR).

Thus, these coffee varieties can confidently widely be promoted for larger commercial production at tested locations and locations with similar agro-ecological conditions.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Modeling the sensitivity of strategic plans to risks and uncertainties in forest management

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**Forest operations are associated with products of a biological source and long-term plans. This makes forest management plans affected by market and environmental uncertainties, which can cause differences between the strategic and operational plans. Risks related to disease spread, adverse weather, timber demand, and available capital can cause a reduction in planted areas. It is necessary that forest management plans aim to optimize production and reduce the effects of restrict planting. In this work, a linear programming model was created for long-term forests planning including a variable to restrict planting area. Prescriptions were defined for 6-, 7- and 8-year rotation considering planting or coppicing, creating 60 scenarios varying intensity, period and time of reductions in the planted areas. All scenarios were evaluated by the NPV at the end of the planning horizon. The results showed that the NPV decreased when the planted area was reduced and when the reductions took place at the beginning of the planning horizon. The differences were between 24.1 and 0.07% in relation to the conventional scenario (no planting restriction). The differences were not higher because prescriptions changed from planting to coppice.**

**Key words:** Linear programming, forest planning, planting reduction.

## INTRODUCTION

Forest operations deal with products of biological sources on a long-term rotation (Campos and Leite, 2017). The implementation of operational (short-term) management plans is directly affected by many factors, including market demand, economic and silvicultural changes, restrictions of access to stand, different return rates from that planned, an uneven proportion of products, and losses by pests or adverse environmental conditions (Nilsson et al., 2012). Decisions based on these unpredictable factors are often time-sensitive, resulting in noncompliance of what was previously intended in the strategic plan (long-term) (Weintraub and Cholaky, 1991; Davis et al., 2000; Messier et al., 2016; Daniel et al., 2017). Generally, the immediate solutions comprise

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reprocessing the long-term model (including the operational contingencies) or reprocessing new scenarios. These actions often lead to sub-optimal solutions if compared to the initial long-term proposal, and they do not consider the impact of changes on the long-term feasibility of the forest enterprise (Pukkala, 1998; Thorsen and Helles, 1998; Weintraub and Davis, 1996; Pasalodos-Tato et al., 2013).

Hierarchical planning (long-, medium-, and short-term), is crucial for the survival of any business since it allows optimal allocation of financial, physical, and human resources (Andersson, 2005; Sterk et al., 2009; Bettinger et al., 2017). Therefore, it is imperative to accurately estimate the impacts of changes in forest operations on production and costs compared to the original plan (Machado, 2014), since this influences resource allocation in the future.

In order to assess the impact of changes occurring at tactical and operational levels in the long-term plan, it is valuable to integrate these levels into a single mathematical model (Leuschner et al., 1975; Pasalodos-Tato et al., 2013; Eriksson, 2006; Rodrigues et al., 2006; Nanang and Hauer, 2008). However, depending on model complexity, integration requires the employment of metaheuristics, resulting in sub-optimal solutions (Murray and Church, 1995; Heinonen and Pukkala, 2004; Rodrigues et al., 2004a, b; Zhu et al., 2007; Nascimento et al., 2013; Borges et al., 2014; Bachmatiuk et al., 2015; Bettinger and Boston, 2017). A simpler alternative is to use a mixed-integer programming model (MIP) (Banhara et al., 2010; Öhman and Eriksson, 2010; Tóth et al., 2012), also metaheuristics which allows the quantification of short-term effects of long-term plans. The objective of this study was to analyze and quantify the impact of planting restrictions on management planning and economic indicators at different times of the planning horizon.

## MATERIALS AND METHODS

### Mathematical model

A mixed-integer programming model was developed using the Regulation Forest Production (RPF) generator (Binoti, 2012). Prescriptions were formulated using planning model type II (Johnson and Scheurman, 1977). Planted areas on plantation prescriptions were modified by varying the planted area (Pk) in the model. This modification allows planting operations assigned on tactical and operational planning levels to differ from the strategic planning previously processed. The constraints for the model were: cut area, minimum and maximum volume, and planting reductions in plantation prescriptions. Since one of the goals was to compare results in scenarios for which planting was not completely done, no forest regulations constrain was implemented.

The planning horizon (PH) was 21 years, and the prescriptions were clear cut with regeneration by planting or coppicing with up to three rotations. Rotations ranged from 6 to 8 years, and the minimum and maximum limits of volumetric demand per period were defined between 65,000 and 90,000 m³. The purchase of timber was considered in periods when production was below the minimum demand.

The mixed-integer programming model developed is as follows:

Objective function:

$$MAX \quad R = \sum_{i=1}^{n}\sum_{j=1}^{m}\sum_{k=1}^{q} X_{ijk} C_{ijk} \tag{1}$$

Subject to:

$$X_{ijk} = A_{ik} \times P_{ij} \tag{2}$$

$$\sum_{j=1}^{m} P_{ij} = 1, \forall i \tag{3}$$

$$D\min_k \leq \sum_{o=1}^{w}\sum_{i=1}^{n}\sum_{j=1}^{m} V_o X_{ijk} \; \{k = 0,1,...,H-1\} \tag{4}$$

$$D\max_k \geq \sum_{o=1}^{w}\sum_{i=1}^{n}\sum_{j=1}^{m} V_o X_{ijk} \; \{k = 0,1,...,H-1\} \tag{5}$$

$$A_k = \sum_{i=1}^{n}\sum_{j=1}^{m} X_{ijk} \; \{k = 0,1,...,H-1\} \tag{6}$$

$$L_k = \sum_{i=1}^{n}\sum_{j=1}^{m} X_{ijk} \; \{k = 0,1,...,H-1\} \tag{7}$$

$$L_k \leq A_k \times P_k \; \{k = 0,1,...,H-1\} \tag{8}$$

$$P_k \in \{0, 0.1, ..., 1\} \tag{9}$$

$$P_{ij} \in \{0,1\} \tag{10}$$

where $R$ = overall net present value (NPV) (€); $n$ = total number of management units; $m$ = total number of management alternatives for the $i^{th}$ management unit; $q$ = period in the planning horizon; $X_{iqjk}$ = area (ha) of management unit i, in number of the periods q coming from the $j^{th}$ prescription in period k; $C_{iqjk}$ = net present value (NPV) per area (ha) of the $i^{th}$ management unit cut in period q, implementing the $j^{th}$ prescription; $A_{ik}$ = area of management unit i in year k; $P_{ij}$ = prescription j for management unit i; $D\min_k$ and $D\max_k$ = respectively, minimum and maximum volumetric demands (m³) in each period k of the planning horizon; $V_0$ = volume per hectare (m³ha$^{-1}$) at age 0, defined by the production table; $A_k$ = areas with prescriptions j in period k; $L_k$ = areas effectively planted on prescription j after cut in period k; $P_k$ = rate of planted area in period k with prescription j.

### Scenario simulation

Data from a model property located in Minas Gerais State, Brazil, with 18 management units (MU) and 1800 ha were used. At the beginning of PH, the area was divided into eight age groups (1, 2, 3, 4, 5, 6, 7 and 8 years), with 225 ha each. The productivity

**Table 1.** Silvicultural operations and harvest costs used for the economic evaluation of the model, and yield table ($m^3ha^{-1}$) by regeneration method and age.

| Year | Cost (€/ha) | Yield ($m^3ha^{-1}$) | |
|---|---|---|---|
| | | Clearcut | Coppice |
| 1st | 1788.12 | 71.1 | 67.54 |
| 2nd | 717.10 | 97.87 | 92.98 |
| 3rd | 338.30 | 132.34 | 125.73 |
| 4th | 38.82 | 174.99 | 166.24 |
| 5th | 38.82 | 225.19 | 213.93 |
| 6th | 38.82 | 280.96 | 266.91 |
| 7th | 38.82 | 339.05 | 322.1 |
| 8th | 38.82 | 395.64 | 375.86 |

Source: Binoti (2010).

**Table 2.** Harvest costs, considering harvesting machine productivity.

| Harvest difficulty | Minimum productivity ($m^3$) | Maximum productivity($m^3$) | Cost (€/$m^3$) |
|---|---|---|---|
| 1 | -1 | 100 | 3.08 |
| 1 | 100 | 200 | 2.64 |
| 1 | 200 | 300 | 2.20 |
| 1 | 300 | 1000 | 1.76 |
| 2 | -1 | 100 | 3.52 |
| 2 | 100 | 200 | 3.08 |
| 2 | 200 | 300 | 2.64 |
| 2 | 300 | 1000 | 2.20 |

Source: Binoti (2010).

(Table 1) was provided by the forestry company where the stands are located. Silviculture and harvesting costs were adapted from a formula developed by Binoti (2010) (Table 1). Harvesting costs were divided into two strata according to the difficulty of harvesting in each plot (Table 2). The amounts were converted from the Brazilian currency to euros (€) using the conversion factor 3.89 (€ 1.00 = R$ 3.89) for 20 December 2017 (European Central Bank, 2019).

The interest rate used was 8% per year, timber price was set at 12.85 (€/$m^3$), and the residual value of timber after the planning horizon was 12.85 (€/$m^3$). The model was processed using *Lingo* (15.0), granted to this study by Lindo Systems Inc. (http://www.lindo.com).

Sixty scenarios were simulated with the variation in the restriction in the planted area (Pk) modifying the plantation prescriptions. In these scenarios, the areas scheduled for planting in the strategic plan differed from the areas where planting was done. This change was based on aspects such as area loss, cost reductions, and planting problems. The net present value (NPV) obtained by the new scenarios was compared and analyzed by gains or losses due to the changes. Furthermore, groups of scenarios were created varying the rate of planted area reduction (e.g., 0% or not planting, 10%, 20%, …, 100% of the area), and the period within the planning horizon of these reductions occurred. Therefore, the options for the reductions in the planted areas were:

(1) Planting entire area, schedule for this purpose, in all years;
(2) Planting 90, 80, ..., 0% of the area in the initial third of the Planning Horizon (PH) (1st to 7th year);
(3) Planting 90, 80, ..., 0% of the area in the middle third of the PH (8th to 14th year);
(4) Planting 90, 80, ..., 0% of the area in the final third of the PH (15th to 21st year);
(5) Planting 90, 80, ..., 0% of the area in the initial half of the PH (1st to 11th year);
(6) Planting 90, 80, ..., 0% of the area in the final half of the PH (12th to 21st year);
(7) Planting 90, 80, ..., 0% of the area in all years.

## RESULTS AND DISCUSSION

All solutions were economically feasible except when the areas were not planted in any period of the PH (100% planting restricted). In this scenario, it was not possible to meet the minimum demand in any period due to the exhaustion of timber stock. Whereas, in the other scenarios, the reduction in planted areas resulted in a modest overall NPV reduction and required timber purchase in some periods of the PH.

In the conventional scenario in which the entire area was planted, timber purchase was not necessary, the NPV was € 6,884,982.01, and timber volume did not significantly variate over the years, even though there were no restrictions accounting for regulation in the

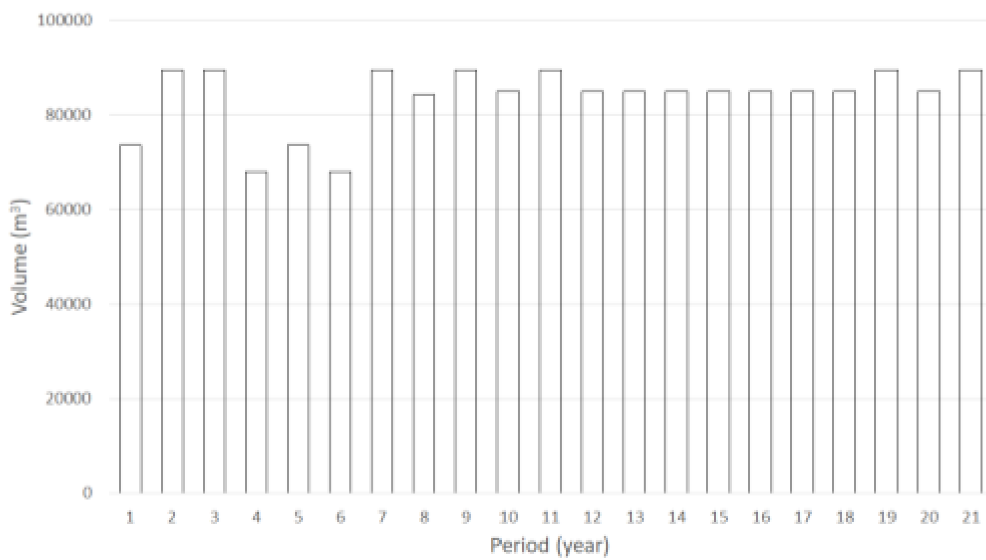


**Figure 1.** Annual cut volume (m³) of the conventional scenario (no planting reduction) distributed within the PH.

model (Figure 1).

The NPV decreased following the restrictions in the planted area (Figure 2). This reduction was driven by the selection of coppicing over planting the stands, considering that sprouts usually yield less.

Planting restrictions at the beginning of the PH result in lower NPV at the end. This is because timber volume production is already compromised at the beginning of the PH due to the selection of coppice prescriptions, yielding less timber volume when compared with plantations. In some groups of scenarios, the NPV is slightly variated. This demonstrates that, even with lower yields in coppice prescriptions, it is an option for periods when there is no availability for planting in harvested areas.

When the restrictions in the planted area variated during the initial third of the PH (1st to 7th year), the NPV gradually decreased. When there was no planting (0% of the area planted), the loss in NPV was 8.06%, whereas when 90% of the area was planted, the difference was €6,246.79, representing a loss of 0.09% (Table 3) if compared to the expected scenario (planting the entire area). In addition, it was necessary to purchase timber in some of the scenarios (for example the scenario with 0% of planting, which had timber purchased in period 11, and the scenario with 30% of planting, where timber was purchased in periods 11 and 18).

Similarly, during the middle third of the PH (8 to 14th year), this group also experienced a gradual reduction in the NPV. Specifically, for the scenario in which there was no planting, the difference from the conventional was €55,781.49, (0.81% loss on investment), while for the minimum reduction (90% of planting area), the difference was €40,843.18 (0.593% loss) (Table 3). These groups did not require timber purchase since all demands were satisfied (Figure 2b).

In the final third of the PH (15th to 21st year), the NPV reduced €38,035.99 (0.552% difference from the conventional scenario) when the planting area reduced from 80 to 0% of the area. The minimum reduction (90% of planting) led to an NPV increase of €622.11 (Table 3).

Scenarios with restrictions in the planted area in the middle third and in the final third of the PH had lower NPV decreases compared to scenarios of the initial third (Table 4). In this group, planting prescriptions occurred at least once, so the timber demand for all periods was satisfied. Similar to that group with planting reductions in the middle third of the PH, timber purchase was not necessary for any period for planting reductions in the final third of the PH (Figure 2c).

The scenarios of planting restrictions in the initial half of the PH ($1^{st}$ to $11^{th}$ year) showed a reduction of 10.14% in the NPV, representing a loss of €697,565.55, with 0% of planting. On reductions from 70 to 0% of the planted area, it was necessary to purchase timber in at least one period, resulting in the highest purchased volumes in 0 and 10% of planting, with 65,000 and 56,634 m³, respectively (Figure 2d).

Planting restrictions in the final half of the PH (12th to 21st year) did not result in large NPV variations, similar to in the middle and final thirds of the PH. The greatest NPV difference in this group was 1.61%, which represents a loss of €115,203.09 (Table 3). The reason timber demand was satisfied in this case was the selection of planting prescriptions at the beginning of the PH. Hence, all timber demands were met for this group, and no timber purchase was required in any period (Figure 2e and f).

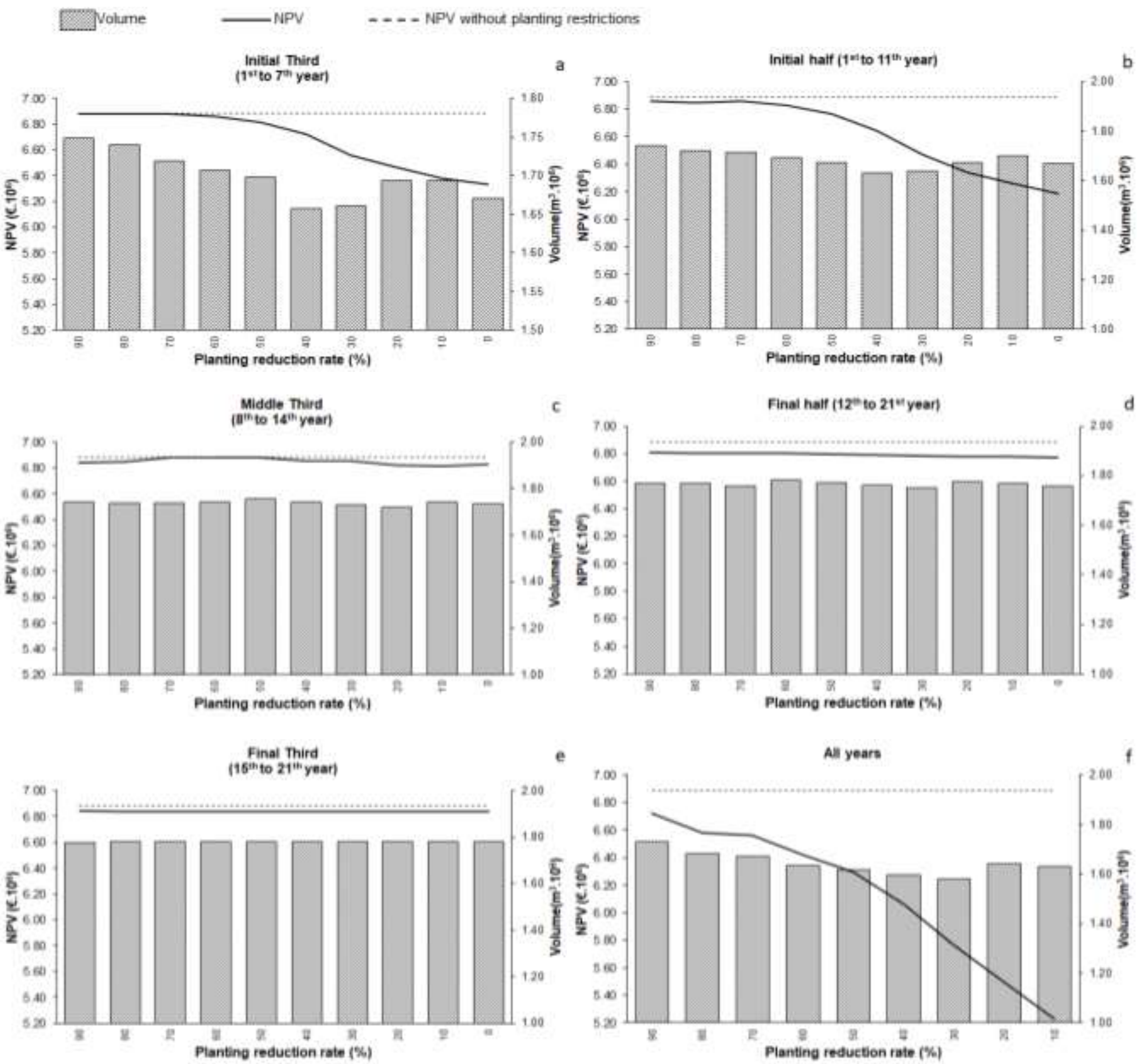


**Figure 2.** Cut timber volume (m³), and net present value (NPV) (€) of the groups of scenarios of planting reductions.

**Table 3.** Maximum and minimum losses in NPV compared to the conventional scenario of planting the entire area in all years of the planning horizon.

| Scenario | NPV | |
|---|---|---|
| | Minimum | Maximum |
| Initial Third | 0.09 | 8.06 |
| Middle third | 0.07 | 1.04 |
| Final Third | 0.552 | 0.6 |
| Initial Half | 0.35 | 10.14 |
| Final Half | 1.16 | 1.61 |
| Every year | 2.33 | 24.1 |

**Table 4.** Net present value (NPV) (€$10^6$) considering variations in the planted area in different periods of the planning horizon.

| Planted area (%) | Period of the planning horizon when planting reduction occurred | | | | | |
|---|---|---|---|---|---|---|
| | Initial third | Middle third | Final third | First half | Final half | All years |
| 0 | 6.33 | 6.83 | 6.85 | 6.19 | 6.77 | |
| 10 | 6.38 | 6.81 | 6.85 | 6.26 | 6.78 | 5.23 |
| 20 | 6.46 | 6.82 | 6.85 | 6.34 | 6.78 | 5.49 |
| 30 | 6.55 | 6.85 | 6.85 | 6.47 | 6.79 | 5.77 |
| 40 | 6.72 | 6.86 | 6.85 | 6.64 | 6.79 | 6.06 |
| 50 | 6.81 | 6.88 | 6.85 | 6.77 | 6.80 | 6.30 |
| 60 | 6.86 | 6.88 | 6.85 | 6.83 | 6.80 | 6.42 |
| 70 | 6.88 | 6.88 | 6.85 | 6.86 | 6.80 | 6.56 |
| 80 | 6.88 | 6.85 | 6.85 | 6.85 | 6.80 | 6.58 |
| 90 | 6.88 | 6.84 | 6.85 | 6.86 | 6.81 | 6.72 |

The scenario with planting restrictions in all periods of the PH resulted in the lowest NPV, indicating that not restoring forest resources can lead to exhaustion of forest timber stock. Although scenarios with planting reduction at the end of the PH resulted in higher NPV, the influence of timber shortage will arise in the next PH. This effect will be either due to the reduction of the planted area or by choosing coppice prescriptions.

Scenarios resulting in the highest NPV losses were those with planting reduction (90 to 0% of the area planted) in all years of the PH. The losses were from 24.1% when planting 10% in all years and from 2.33% when planting 90% in all years. In scenarios with 0 to 70% of planting, it was necessary to purchase timber in at least one period (Figure 2).

Application of operational research tools in hierarchical forest planning can contribute meaningfully to decision-making (Fonseca et al., 2012; Martins et al., 2014; Hahn et al., 2014; Garcia-Gonzalo et al., 2016; Carvalho et al., 2016). However, because of the relatively large PH, there are several risks and uncertainties that this model is unable to predict (e.g. economic fluctuations, area loss by wildfire or disease, and budget cutbacks) (Kangas and Kangas, 2004; Eyvindson and Kangas, 2018). In these cases, there is an impact on the optimization response of the model at the strategic, tactical, and operational levels.

The restrictions in planted areas decreased the NPV in all scenarios in relation to the three dimensions of the decision variable: intensity (90 to 0% of the area), time, and period. Changes in all dimensions showed a tendency to exhaust forest resources that are withdrawn and not restored. Regarding intensity, it was verified in all scenarios that, the greater the reduction in the planted area, the lower the NPV. This indicates that, although planning can absorb part of the reductions by selecting coppice prescriptions, the yield will be lower compared to those when the areas were completely planted. Temporally, NPV losses were greater when they occurred earlier in the PH because of the reduction of areas available for production and future harvest. However, losses with reductions at the end of the PH will be incurred after the transition period due to the timber shortage failing to supply the minimum demand during the next first periods. The period when restrictions occurred also had a direct relationship to NPV decrease. Incomplete planting in one third, half, or in all periods of the PH was directly related to the reduction of NPV when compared with the scenario where there was no change in planting.

## Conclusion

The model proposed in this study is effective in verifying the impact of restrictions in the planted area on forest planning and can be used as decision criteria as it shows the effects of not investing in planting after harvesting the stand. Although the model was tested only with reductions in planted areas, it can be used in situations where there are uncertainties such as an increase/decrease in planted areas or in timber volume demand.

Suggestions for future studies include: (1) simulate planting restrictions scenarios in interleaved years and verify whether the final revenue variation is lower; (2) simulate scenarios of increase or decrease in planting areas and verify whether planting new areas can absorb the revenue loss emerged from the restrictions; and (3) simulate variations in interest rate, stumpage, forest yield, and timber purchase price, modifying the objective function to maximize production and reduce cost.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Successive off take of elements by maize grown in pure basalt powder

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**Basalt powder wastes from mining activities have potential to be used as a natural fertilizer. Basalt minerals in agricultural soils may release plant nutrients and increase soil negative charge. In this work, the weathering of basalt promoted by maize rhizosphere was investigated. We studied the chemical and mineralogical composition of basalt, including cation exchange capacity, as well as the rate of elements offtake by maize grown in a pure basalt powder during seven successive growth cycles. A pot experiment was carried out under controlled environmental conditions; plant and rock materials were evaluated at the end of successive growth cycles. X-ray powder diffraction analysis showed diopside and andesine as main minerals of basalt, and smectite. Scanning electron microscopy images evidenced new amorphous components resulting from rhizosphere-induced weathering. The elements K, Ca, Mg, Al, B, Cu, Fe, Mn and Zn were measured in plant tissue, and related to the weathering of basalt minerals. The studied basalt, therefore, provides nutrients to plants and exhibits physicochemical properties, such as cation exchange capacity, especially important for highly weathered soils presenting low cation exchange capacity, such as Oxisols.**

**Key words:** Bioweathering, natural fertilizer, mining waste, basalt minerals, cation exchange, nutrient availability.

## INTRODUCTION

Some silicate minerals contain high concentration of nutrients, which are required by plants for growth. These minerals have been used as agricultural fertilizers, releasing its nutrients slowly (Ciceri and Allanore, 2019; Manning et al., 2017; Zorb et al., 2014). In the recent years, many studies where mining by-products were applied to soils have emerged, turning mining waste in products as a soil fertilizer or remineralizer. The approach

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is an attempt to reduce agricultural costs and dependence on imported fertilizers. Many works showed positive results for crop productivity and increases in soil quality, whereby basalt powder, for its composition and abundance in spread areas over the world, may assist massively in quality building of soils (Anda et al., 2015; Nunes et al., 2014; Silva et al., 2017). In Brazil, an existing network of basalt quarries, already producing construction aggregates at low cost, has the potential to supply crushed material to agricultural regions (Lefebvre et al., 2019).

Basalts are among the most studied rocks because it provides nutrients for plants, especially calcium (Ca), magnesium (Mg), potassium (K) and micronutrients, such as boron (B), copper (Cu), manganese (Mn) and zinc (Zn) (Anda et al., 2015; Chathurika et al., 2015; Ramos et al., 2015). Plants rhizosphere and their associated microbial populations play a major role in the silicate minerals weathering (bioweathering) by increasing acidity, absorbing and releasing elements and organic ligands, as well as siderophores (Burghelea et al., 2015). Plant roots, ultimately, contribute to increase the dissolution rates of Ca and Mg silicates present in basaltic minerals (Akter and Akagi, 2005; Anda et al., 2015; Hinsinger et al., 2001; Silva et al., 2017).

Basalt minerals undergo a congruent dissolution whenever its bulk chemical composition is rich in iron (Fe), and alkaline earth elements, such as Mg and Ca (Silva et al., 2017). The susceptibility of basalt minerals to weathering, generally follows the sequence: glass > olivine > pyroxene > amphibole > plagioclase > K-feldspar (Eggleton et al., 1987). The Fe(II) oxidation is a driving force in primary mineral weathering (Essington, 2003). As an intermediate step, at an early stage of weathering of basic silicate minerals, the rapid oxidation of Fe(II) can form amorphous phases and low crystallinity minerals, such as ferrihydrite (Colombo et al., 2014; Yu et al., 2017), which is formed from solution precipitates. In time, the weathering of these minerals may become a mixture of ferrihydrite, iron oxide-hydroxides and clay minerals. Some phases may be slowly recrystallized forming secondary minerals. During weathering, some of the basalt minerals may be converted into smectites. Ferromagnesian minerals form trioctahedral smectite, whereas plagioclase alters to dioctahedral smectite.

In weathered basalts, pyroxenes also weather via a mechanism involving a high degree of structural inheritance. The mechanism can be induced by grinding particles to an ultra-fine size (Berner and Schott, 1982). When the surface of these particles, in diopside, suffers cation depletion followed by deprotonation, an incongruent dissolution takes place, and secondary minerals, normally smectite, are formed (Berner and Schott, 1982).

The application of a finely ground basalt in a highly weathered soil increases its cation exchange capacity. Anda et al. (2015) verified, after a high dose (80 t basalt $ha^{-1}$) was applied in a Malaysian Oxisol, a sharp increase in the soil's net negative charge was observed from 1.5 to 6.3 $cmol_c$ $kg^{-1}$ in a 12-month incubation period, and to 10.1 $cmol_c$ $kg^{-1}$, after 24 months. The increasing net negative charge rate suggest that smectite like minerals or low crystallinity minerals, which is a source of permanent negative charge, were formed. An increase in permanent negative charge in Oxisols is an invaluable gain for its quality. However, basalt composition is very dissimilar among different source locations and its charge contribution to soils, as well as rate of transformation or dissolution will depend on the basalt composition and texture, and on abiotic and biotic conditions.

Most natural tropical soils - such as Brazilian Oxisols - due to the strong weathering and intense leaching processes become acidic, with low fertility and low cation exchange capacity. Thus, the management of these soils with crushed rocks should be focused on increased surface charge characteristics and cation retention. We hypothesize that crushed basalt applied to agrosystems release beneficial elements for crop growth and generate new negative charge sites in the soil. Hence, the knowledge of physicochemical properties of basalt powder and how the rhizosphere of cultivated plants affects the weathering of basalt minerals is a requirement to understand its potential benefits to agricultural soils. The aim of this work was to investigate changes in the chemical and mineralogical composition of basalt powder, including cation exchange capacity, as affected by the rhizosphere of maize, as well as the rate of elements offtake.

## MATERIALS AND METHODS

### Sampling

A basalt sample was collected from piles located at Araguari, Minas Gerais State, Brazil (Moraes et al., 2018). This is a by-product that originated from the production of gravel for civil construction. The sample was air dried and was homogenized using the cone-and-quartering reduction method (Campos and Campos, 2017). This procedure was repeated several times to ensure complete homogenization of material, forming the bulk sample.

### Greenhouse experiment

The pure bulk basalt sample was placed into 500 mL pots under controlled environmental conditions in a greenhouse. Two plants of maize (*Zea mays*) per pot were grown in sets of three pots, repeated for seven growth cycles, totaling 21 pots. Each growth cycle was 45 days. Seven extra pots without plants were prepared, as a control treatment. At every two days, all pots were watered with deionized water. Pots were fertilized with nutrient solution (92.76 mg $pot^{-1}$ $NH_4H_2PO_4$) in the $15^{th}$ and $30^{th}$ days of growth of each cycle.

At the end of each 45-days cycle, whole plants from all pots were harvested. The basalt content from one set of three pots previously grown with plants, and another, from one pot of the control set were removed from the greenhouse. The pots content was used for the laboratory analyses. The remaining sets were re-sown for a new growth cycle.

### Plant analysis

The harvested plants were oven dried at 65°C until constant weight. The dry biomass was taken. Major (K, Ca, Mg, Al, Fe) and minor elements (B, Cu Mn e Zn) in dry biomass (total dry mass comprising shoots and roots) were extracted by $HNO_3$:$HClO_4$ in a digestion block, according to Embrapa (2017) and determined by inductively coupled plasma-optical emission spectrometry (ICP-OES).

Elements offtake (plant element concentration x dry mass) along the cycles were modelled and the equations were selected according to the analysis of variance (ANOVA) and, thereafter, were tested for normality (Shapiro-Wilk) and constant variance test (homoscedasticity). These statistical analysis were performed using Sigma Plot 12.0 software (Sigma Plot Software; San Jose, California, USA).

Dry mass and elements offtake were analyzed by Principal Component Analysis (PCA), using standardized scores. The RStudio software (version 3.4.0) was used along with its PCA packages FactoMineR and factoextra.

### Rock material analysis

The basalt pots removed from the glasshouse at the end of each growth cycle were dismantled and its contents were wet sieved to obtain four size fractions: < 53 µm, 53-300 µm, 300-1000 µm and > 1000 µm.

The main chemical elements of basalt were analyzed using the multi-acid solution method, where: 500 mg of sample was digested in 2:3:2:1 ratio of HCl:$HNO_3$:HF:$HClO_4$; 10:15:10:5 mL, respectively, determined by ICP-OES (SGS Geosol Laboratórios Ltda). Major elements were determined by wavelength dispersive X-Ray Fluorescence (XRF) spectroscopy, on fused glass discs, 40 mm-diameter, prepared from 0.8 g of sample powder mixed with 4.5 g lithium tetraborate flux and fused in Pt-5% Au crucibles at 1120°C (SGS Geosol Laboratórios Ltda). The loss on ignition was determined after heating samples overnight at 105°C to remove water. The weight loss was measured after calcination of samples at 1,000°C for approximately 2 h.

The mineralogical composition of fractions < 53 µm was analyzed by X-ray diffraction analysis (XRD) using a PANalytical Empyrean (PW3050/60) diffractometer, using the powder method in the range of 5º < 2θ < 75º. CoKα radiation (40 kV; 40 mA) was applied, and the 2θ scanning speed was set at 0.02° $s^{-1}$. Data was acquired using the software X'Pert Data Collector 4.0 and the data were treated on X´Pert HighScore 3.0 (PANalytical). Minerals were identified by comparing the obtained diffractogram with the ICDD-PDF (International Center for Diffraction Data) database.

The X-ray diffraction pattern of the clay fraction (oriented sample) wase obtained three times: the first was air-dried, the second was after treatment with ethylene glycol, and the third was after heating at 550°C for 2 h.

The mineralogical composition in each basalt size fraction was estimated by the stoichiometric method, also known as rational calculation, which is based on the relationship of the experimental chemical composition with the chemical formulas of the minerals, establishing logical considerations based on the qualitative (XRD) and quantitative (XRF) analytical data. An already well-known software used to perform the rational calculation is the ModAn (Paktunk, 1998), which was used in this work.

The morphology of the basalt bulk samples was examined by scanning electron microscopy (SEM) using a Zeiss field emission microscope model SIGMA HV using the InLens detector. A thin conductive layer of gold (10 nm) was deposited over the samples using the Q150T-ES sputter (Quorum Technologies). The chemical composition of selected mineral particles was evaluated by Energy-dispersive X-ray spectroscopy (EDS).

Cation exchange capacity on the fractions < 53 µm was determined by magnesium sorption (BS EN ISO 11260, 2011). Measurements of the CEC followed the methodology: 3.5 g of sample were placed in 50 mL polyethylene tubes, and leached with 30 mL 0.1 mol $L^{-1}$ barium chloride dihydrate for 1 h. Tubes were centrifuged and the supernatant was removed. The procedure was repeated three times. Samples were, then, equilibrated with 30 mL 0.01 mol $L^{-1}$ barium chloride solution, and shaken for 12 h. Tubes were centrifuged and the supernatants were removed. Subsequently, 30 mL 0.02 mol $L^{-1}$ magnesium sulfate heptahydrate was added and shaken for 12 h. Tubes were centrifuged and supernatant was collected for analysis. The excess magnesium was determined by flame atomic absorption spectrometry (FAAS AA-6300 Shimadzu). Triplicates were used.

## RESULTS AND DISCUSSION

The X-ray fluorescence (XRF) analysis showed that the chemical composition in basalt fractions (Table 1) was nearly uniform (Tables 2 and 3). The amount of $SiO_2$ and $Al_2O_3$ reflects the presence of minerals such as andesine [$(Ca,Na)(Al,Si)_4O_8$], a plagioclase feldspar, and diopside [$MgCaSi_2O_6$], a monoclinic pyroxene. A high content of $Fe_2O_3$ comes from diopside and ilmenite [$FeTiO_3$]. Significant concentration of CaO and MgO was found in all grain sizes, when compared to the basalt composition applied as fertilizers in another studies (Nunes et al., 2014; Ramos et al., 2015), even though basalt chemical composition varies widely.

Basalt mineralogy is dominated by andesine and diopside (Figure 1). After 315 days in presence or absence of plants, basalt mineralogy remained unchanged. The reflections of andesine and diopside minerals showed little changes in the X-ray patterns and there were detected no secondary crystalline minerals after seven maize growth cycles.

The XRD analysis of the clay fraction as analyzed on oriented mount (Figure 2) revealed the typical shift of the $d_{001}$ peak from 15.9 Å (in the air-dried state) to 17.7 Å (in the ethylene glycol state), indicating the presence of smectite, which was found in different proportions among the basalt fractions (Table 3). After heating, the typical collapse to 9.9 Å is observed.

An important parameter in the dissolution rate and nutrient release of primary minerals is the grain size and its relationship with the exposed reactive surface of minerals and with the chemical composition. The smallest grain sizes are the most reactive fractions (Basak, 2018; Bray et al., 2015).

The crystalline phases on < 53 µm size fraction of the mineral were composed of 48.2% of andesine that has also calcium in its structure (Table 3 and Figure 3). However, andesine has high structural stability and is not easily weathered as it presents a great proportion (Al/Si = 0.5 – 0.66) of Al in its structure. It would be expected that the dissolution of andesine was incongruent, releasing alkalies and alkaline earths relative to silica and alumina.

Diopside represents 20.5% of <53 µm sample. Depending on milling size, and environmental conditions,

**Table 1.** Particle size distribution of the basalt powder used in the experiment.

| Sample | Particle size distribution (µm) | | | | Total (g $pot^{-1}$) |
|---|---|---|---|---|---|
| | < 53 | 53 - 300 | 300 - 1000 | > 1000 | |
| | ----------%---------- | | | | |
| Basalt powder | 10.67 | 12.44 | 26.5 | 50.39 | 663.69 |

**Table 2.** Chemical composition of the basalt powder (fractions and bulk) determined by XRF and ICP-OES.[1]

| Fraction (µm) | $SiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | CaO | MgO | $TiO_2$ | $P_2O_5$ | $Na_2O$ | $K_2O$ | MnO | BaO | $Cr_2O_3$ | Cu | Mo | Zn | LOI[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ----------%---------- | | | | | | | | | | | | ----- mg $kg^{-1}$ ----- | | | |
| < 53 | 49.4 | 12 | 16.5 | 7.38 | 6.35 | 2.74 | 0.35 | 1.72 | 0.97 | 0.16 | 0.06 | <0,01 | 206 | <3 | 116 | 2.57 |
| 53 - 300 | 49 | 11.3 | 16.2 | 7.91 | 5.91 | 2.84 | 0.36 | 1.73 | 0.95 | 0.19 | 0.06 | 0.01 | 196 | <3 | 131 | 1.55 |
| 300 - 1000 | 49.4 | 12.2 | 15.5 | 8.53 | 5.4 | 3.24 | 0.4 | 1.95 | 0.98 | 0.19 | 0.06 | 0.01 | 194 | <3 | 134 | 0.93 |
| > 1000 | 49.4 | 12.7 | 15.1 | 8.85 | 4.9 | 3.38 | 0.4 | 2.46 | 0.93 | 0.2 | 0.05 | 0.01 | 211 | <3 | 149 | 0.58 |
| Content bulk[3] | 49 | 12.6 | 15.4 | 8.73 | 5.28 | 3.2 | 0.43 | 2.06 | 0.9 | 0.2 | 0.06 | <0,01 | 208 | <3 | 139 | 1.32 |

[1]SGS Geosol Laboratórios Ltda; [2]Loss on ignition; [3]Original sample.

**Table 3.** Mineralogical composition of the basalt powder (fractions and bulk).

| Fraction (µm) | Andesine (%) | Diopside (%) | Ilmenite (%) | Smectite (%) |
|---|---|---|---|---|
| < 53 | 48.2 | 20.5 | 18.0 | 13.2 |
| 53 - 300 | 47.0 | 24.5 | 18.3 | 10.2 |
| 300 - 1000 | 50.7 | 24.9 | 18.0 | 6.3 |
| > 1000 | 53.1 | 25.7 | 17.9 | 3.3 |
| Content bulk[1] | 51.9 | 25.7 | 18.1 | 4.3 |

[1]Original sample.

the diopside is relatively, an easily weatherable mineral, and after being applied to agricultural soils, may become a source of Ca and Mg for crops in diopside rich basalts (Figure 3). Moreover, diopside is unstable under acidic conditions and may dissolve congruently by weathering in such conditions (Wilson, 2004). The rhizosphere is the environment where diopside dissolution is likely to occur. The dissolution of pyroxenes is controlled by reactions at the mineral surface. By structural reasons, Ca, Mg and Fe are released preferentially at the beginning, and, lately, the dissolution becomes congruent and linear, as the weathering proceeds. The dissolution products could be precipitated as amorphous compounds, not detectable by XRD (Berner and Schott, 1982).

Diopside and ilmenite (18% of <53 µm fraction) comprise the major iron-containing minerals in the basalt sample (Figures 1 and 3). Iron rich minerals are also rich in micronutrients such as Mn, B, Cu, and Zn.

A representative amount of a smectite (13.2%), mainly in the <53 µm fraction was present in the basalt. Smectite is an expansive clay that has a high cationic exchange capacity (CEC). As particle size increases, the presence of smectite decreases (Table 3). Probably, the weakest cleavage faces on basalt particles are those richer in smectite. Non-weathered samples from basalt presented 24.85 (±1.16) $cmol_c$ $kg^{-1}$ on < 53 µm size fraction. The smectite is a secondary mineral of the phyllosilicate class responsible for most of the reactivity of the studied basalt. As a matter of comparison, the CEC values of the montmorillonites range between 70 and 120 $cmol_c$ $kg^{-1}$, while the CEC values for the vermiculites range between 130 and 210 $cmol_c$ $kg^{-1}$ (Essington, 2003). Also, Oxisols from Cerrado present CEC ~9.4 $cmol_c$ $kg^{-1}$ (STD = 3.9), and some are as low as 4.1 $cmol_c$ $kg^{-1}$ at pH 7.0 (Marchi et al., 2015). These soils present variable charge and natural pH is lower than 5, with effective CEC to even lower values. Therefore, inputs materials containing elevated surface area and permanent negative charge in these soils may represent a great gain in quality.

Total plant biomass production decreased along each successive cycle (Equation 1), with highest production in the first crop cycle (4.15 g of dry matter) and the lowest in

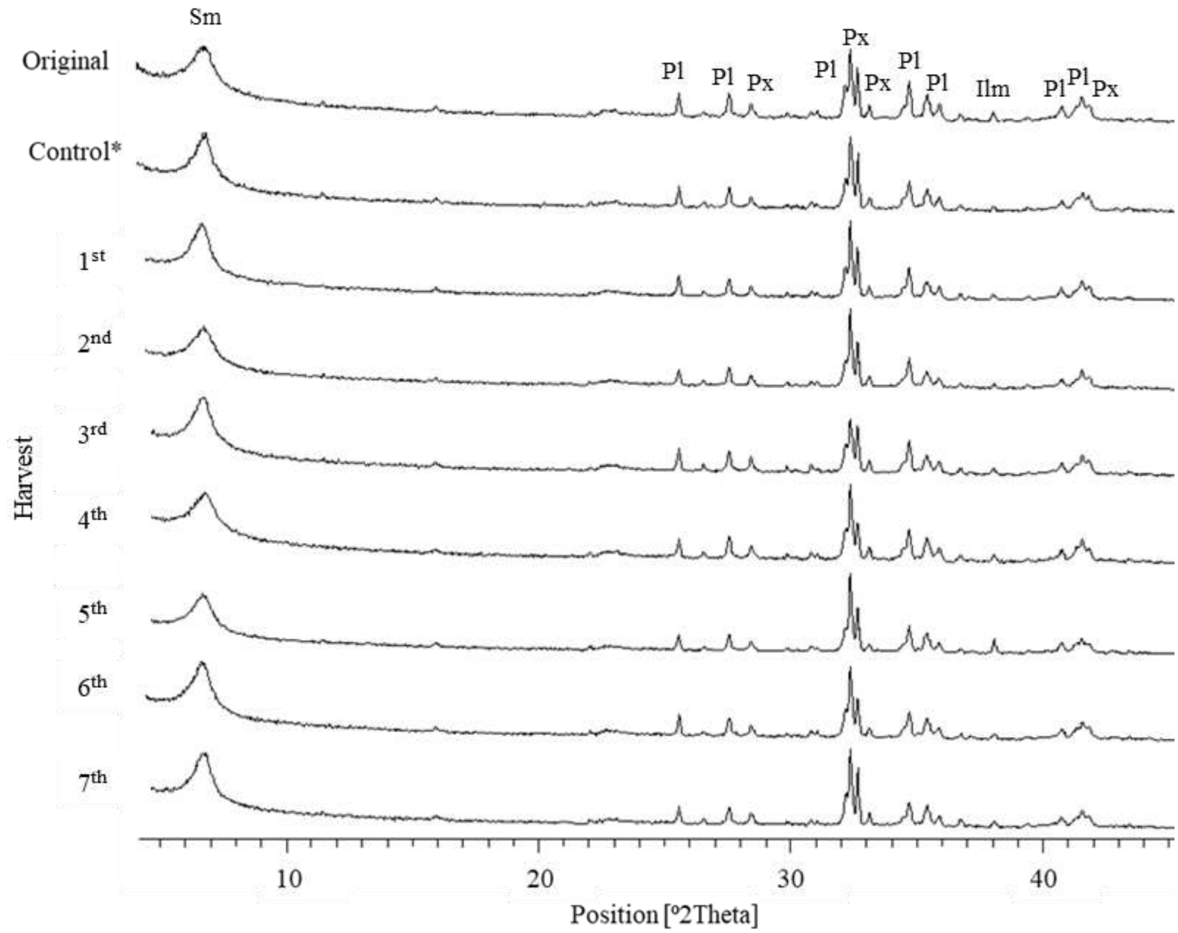


**Figure 1**. X-ray diffraction patterns of <53 μm size particles of basalt after interaction with maize rhizosphere evidencing andesine (Pl), diopside (Px), smectite (Sm) and ilmenite (Ilm) minerals during 7 harvest crops; *Control is the basalt collected from the pot without plants at the end of the experiment.

the last cycle (1.22 g of dry matter; Figure 4). Although plants were grown in pure rock, they were able to grow and take up some macro and micronutrients from basalt in its natural pH (9.04 - 6.99).

$$\text{Dry mass}_{\text{(g per pot)}} = 4.54^{**} - 0.44^{**}(\text{cycle}),\ R^2 = 0.96 \quad (1)$$

The amount of nutrients taken up from basalt by plants varied over each crop cycle (Figure 4). These differences in element mobilization were related to dissolution kinetics of specific minerals. Potassium uptake showed a diverse dynamic than the other elements (Figure 5). Probably, K was extracted by plant roots from non-exchangeable sites from interlayers of smectite minerals, other than by dissolution, such as Ca and Mg. Slowly available potassium, which is fixed and non-exchangeable, is trapped between the layers or sheets of K-rich 2:1 minerals. The idea was clarified statistically by the principal component analysis (PCA) where K, with similar statistical contribution than the other elements to describe results, pulls up toward the y-axis, pulling dry matter in between (Figure 6). The PCA indicated that K presents a different mechanism of release from rock and it reflects on the interaction among the other nutrients.

After applied to soils, the layers from 2:1 minerals may adsorb and release cations, but their efficacy will also depend on the mineral particle size. Indeed, several studies show the presence of 2:1 clay size minerals, even in subsidiary quantities, increases effectively the retention of cations in soil (Raheb and Heidari, 2011).

Plants have developed several highly specific mechanisms to acquire K from minerals (Samal et al., 2010). Wang et al. (2000) investigated the ability of plant types to extract and uptake K from slightly weathered gneiss of differing particle sizes. The authors showed that, among the studied plant species, maize was the one that extracted the highest amount of K.

Concentrations of K in maize dry mass varied from 2.6 to 15 g K $kg^{-1}$ of dry weight. The results demonstrate that the plants were able to access some K from basalt to sustain growth. The K requirement for optimal maize growth range is 17.5 to 22.5 g $kg^{-1}$ in vegetative parts

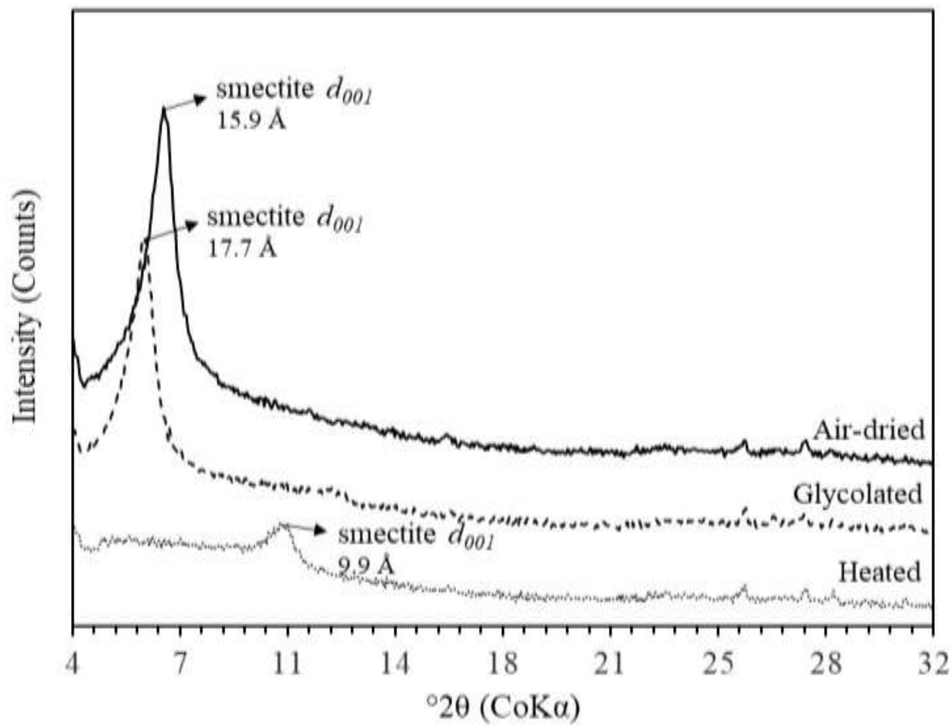


**Figure 2**. X-ray diffraction patterns of the clay fraction (oriented sample) obtained three times: air-dried, after treatment with ethylene glycol, and heated at 550°C.

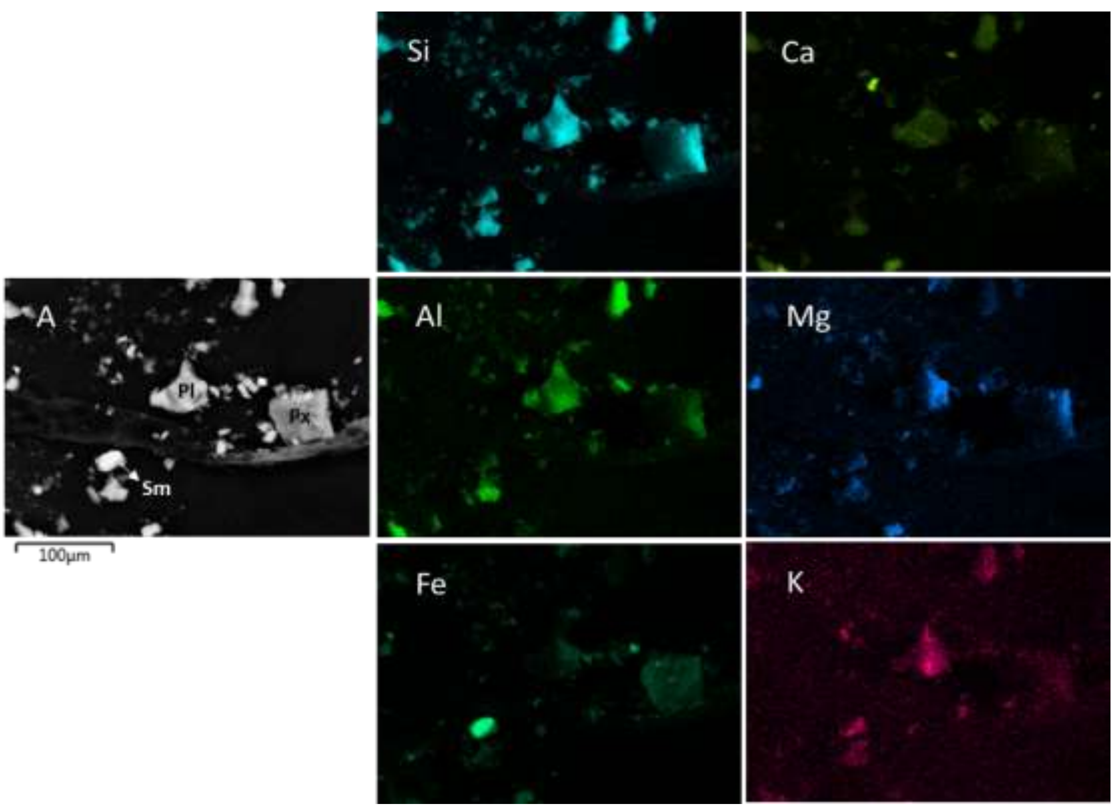


**Figure 3.** Energy-dispersive X-ray spectroscopy (EDS) images of basalt mineral compounds around maize root. A: secondary electrons image evidencing andesine (Pl), diopside (Px) and smectite (Sm) minerals; Si: silicon map; Al: aluminium map; Fe: iron map; Ca: calcium map; Mg: magnesium map; and K: potassium map.

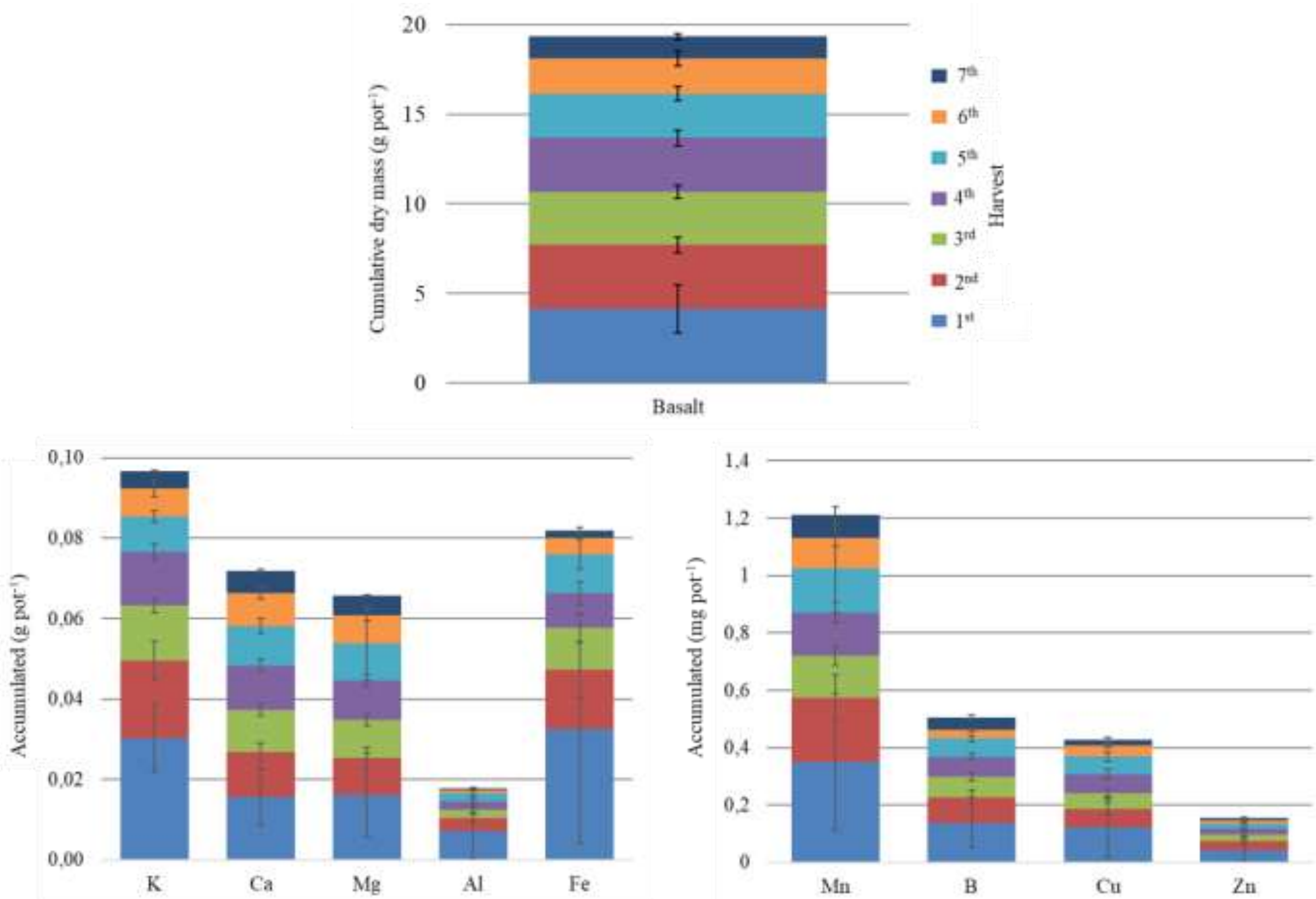


**Figure 4**. Cumulative dry mass and elements offtake[1]by maize cultivated in basalt power after seven cycles. [1]The number of samples per cycle used to calculate the mean (*n*), from the 1st to the 7th cycle, was: *n* = 24 - i; where i = cycle number * -3. Error bars for each cycle were the standard deviation of *n*.

(Malavolta et al., 1997). K offtake were in range between 0.03 g pot$^{-1}$ from the first cycle to 0.004 g pot$^{-1}$ to the last cycle (Figure 4, Equation 2).

$$K_{offtake\ (g\ per\ pot)} = 0.03^{**} - 0.0049^{**}(cycle),\ R^2 = 0.81 \quad (2)$$

Contents of Ca, Mg, Al and Fe in the maize tissue along the seven growth cycles indicated that nutrients were released from basalt minerals, and that basalt minerals were dissolved, partly due to a preferential dissolution of diopside.

The weathering of diopside and andesine in maize rhizosphere provided calcium and magnesium for plants. The nutrient concentration in dry mass ranged from 2.3 to 5.3 g Ca kg$^{-1}$ and from 1.3 to 6.6 g Mg kg$^{-1}$. Calcium and magnesium requirement for maize growth was reached as concentration in maize leaves for optimum growth varies from 2.5 to 4.0 g Ca kg$^{-1}$ (Malavolta et al., 1997), and from 2.5 to 4.0 g Mg kg$^{-1}$ (Hawkesford et al., 2012). Calcium offtake was from 0.015 to 0.005 g pot$^{-1}$ and Mg was from 0.016 to 0.004 g pot$^{-1}$, from the first to the last cycle, respectively (Figure 4, Equations 3 and 4).

$$Ca_{offtake\ (g\ per\ pot)} = 0.015^{**} - 0.0042^{**}\ln(cycle),\ R^2 = 0.85 \quad (3)$$

$$Mg_{offtake\ (g\ per\ pot)} = 0.014^{**} - 0.0046^{**}\ln(cycle),\ R^2 = 0.80 \quad (4)$$

The decay in maize growth and in the offtake of elements is probably due to the weathering of surfaces and dissolution of very small mineral particles in the initial cycles, where nutrients were easily extractable, while in the subsequent cycles a higher effort seems to be made to extract and acquire nutrients from basalt minerals. The rhizosphere has an intrinsic role in basalt dissolution and release of elements. Akter and Akagi (2005) evaluated the active role of rhizosphere in basalt and showed that maize increased the Ca and Mg extraction by a factor of 3 - 4 and 15 - 75, respectively, when compared with the control pots with no plants. According to Hinsinger et al. (2001), the amounts of Ca, Mg and Na released from

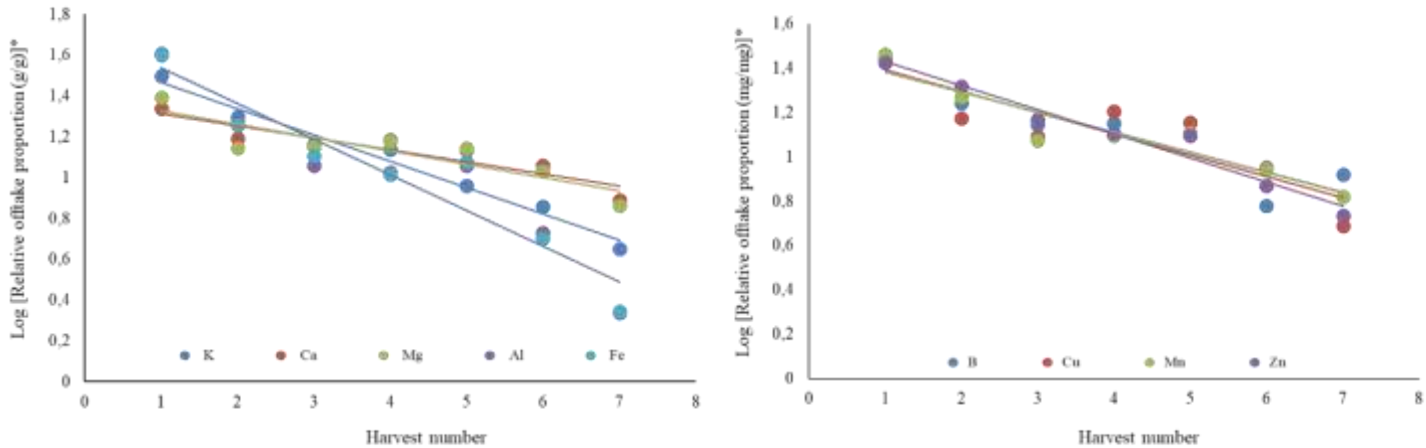


**Figure 5.** Elements offtake ratio from basalt by maize. *Relative offtake proportion = $Log\,[\frac{offtake.100}{\sum_{c=7}^{1}(\frac{offtake}{c})}]$; c = harvest number.

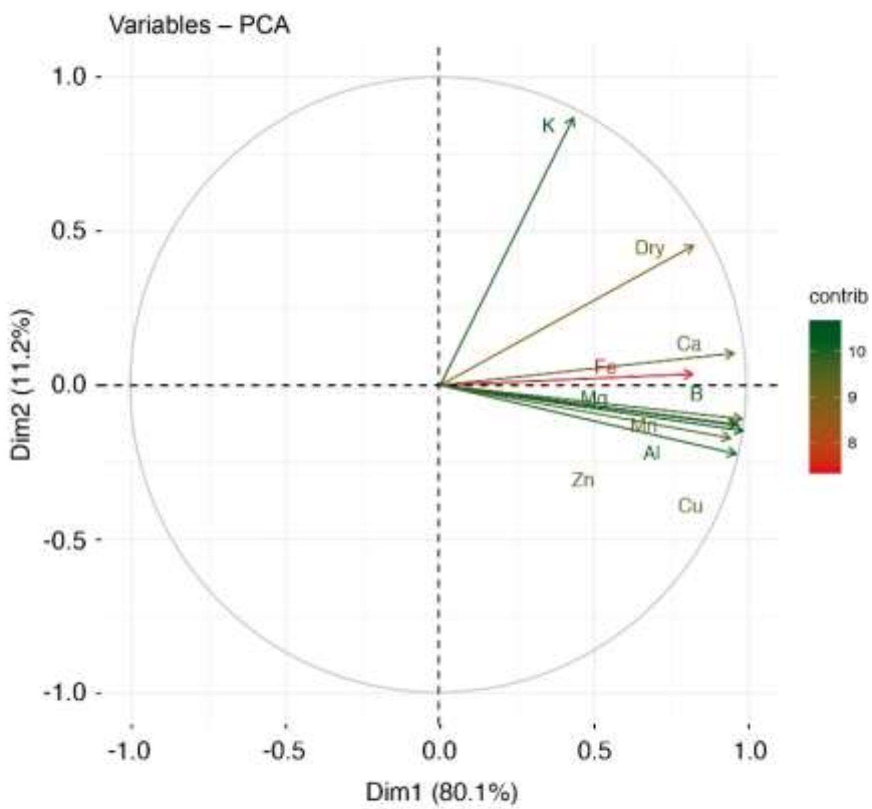


**Figure 6.** Principal component analysis of K, Ca, Mg, Fe, Al, Mn, B, Cu, and Zn maize offtake from basalt and cumulative. Dry = maize dry matter; Dim = dimension; Contrib = contribution.

basalt under leaching conditions in the laboratory increased by a factor ranging from 1 to 5 in the presence of crop plants.

The Al concentration in dry mass ranged from 0.2 to 3.5 g Al $kg^{-1}$.Aluminum offtake along the cycles, although in a different scale, presented the same rate of Fe offtake (Figures 4 and 5). The Fe concentration ranged from 0.8 to 21.5 mg Fe $kg^{-1}$ and the concentration in maize leaves for optimum growth varies from 50 – 250 mg Fe $kg^{-1}$ (Malavolta et al., 1997). In the course of the experiment, the offtake of these elements decayed (Equations 5 and 6). It suggests that ilmenite and diopside dissolution starts from iron oxidation during weathering, mainly from small particles and surfaces of lower crystallinity. As dissolution of easily weatherable minerals proceeds, along the cycles, Al, and Fe offtake decreases. Silva (2016) compared the dissolution of basalt at different grain sizes, despite not verifying the formation of new solid phases, confirmed the idea that the finer fractions are responsible for faster dissolution, while the coarser fractions dissolved slowly.

$$Al_{offtake\ (g\ per\ pot)} = 0.01^{**}e^{(-0.47^{**}cycle)},\ R^2 = 0.91 \quad (5)$$

$$Fe_{offtake\ (g\ per\ pot)} = 0.047^{**}e^{(-0.45^{**}cycle)},\ R^2 = 0.91 \quad (6)$$

The dissolution of small inclusions of ilmenite and diopside in basalt releases Fe. Strains of Fe-oxidizing bacteria are able to grow using the Fe(II) derived from ilmenite of basaltic rocks (Navarrete et al., 2013). This effect may be strongly influenced by the presence of organic acids from plants rhizosphere (Dontsova et al., 2014).

The water solubility of minerals containing Fe in soil is usually very low; however, plant and microbes, in the presence of organic substances, may work together for the oxidation and extraction of Fe(II) complexes, increasing Fe availability for plant growth (Colombo et al., 2014). In particular, gramineous species may have evolved, developing a very efficient mechanism to mobilize Fe (Broadley et al., 2012). According to Hinsinger et al. (2001), the amount of Fe released from basalt under leaching conditions using maize plants reached a maximum increase of about 100-500 times the release without plants (control). Non-absorbed iron ions in solution are then precipitated as low crystallinity iron oxides (especially ferrihydrite and amorphous ferric hydroxide) (Silva et al., 2017).

Images of basalt samples obtained by SEM after the last crop cycle show the interaction between roots and mineral particles, including low crystalline structures (Figure 7). These structures are known as short-range ordered (SRO) minerals like ferrihydrite. Long-term field studies conducted by Yu et al. (2017) demonstrated that the presence of roots significantly increased Al and Fe availability from soils.

This result challenges the conceptual view that the weathering and the formation of SRO minerals are very slow processes and cannot be detected in the short-term (Colombo et al., 2014; Yu et al., 2017). It suggests that the dissolution of minerals can be accelerated by plant roots.

Offtake of manganese, boron, copper, and zinc showed a similar trend when normalized (Figures 4 and 5), although were taken up in different proportions by plants.

The micronutrients Mn, B and Zn are present indistinctively in all primary minerals that compose this basalt, especially diopside (Moraes et al., 2018), while Cu is accumulated in smectite structure, formed by hydrothermal process (Baggio et al., 2016).

Manganese undertakes a similar oxidation process than Fe in mineral structures during weathering, releasing other elements. Manganese concentration values in maize tissue varied from 35 to 159 mg Mn $kg^{-1}$, while the sufficiency range for maize is from 50 to 150 mg Mn $kg^{-1}$ (Malavolta et al., 1997). The Mn offtake varied from 0.35 to 0.07 mg $pot^{-1}$, from the first to the last cycle, respectively (Figure 4), and decayed according to the Equation 7.

$$Mn_{offtake\ (mg\ per\ pot)} = 0.32^{**} - 0.12^{**}\ln(cycle),\ R^2 = 0.92 \quad (7)$$

Boron concentration values in maize ranged between 13.5 to 76.7 mg B $kg^{-1}$, while the adequate level for maize is from 15 to 20 mg B $kg^{-1}$ (Malavolta et al., 1997). For maize, the critical toxicity concentrations in leaves are in the range of 100 mg $kg^{-1}$ (Broadley et al., 2012); therefore, in the case where the basalt is applied to soils, it does not represent any risk of soil contamination with B, but a source of nutrient. In the course of experiment, B offtake ranged from 0.13 to 0.04 mg $pot^{-1}$ from first and the last cycle (Figure 4) and decayed according to the Equation 8.

$$B_{offtake\ (mg\ per\ pot)} = 0.13^{**} - 0.049^{**}\ln(cycle),\ R^2 = 0.92 \quad (8)$$

Copper offtake was higher in the first cycle, media of 120 mg $pot^{-1}$, and decreased to 20 mg $pot^{-1}$ in the last (Figure 4, Equation 9) but shows an adequate Cu concentration in all cycles (mean 23.5 mg Cu $kg^{-1}$). The critical level concentration of Cu in maize is generally between 6 to 20 mg Cu $kg^{-1}$ (Malavolta et al., 1997) and no visible toxicity symptoms of Cu were noticed.

$$Cu_{offtake\ (mg\ per\ pot)} = 0.11^{**} - 0.041^{**}\ln(cycle),\ R^2 = 0.79 \quad (9)$$

Zinc concentration in maize dry matter were below the critical level [15 – 50 mg Zn $kg^{-1}$ (Broadley et al., 2012)]. Zinc offtake was the lowest among micronutrients, reflecting the content of the element in the basalt. Zinc offtake decayed from 0.04 to 0.08 mg $pot^{-1}$, from the first to the last cycle (Figure 4), following the Equation 10.

$$Zn_{offtake\ (mg\ per\ pot)} = 0.04^{**} - 0.016^{**}\ln(cycle),\ R^2 = 0.97 \quad (10)$$

## Conclusion

The basalt mineralogical composition did not change along the experiment, because the dissolution of basalt in maize rhizosphere was congruent. The smectite present in the basalt contributed to the high cationic exchange

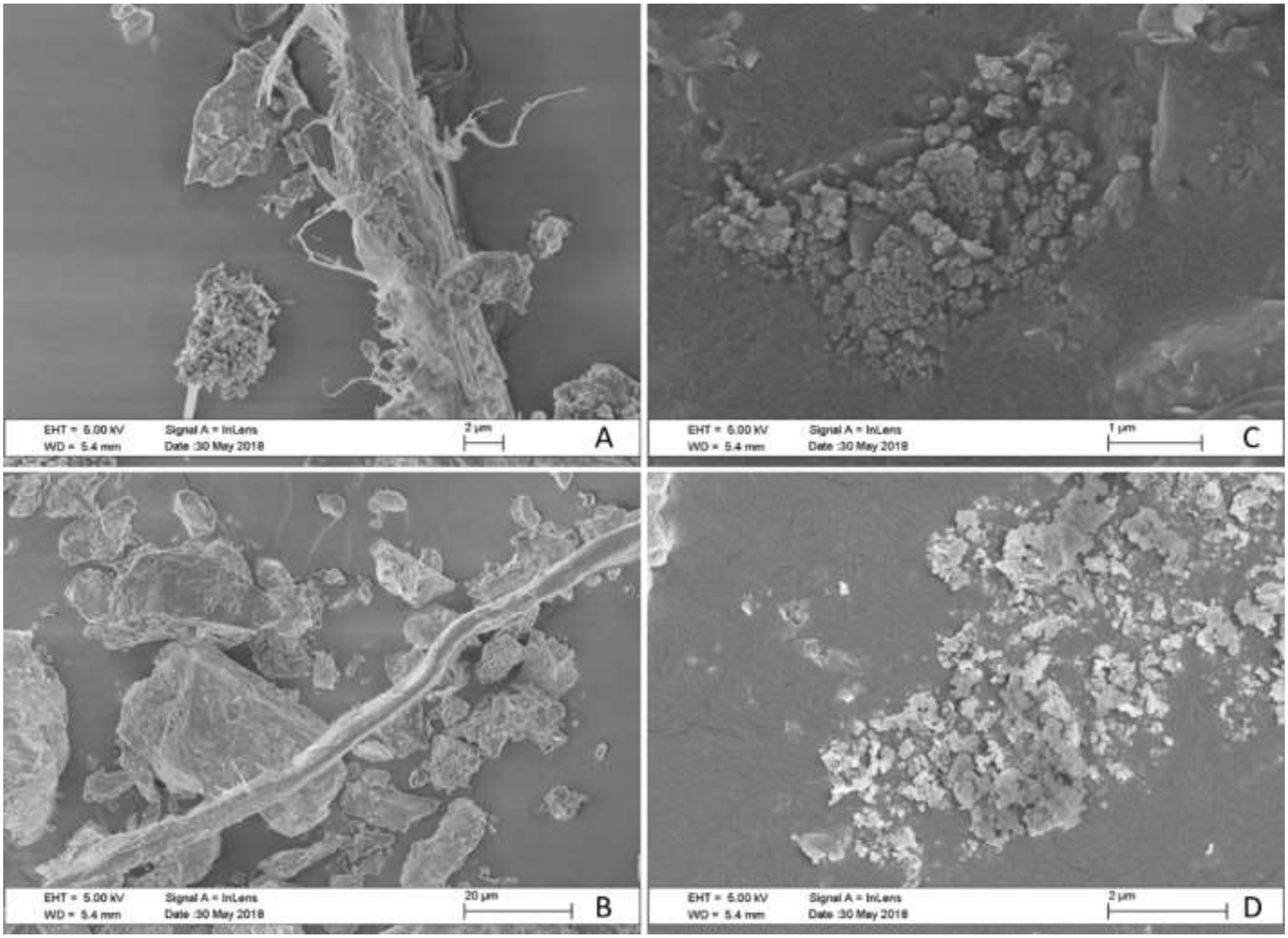


**Figure 7.** Scanning electron microscopy images of basalt samples after seven maize growth cycles. A and B: interaction of roots with basalt minerals; C and D: deposits of low crystallinity minerals.

capacity, indicating a possible ability to change cations into soil environment. Therefore, applications of basalt in Cerrado Oxisols can improve soil quality by adding permanent charge and by increasing its overall CEC. Basalt was able to provide nutrients to maize plants in a short period. Additionally, the offtake rate of elements from basalt was described by equations, which may be used for estimating nutrients release from basalt after being applied in agricultural soils.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Relation of macrofauna diversity and chemical soil properties in rice field ecosystem, Dukuhseti district, Pati regency, Indonesia

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**Indonesia is a country with the third largest biodiversity after Brazil and Madagascar. Of the about 325.530 species of flora and fauna globally, an estimated 25% of the world's species are found in Indonesia. Macrofauna, a land animal that plays a role in influencing the soil ecosystem has specific environmental requirements that enables it to be used as biological indicators of ecosystem conditions, mainly rice paddy ecosystem. This research is necessary owing to the demand for an easy and accurate indicator in predicting soil fertility for farmers. In addition, this study conducted in July TO August 2018 can be used as an inventory of macrofauna indigenous species that may still remain in the paddy field, Dukuhseti district. The implementation of observations was done in paddy fields in the Dukuhseti district in Pati regency, Central Java. Macro fauna specimens and soil samples were taken at various points. 121 individual macro faunas were found and divided into three phyla and 10 Order. The results showed that macro fauna diversity was not always positively correlated with soil chemical properties. Total N, available P, organic C and pH is not directly proportional to the increasingly diverse types of macro fauna in a rice field. The soil's chemical nature in the form of base saturation has a positive correlation with macro fauna diversity that makes it serve as an indicator of fertility. Base saturation in the fields can be associated with the presence of different kinds of macrofauna decomposers such as earthworms, millipedes and denitrivor. Indigenous macrofauna species was not found in the paddy fields in the Dukuhseti district due to intensive land management.**

**Key words:** Macrofauna, soil fertility, soil biology.

## INTRODUCTION

Biodiversity is the number of life contained in a region (Sugiyanto et al., 2007) and Indonesia is the third largest country with the amount of biological diversity after Brazil and Madagascar. Of the about 325.530 species of flora

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and fauna globally, an estimated 25% of the world's species are found in Indonesia (Rahmawati, 2013). Soil macrofauna have a very important role in the ecosystem, which is to keep the soil macrofauna soil fertility through overhaul of organic matter, nutrient distribution, increase soil aeration and many more. Soil macrofauna is responsible for chemical transformations in the soil. mineralization rates getting higher and concentrations of mineral nutrients in ant nests suggest that ants alter the abundance, and perhaps the taxonomic identity (Petal, 1980; Friese and Allen, 1993). Soil macrofauna such as herbivor, denitrivor and predator had multiple roles in soil ecosystem, which is to manage the nutrient cycle in soil ecosystem (Melman et al., 2019). Nutrient cycle is one of the many roles to determine productivity on agricultural practice. Soil macrofauna was proofen to giving an impact for plant growth productivity and other soil processes (van Groenigen et al., 2014).

Value of soil macrofauna species diversity tends to increase at lower soil temperatures and largest percent dense cover. Soil organic matter content could be expected to affect the level of soil macrofauna diversity. Soil organic matter content of the higher have a tendency to increase the diversity of soil macrofauna (Cahyo and Shamsuddin, 2017). Ants can be used to estimate the extent of forest recovery stage. Classification stage of ant genera has the advantage of a faster and cheaper method than that of determining the physical, chemical and biological soil atrributes (Julia et al., 2019).

Soil macrofauna is the most sensitive indicator to changes in land use, so it can be used to predict the quality of the land (Rousseau et al., 2012). In carrying out his activities, soil macrofauna require specific requirements. The environmental conditions are the main factors that determine the survival, namely: climate (rainfall, temperature), soil (acidity, humidity, soil temperature, nutrients), vegetation (forest, meadow) and sunlight (Sugiyarto et al., 2007).

This research needs to be done considering the use of intensive management, thus affecting biodiversity in the soil of rice field. Local farmer in dukuhseti had used intensification practices for many years and it affect their land biodoversity. This study can also be used as an indicator of the status of soil fertility in the soil macrofauna biodiversity observation and an indication of the indigenous macrofauna that may still remain in place. The study could be a reference to the local farmer and given the knowledge advantage and hopefully improving better agricultural practices.

## MATERIALS AND METHODS

### Sampling macro fauna

This research was conducted in July to August 2018 and its focus is on the implementation of observations done in paddy fields at the Dukuhseti district in Pati Regency, Central Java location. Rice field is located in the Dukuhseti district, Pati Regency, Central Java province. The extent of rice fields and uniform type of vegetation causes of the sample was determined to be 10 point locations. The sampling method used, among others, is purposive sampling method.

Interviews with farmers' groups were conducted to determine the type of management that applies to land, because it is highly sensitive to biological indicators impact by land management factors, especially against mechanical ploughing treatment and fertilization (Setälä et al., 1990).

Soil fauna is picked up from soil using Pitfall Trap methods. Pitfall traps procedure involves the use of chemical to isolate the fauna that runs on land and soil depth <5 cm (Setälä et al., 1990). The collection of primary data including data type of the plant was taken using purposive sampling method. A basis for determining the sampling point is through consultation with local residents about the condition of the land in the area, so that treatment used on the land can be known. Same treatment such as plowed land use mechanical machines and the use of chemical fertilizers was employed. At a point, one plot per observation area was made with two replications. Plot observations were made amounting to two plots in each field observations. Observation plots have a size of 25 cm × 25 cm that is based on visual observations by focusing on sites that have sufficient moisture and enough shade.

The sampling procedure for soil macro fauna advanced in all the litter in the observation plots was quickly removed and transferred into clear trash bag. Further, a layer of surface soil (0-10 cm) on plots were dug quickly and then transferred into another clear trash bag. Thereafter, clear trash bag was removed from each block where the discovery observations annotated (soil/litter), swath number, and location of the plot (Cahyo and Shamsuddin, 2017). Soil surface macrofauna was collected using Pitfall traps methods (Decoy trap).

### *Decoy trap method*

Random sampling point was made on the plot to obtain a hole-sized glass trap. Glass trap containing 4% formalin was inserted into the hole, and the glass (rims) surface was made parallel or flat to the ground. Thus, rain water does not enter the glass trap through the glass trap roof. Traps were left for 24 h. Subsequently, trapped macrofauna were identified in the laboratory (Suin, 2012).
Soil macrofauna caught from litter and soil material were identified up to the family level. Books such as *Keys to The Terrestrial Invertebrates* (Mohamed, 1999) and *Introduction to Lesson Insects Edition 6* (Borror et al., 1996) were used for identification of macrofauna soil. Species diversity was calculated using Shannon-Wienner diversity index (Odum, 1993) using the following formula:

$$H' = -\Sigma \, Pi \ln (Pi),$$

where Pi = (ni / N); H '= Shannon-Wienner diversity index; ni = Number of individual types of I and
N = Number of individuals of all species

Shannon diversity index value criteria - Wiener (H ') are as follows:

H '<1: lower diversity
1 <H'≤3: diversity is being
H '> 3: high diversity

### Laboratory analysis

Chemical properties of the samples were examined such as soil total N, available P, organic carbon, base saturation and pH. Sampling at each point is done at a depth of 0-20 cm by taking a 5-sub point then composited. Sampling was conducted by composite soil because it can represent the condition of each sampling point (Supriyadi et al., 2015). Analysis of the laboratory was carried out in the Laboratory of Chemistry and Soil Fertility, Soil Science Department, Faculty of Agriculture, University Sebelas Maret, Indonesia. This research was conducted from July 2018 to September 2018. Analysis of soil fertility indicators include pH $H_2O$ (Volumetric Elektrometrik, C-Organic by Walkley and Black Method (Balittan, 2009) total N with Kjeldahl Method (Balittan, 2009), and available P using Olsen method (Balittan, 2009).

### Data analysis

Normality test was performed with SPSS software version 21 which showed normal data distribution. Tests carried out by the correlation between diversity index of macrofauna with the chemical properties of soil fertility was performed using Pearson correlation test with an accuracy level of 95%. Normality test was done using Kolmogorov-Smirnov test (De Vaus, 1991)

## RESULTS

### Importance value index on macro fauna at sampling location

Test for normality using Kolmogorov Smirnov was carried out and the results obtained showed that normal data distribution and standard deviation of diversity index is (+) 0.17197 with an average of 1.3780. Standard deviation (+) 2.24343 with an average of 9.1470 using a 95% confidence level indicates the number -0.412 which means below -0.500 (Table 1).

It states that P availability is not significantly correlated with macrofauna diversity index (H). The results, according to the theory of P availability, have been disposed of through regular mineralization cycle, regardless of fluctuations in biological activity. Pearson correlation test between variables method with variable Diversity Index, Total soil N, Standard deviation (+) 0,3507 0,07087 and the 95% average use rate shows that the value 0,412 thus declared revealed no significant correlation between the diversity index (H) with total soil N levels (N) (Table 2).

The results of Pearson correlation test between the method of data macrofauna diversity index (H) with Base saturation levels (KB), standard deviation (+) 27,5207 4,91392 and 95% the average use level showed significant figure above -0,654 × 0.500 ($P > 0.05$) which stated that among the variables with base saturation diversity index there was a positive correlation. The results of Pearson correlation test method with a confidence level of 5% shows the results -0, 491 which implies that no correlation was found between the diversity index of soil organic C content. This indicates that the diversity of macrofauna in the fields, do not necessarily lead to higher soil organic C. The results of Pearson correlation test between the method of data macrofauna diversity index (H) was obtained with a pH of standard deviation (+) 0,96375 and an average 6 (Figures 1 and 2).

## DISCUSSION

### Total soil nitrogen levels

No significant correlation was found between the variable diversity index (H) with total soil N content (N). Lowest total N measured was around 0,28 in accordance with Balitan clasiffication (2007) classified as medium level/degree. Highest levels of total N was measured at 0,3 which means that the entry (2007) is classified as being medium level. All sample points have classified their nitrogen levels as the medium level, where it is suspected because of the intensive use of inorganic fertilizers.

Macrofauna role in the decomposition of organic matter, among others, is the process of fractionation (separation into smaller fractions OM), Distribution (Translocation fraction) and a medium for other organisms. Existence of some macrofauna species can be attracted to the others since macrofauna functions either as predator or antipredator. This causes an increase in the macrofauna diversity index when the rate of decomposition increases (Lavelle et al., 1994; Brussaard, 1998).

Minimum raw organic matter field conditions (litter thickness = 0) impacted on low diversity of macrofauna in this study. Micro-climatic conditions such as high temperatures causing nitrogen content changed into gas, because evapotranspiration is directly proportional to the rate of evaporation of nitrogen (Susanti and Halwany, 2017).

Temperatures measured in the field when the research was held ranges from 30-36°C. The soil temperature is measured at the range of 28 to 37°C. A constant temperature of 15°C causing the growth of certain macrofauna rapidly increased, raising macrofauna growth and resulting to increased diversity of macrofauna (Sukarsono, 2009). The dominant condition of dry rice fields, causing the insect class of ants had bigger dominance than insects that require high environmental humidity levels (molluscs, arthropods, centipede) levels. Higher nitrogen content was found on land that had been used as a nest of ants. This indicates that within the ant nest, there were conditions causing probable mineralization of N to happen compared to humification (Petal and Kounsiska, 1994). The temprature shouldve been controlled with macrofauna and nitrogen keep up at their optimum phase.

### Available soil P levels

P element was not only released on the biological cycle, but also plays a role in chemical reactions, in contrast to N elements that almost fully rely on the biological cycle (Stout et al., 1976). Moreover, previous research by Cline et al, (1985) reported that in alluvial soil, mineralization of P was only caused by the chemical transformation

**Table 1.** Importance Value Indeks (IVI) Macrofauna Species in Dukuhseti Rice Fields.

| Name | Phylum | Order | IVI (%) |
|---|---|---|---|
| *Pila ampullacea* | Mollusca | Gastropoda | 13 |
| *Chrysolina* sp. | Arthropoda | Coleoptera | 11 |
| *Camponotus* sp. | Arthropoda | Hymenoptera | 45 |
| *Polyrarchis* sp. | Arthropoda | Hymenoptora | 36 |
| *Lycosa* sp | Arthropoda | Araneae | 24 |
| *Coenagrionidae* sp. | Arthropoda | Odonata | 3 |
| *Atractomorpha* sp. | Arthropoda | Orthoptera | 4 |
| *Ortherum* sp. | Arthropoda | Odonata | 6 |
| *Ortherum* sp. | Arthropoda | Odonata | 1 |
| *Gryllidae* | Arthropoda | Orthoptera | 1 |
| *Dermaptera* sp. | Arthropoda | Dermaptera | 6 |
| *Lumbricus* sp. | Annelida | Megadrilacea | 20 |
| *Coccinellidae* | Arthropoda | Coleoptera | 1 |
| *Culex* sp. | Arthropoda | Diptera | 17 |
| *Siphanta* sp. | Arthropoda | Hemiptera | 1 |
| *Monomorium* sp | Arthropoda | Hymenoptora | 24 |

**Table 2.** Soil lab analysis results on all mean sample and category according to Balitan (2009).

| Attribute | Value | Category |
|---|---|---|
| N Content | 0.35 cmol/kg | Medium |
| Available P | 9.15 ppm | Medium |
| Base saturation | 27.52% | Low |
| Organic C | 2.16% | Medium |
| PH | 6.11 | Pretty acid |

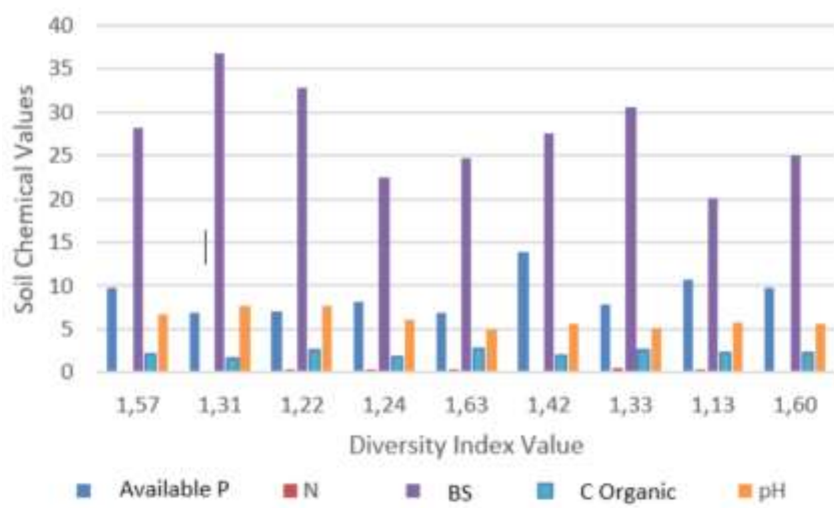


**Figure 1.** Diversity Index Value chart on each site sampling, and their soil chemical properties value that had been tested on each sample.

compared with biological transformation.

Although the diversity of macrofauna was not significantly correlated with the availability of P in the soil in this study, According to Anderson and Ineson (1984) in Ingham et al. (1985), most nematodes and protozoa accelerate mineralization P, while the worm does not

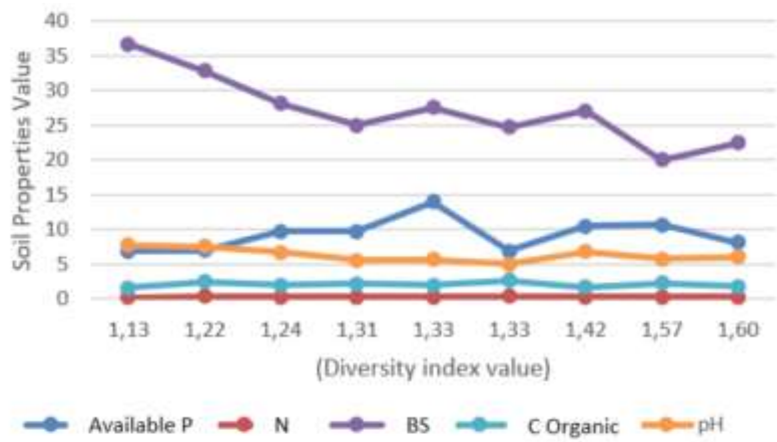


**Figure 2.** Diversity Index Value had been sorted from his lowest to highest, Each Chemical Soil Properties showing their trend.

affect the P but can be extracted from KCl in the pine forest litter (Setälä et al, 1990). McBrayer (1977) emphasizes the utilization of land mesofauna as immobilization agent P and invertebrates as ion releasing agent at ongoing basis to maintain the fertility of conifer forest ecosystems with the goal of long-term plantations (forest).

Research conducted by Schipper et al. (2009) states that there is no real influence on the environment with epigeic worm to soil organic matter content although comparison of the addition of phosphate fertilizers was observed. This shows that P did not relate significantly to the macrofauna dynamics in the soil. Directly, macrofauna diversity is positively correlated with soil physical attributes. Soil physical attributes lead to improved quality of chemical and biological attributes of soil, especially soil biological attributes that are directly influenced by the fauna. Macrofauna diversity causes an increased mikrofauna cluster so that the cycling of nutrients process becomes faster (Manyanga et al., 2014). There is no significant correlation between available P and diversity macrofauna proofen in this study, because P available more influenced by microfauna (Setälä et al, 1990)

## Base saturation levels of the soil

The results of Pearson correlation method test between the data of macrofauna diversity index (H) and Base saturation levels (KB) using a 95% confidence level indicates the number -0,654* which means above -0,500 (P> 0.05). Soil fauna which proved instrumental in base saturation levels of most of the types is macroinvertebrata. Macroinvertebrata on calcaerous ecosystems had a role in actively generating mull (humus formed on the ecosystem of non-acid) by increasing the activity of microbes (Scheu, 1990) where cutting litter becomes smaller and burial of organic material was noticed (Van der Drift, 1963; Hirschenberger and Bauer, 1994).

At the study site, the type of macroinvertebrata encountered was predominantly of three order, the Hymnenoptora, Annelida and Areneae. Macrofauna that resulted from order Hymenoptora is from formidae tribe like ant with an IVI (Importance Value Index) of 81%, the most in a rice paddy ecosystem here. Profit from the order Araneae was found with IVI by 24%. From the order Annelida, earthworms with an IVI of 20% was found and it can be concluded that macroinvertebrata have a considerable role in the ecosystem of the fields here.

According to Ondina et al. (2004), an estimated few specific insect species which include rove beetles (*Tachyporus dispar*), land snails (*Cochlicopa lubrica*) and *Arion circumcriptus* can be an indicator for positive correlation with base saturation content. Barros et al. (2002) also support this statement, he stated that the litter arthropods, especially *Isopoda* and *Diplopoda* groups most commonly appear in soil that has a high base saturation.

Paddy soil management factors showed that using tractor mechanics leads to lower macrofauna IVI class *Gastropoda*, *Diplopoda* and *Isopoda* which is only for an amount equal to 14% because the management of an inch layer of soil causes macroinvertebrata leading to shrinkage (Barros et al., 2002). Land management practices need to be reduced in such intensity to maintain the diversity of macrofauna, because the increased macrofauna is a way to consider improving and maintaining the paddy soil base saturation levels.

## Levels of soil organic C

Availability of organic material can be estimated by comparing the levels of organic C and N total, thus obtained C/N ratio can be used to predict the avaiLabileity of nutrients from organic matter mineralization (Sukmawati, 2015). Application of intensive agriculture leads to decline in soil organic C levels. The content of soil organic matter can be maintained at 2% level when given minimal input of organic materials as much as 8-9 tonnes/ha (Young, 1989).

Macrofauna have a role in recycling and storage of carbon (C). Low soil organic C is caused by the nature of movement of carbon cycle into the biomass in the organism so that it becomes immobilized. A high diversity causes the displacement of carbon into biomass in bulk (Moco et al., 2010).

Although this study did not find a correlation between diversity and levels of organic Carbon (C), some types of macrofauna are known to have an influence on the decomposition of organic matter, cycling of nutrients (nitrogen, phosphorus and carbon), better productivity of agriculture and improvement of the physical, biological and chemical soil (Brussaard et al, 2007). Macrofauna digestive activity causes the formation of aggregates and changes recalcitrant into simpler forms, thus improving the quality of soil physical properties.

Macrofauna dominance in rice field sample point is not balanced. Family important value index represented in annelid worm ranks second after the family Formicidae. The spread of organic C in the soil has a correlation that is important to earthworms, which revealed that the isotope levels of $^{14}C$ being introduced has moving and average concentrations in soil depth that contained earthworm activity as compared to that without earthworm activity (Stout and Goh, 1980).

Soil organic C content is also indicated by the presence of macrofauna and larvae type groups that are decomposers, such as coleopterae that indicates high soil C content (Rousseau et al, 2012). Research located in countries having cocoa forests like Brazil also shows that coleoptera larvae is associated with lignin and wood-consuming, resulting in the efficiency of nutrient cycling and carbon (C) (Moco et al, 2010). Macrofauna diversity could be a solution in order to keep soil organic carbon at optimum phase.

## Soil pH levels

It can be concluded that no correlation was found between the diversity of macrofauna with soil pH. According Suin (2012), soil fauna pH have linkage relationships of mutual influence. Soil pH levels affect soil macrofauna density, due to the pH of determining levels of nutrient avaiLabileity in the soil (Suin, 2012). A known pH 6-7 range is found to cause optimal nutrient availability in the soil (Hairiah et al., 2004). pH is not an absolute determining factor of macrofauna existence, because according to Suin (2012), certain types of soil fauna can survive in extreme circumstances like acidic or alkaline.

The decomposition process of macrofauna have been affected by pH. According to Notopriwihardjo (2000), temperatures for slightly acidic to slightly alkaline pH range are good in accelerating the decomposition of organic matter. The role of pH in this case is to provide a good environment for organisms that decompose organic material. pH range >5.5 could provide a good environment for N fixation bacteria, phosphate solvent bacteria and nitrification bacteria (Hardjowigeno, 1995).

It is known that the pH has a positive influence, directly or indirectly, especially against predatory macrofauna according to research results by Moco et al. (2010) in cocoa plantations and forest in Brazil. In addition, Lavelle et al. (2003) confirms that the environment shows a high population diversity of *diplopoda* and *Diptera* species of larvae, which are classified as decomposers of litter. This explains the abundance of macrofauna predators in the ecosytem. Young (1986) stated that the Diptera larvae grow well in alkaline pH conditions. The discovery of macrofauna samples with little population are not well saturated due to the continuous changes in ecosystems on this macrofauna habitat. Systematically, flooding treatment on rice field causes rapid ecosystem changes and resulting periodic overhaul of macrofauna population. According to Folgarait et al. (2003), the environment has the same mechanism of the fauna species.

Ecosystem, have a tendency to adopt the behavior of new species with the same role when they lose a species rather than adopting various new species all the time. As a result, the diversity of fauna ecosystems never returned exactly the same after the changes in habitat. Case studies in Argentina showed that after natural grasslands were changed to rice fields, the ecosystem does not return to its original state (as natural grasslands) despite being rehabilitated within a period of 15 years (Thomas et al, 2004). Macrofauna couldnt be a precise indicator to fortell soil pH level because macrofauna diversity and soil pH level had mutual relation.

## Conclusion

Macrofauna diversity have a positive correlation with soil chemical properties, including the base saturation. Macrofauna cannot be used as the sole indicator of soil fertility in a particular place; this is because macrofauna are highly dependent on microclimates and microecosystem. Fauna found on the same land use in different places are not necessarily identical; macrofauna possess the interplay of the soil pH. Soil pH caused optimal conditions for the growth of macrofauna which helps maintain balanced pH levels.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Extent of rumen contents use in livestock diets among farmers in Uganda

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**This study was conducted with the aim of finding how rumen contents are used in livestock diets, problems encountered and areas that needs improvement to enhance livestock production. One hundred livestock farmers from Kampala, Wakiso and Mukono districts were interviewed using a structured questionnaire. The results showed that majority of the work force involved in livestock farming were middle aged adults between 30 and 45 years contributing 37% of total work force; this was followed by young adults between 20 and 30 years contributing 26% of the work force. The highest household (HH) size was (1-5) people contributing 68% of the total HH structure. Poultry farming, indigenous birds in particular were the most practiced enterprise among the respondents. High feed input prices (67%) were reported as the biggest problem faced by livestock farmers, followed by feed adulteration (44%). The use of peels and industrial by-products was reported as the most commonly used alternative feeding strategies to increasing feed prices. The use of rumen contents was still low and limited to pigs and layers. Inadequate knowledge in relation to rumen content inclusion rates in livestock diets was reported as the major hindrance to utilization of rumen contents in livestock. In general, farmers need sensitization from extension staff and research scientists with regard to efficient use of rumen contents in livestock diets.**

**Key words:** Feed scarcity, inclusion levels, rumen content processing.

## INTRODUCTION

Rumen content is partially digested feed found in the fore stomach of ruminants. They are fairly rich in crude protein as they contain microbial protein from bacteria, fungi, and protozoa (Agbabiaka et al., 2012). Rumen contents are also important source of energy, minerals and vitamins, especially vitamin B complex (Ravindra et al., 2017; Sakaba et al., 2017). These attributes make rumen contents a potential candidate feed ingredient for livestock (Cherdthong, 2019) and could also be vital in reducing the competition between man and animal for food. Despite these attributes that make rumen content a potential livestock feed ingredient, it is still largely underutilized which complicates its efficient disposal and therefore making it a potential environmental pollutant.

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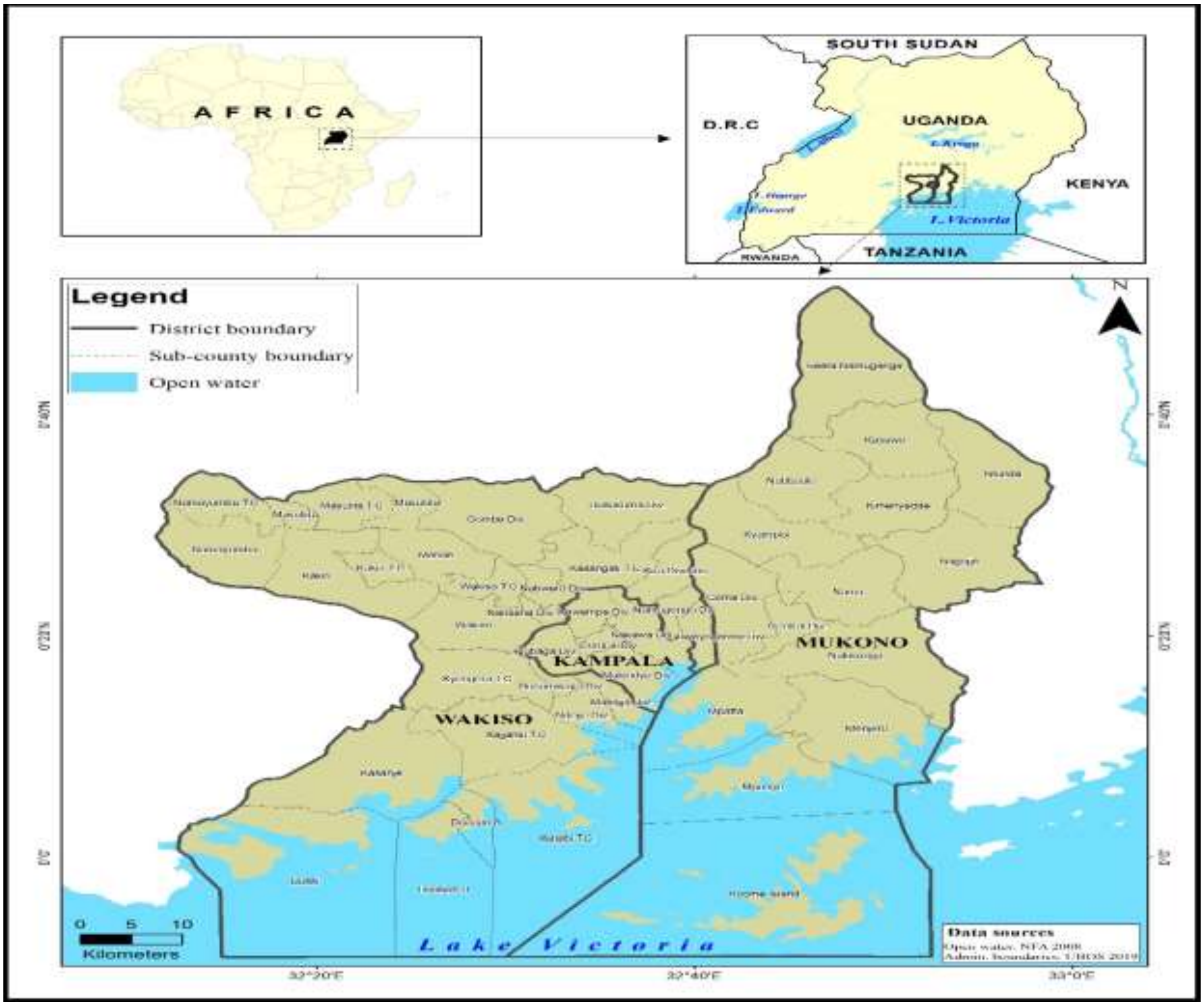


**Figure 1.** Map showing the location of study area, source of the map.
Source: UBOS (2016).

Enormous volumes of rumen content wastes are being generated on daily basis from slaughterhouse operations in the urban settings which creates disposal challenges (Uddin et al., 2018). Improper disposal of rumen contents can lead to environmental pollution which negatively impacts on people's wellbeing. Feeding livestock with slaughterhouse wastes would not only result in reducing feed costs but also immensely contribute to the safe disposal of wastes through recycling (Dairo et al., 2005; Esonu et al., 2006). It has also been reported that when incorporated in livestock diets, rumen content has no adverse effects on animals as long as critical care is taken for balanced feed formulation that meets the animal's nutrition requirements. In this study, the extent of rumen content usage in livestock diets among the farming communities in Uganda and the associated constraints in order to boost livestock production was investigated.

## METHODOLOGY

### Description of the study areas

The study was conducted in three Districts (Wakiso, Mukono and Kampala) in central Uganda. Kampala city is located 45 km north of the Equator at 0°19'6"N and 32°34'60"E (Figure 1). Wakiso district is located at approximately 20 km northwest of Kampala, at 00°24'N, 32°29'E coordinates, while Mukono district is located 27 km from Kampala at 00°20'N, 32°45'E. The districts were chosen for the study because of their close proximity to the city center and being one of the fastest growing peri-urban areas in Uganda.

### Data collection

Interviews were conducted targeting people who knew more about the area (key informants). These included veterinary doctors, health inspectors, abattoir chair persons and elders. The sample size was determined according to formula by Yamane (1967).

n= $N/(1+N*(e)2$

Where, n is the sample size, N is the population size, and e is the acceptable standard error; e=0.05.

The sample size was calculated based on 95% confidence level. Considering a population of about 130 livestock farmers who utilize rumen contents in livestock diets around the central districts in Uganda, a total of 100 participants were interviewed, 30 from Kampala, 33 from Mukono and 37 from Wakiso.

Both qualitative and quantitative data were collected using a structured questionnaire with both open and closed end questions. Qualitative data included, sex, marital, education status, feeding and feed resource utilization, potential constraints, use of animal wastes in livestock feeds and other alternative feeding strategies. On the other hand, quantitative data were family size, flock size and proportion of rumen content use in the livestock diets. Focus group discussions were also carried out with key informants in order to get deep understanding of people's feeling about the subject matter.

#### Data analysis

The filled questionnaires were coded and entered into the SPSS version 22 computer software (IBM SPSS statistics). The data was then subjected to descriptive statistics, cross tabulations and Pearson's Chi square of association.

## RESULTS

### Demographic characteristics of the respondents

The results of demographic characteristics of the respondents are shown in Table 1. The result shows that majority of the work force involved in livestock farming are middle-aged adults between 30 and 45 years contributing 37% of total work force, followed by young adults between 20-30 years contributing 26% of the labor force. The young (10-20 years) and old people (60-90 years) contributed the least to the labor force at livestock farm with 2 and 6% respectively.

In relation to family position, the highest percentage of the work force in livestock farming operations (51%) is contributed by the fathers while mothers contributed 46%.

Household (HH) size was grouped in three categories: small (1-5), medium (5-10) and large (>10) people. The highest percentage of HH size was (1-5) people accounting for 68%, followed by (5-10) people category contributing 23%, while the least was the >10 category with least 8%.

In relation to education level, the highest percentage of the respondents had attained at least secondary education (48%), followed by people who attained primary education, and then university education and advanced level of education. People who did not go to school contributed the lowest percentage.

### Animal types kept by the farmers

The kind of livestock kept by the respondents is shown in Table 2. The highest number of respondents had indigenous chicken, followed by dairy cattle and small ruminants (sheep and goat). The number of respondents with pigs and layers was similar.

### Challenges faced by farmers

The challenges faced by the farmers that hinder their smooth operations are shown in Table 3. High prices of inputs was reported as the biggest problem farmers experienced followed by adulteration of feeds, feed scarcity and limited land.

### Alternative feeding strategies used by farmers

In order to cope with the feed related problems, farmers employ various strategies as shown in Table 4. Use of peelings was the most widely employed strategy by the farmers with 44%, followed by use of industrial by-products 34%, others 28%, use of kitchen swill 23%, and use of forages and concentrates were the least used at 21%.

### Use of rumen content in livestock diets

Rumen contents were used in pig rations (23%), followed by layers (13%) at $P<0.05$. Use of rumen contents in indigenous chickens was not a common practice among respondents. However, no respondent reported use of rumen contents in broiler rations (Table 5).

### Degree of rumen contents use in the study area

Level of use of rumen contents among farmers in the study area differed significantly ($p<0.001$), with Kampala district having the highest number of farmers (18) using rumen contents in livestock diets, followed by Wakiso (16) and Mukono (4) (Table 6).

### Proportion of rumen content use in different livestock

The different levels of rumen content use in livestock diets as revealed by the respondents is shown in Table 7. Overall rumen contents were mostly used in pigs and layer diets. In layers diets, most of the respondents (85.8%) incorporated rumen contents at 20% inclusion level. In pig diets, a big percentage of the respondents (77.4%) could not quantify the amount of rumen contents they used in pig rations.

### Benefits realized by the farmers with use of rumen content in livestock diets

Farmers who used rumen contents in pig and layer diets revealed several benefits as shown in Table 8.

**Table 1.** Distribution of respondent's demographic characteristics**.**

| Demographic characteristic | Livestock farmers (n=100) | |
|---|---|---|
| | Frequency | % |
| **Age (years)** | | |
| 10-20 | 2 | 2.0 |
| 20-30 | 26 | 26.0 |
| 30-45 | 37 | 37.0 |
| 45-60 | 25 | 25.0 |
| 60-90 | 6 | 6.0 |
| **Position in family** | | |
| Head | 51 | 51.0 |
| Mother | 46 | 46.0 |
| Son | 2 | 2.0 |
| Daughter | 1 | 1 |
| **Marital status** | | |
| Single | 5 | 5.0 |
| Married | 89 | 89.0 |
| Divorced | 1 | 1.0 |
| Widowed | 3 | 3.0 |
| **House hold size** | | |
| 1-5 | 68 | 68.0 |
| 5-10 | 23 | 23.0 |
| >10 | 8 | 8.0 |
| **Education level** | | |
| None | 5 | 5.0 |
| Primary | 24 | 24.0 |
| Secondary | 48 | 48.0 |
| Advanced level | 10 | 10.0 |
| University | 10 | 10.0 |

**Table 2.** Animals kept among livestock farmers.

| Animal type | N | Minimum | Maximum | Mean | SEM |
|---|---|---|---|---|---|
| Dairy | 43 | 1 | 20 | 3.28 | 0.48 |
| Sheep and goats | 36 | 1 | 10 | 4.25 | 0.41 |
| Layers | 33 | 300 | 10000 | 1780.30 | 496.88 |
| Broilers | 25 | 100 | 5000 | 724.00 | 190.18 |
| Pigs | 33 | 1 | 100 | 9.70 | 2.98 |
| Indigenous chicken | 46 | 1 | 100 | 17.46 | 2.64 |

SEM; Standard error of the mean.

## Problems encountered with use of rumen contents

Appropriate rumen content inclusion rates, drying, rumen content offensive smell and rumen content contaminants in order of importance were the challenges encountered by the farmers with use of rumen contents in livestock diets.

## Advice farmers needed for efficient utilization of rumen contents in livestock diets

Farmers stated several areas where they needed advice in relation to efficient use of rumen content for improved livestock production. Overall, most farmers needed advice in pig ration formulation, followed by advice in

**Table 3.** Challenges faced by the farmers**.**

| Parameter | Frequency | %* |
|---|---|---|
| Limited land | 10 | 10.0 |
| Scarcity of feeds | 34 | 34.0 |
| High prices of inputs | 67 | 67.0 |
| Adulteration of feeds | 44 | 44.0 |
| Drought | 32 | 32.0 |
| Limited water supply | 8 | 8.0 |

*Percentage more than 100 because farmers stated more than one problem.

**Table 4.** Alternative feeding strategies employed in livestock feeding.

| Strategy | Frequency | %* |
|---|---|---|
| Peels | 44 | 44.0 |
| Forages | 21 | 21.0 |
| Concentrates | 21 | 21.0 |
| Industrial by products | 34 | 34.0 |
| Left overs | 23 | 23.0 |
| Others | 28 | 28.0 |

*Percentage more than 100 because farmers stated more than one feeding strategy used.

**Table 5.** Use of rumen contents in livestock diets.

| Type of animal | Use (%) | Do not use (%) | P-value |
|---|---|---|---|
| Pigs | 23 | 15 | 0.0001 |
| Layers | 13 | 25 | 0.0006 |
| Indigenous chicken | 1 | 37 | 0.38 |
| Other animals | 2 | 36 | 0.142 |

poultry feed formulation. The kind of advice needed include level of rumen content inclusion in the diets, rumen content processing methods and its storage in order of importance.

## DISCUSSION

### Demographic characteristics of the respondents

The study showed livestock farming in the area of study was dominated by the young age group (30-45 years). This implies that people within this age bracket are energetically fit to execute the required duties as opposed to older people. Household (HH) size (1-5 people) was the highest, and is line with the findings of UBOS (2018) which revealed an average of 4.5 persons per household. Of the total respondents, the majority (48%) had attained at least secondary education. These results despite being lower, agree with the findings of Katongole et al. (2012) while investigating strategies for coping with feed scarcity among urban and peri-urban livestock farmers in Kampala, Uganda.

### Animal types kept by the farmers

Poultry enterprise (layers, broilers indigenous chicken) was the predominantly practiced among the livestock farmers. This may be due to the fact that chicken is easier to rear and can survive with minimal input at household level (Kperegbeyi et al., 2009). More so, according to FAOSTAT (2016), poultry meat and eggs are among the most commonly consumed animal food source as it is not discriminated among cultures and religions, thus making it a key component in food security and nutrition of most households in the study area. Poultry is mostly crucial among smallholder farmers, resource poor people in the urban and rural areas and is also mainly produced in large scale and intensive operations, which thus makes it one of the fastest growing subsectors globally. Poultry also has a short reproduction and production cycle and can be sold off quickly in case of a need; more so, because poultry convert household wastes into edible products like meat and eggs, could be one of the reasons they are found in almost every household (FAO-AGAL, 2016).

Most respondents kept indigenous chicken followed by dairy, sheep and goat. This finding differs from that of Katongole et al. (2012) who reported dairy cattle as the most reared livestock specie. The reason for this could be as a result of change in land tenure, increasing urbanization of what used to be peri-urban districts surrounding Kampala, the capital city of Uganda. This change in land use has eroded most agricultural land (Sabiti and Katongole, 2016). As a consequence, most urban dwellers have been left with small pieces of land which has forced many to keep birds that require small area of land as opposed to large ruminants. This is also emphasized by the United Nations report of 2011 that urbanization presents unprecedented environmental, social, economic and political challenges. Globally, expansion of cities not only leads to loss of agricultural land but also changes in hydrology and natural habitat (UN, 2011).

### Challenges faced by livestock farmers

High prices of agricultural inputs remain the biggest problem encountered by livestock farmers, followed by adulteration of animal feeds, feed scarcity and limited land. High feed prices are not unique to Uganda but a major problem facing most developing countries. In Uganda, fish meal and maize are the predominant protein and energy feed ingredients used in livestock ration

**Table 6.** Use of rumen contents in the study area.

| Kampala {n (%)} | Mukono {n (%)} | Wakiso {n (%)} | P-value |
|---|---|---|---|
| 18(47.4) | 4(10.5) | 16(42.1) | 0.0001 |

**Table 7.** Proportion of rumen contents used in pigs and layers.

| Inclusion level | Pigs (%) | Layers (%) |
|---|---|---|
| 5% | 9.7 | 7.1 |
| 15% | 3.2 | 7.1 |
| 20% | 9.7 | 85.8 |
| No limit | 77.4 | 0 |

**Table 8.** Benefits realized by the farmers with use of rumen contents in livestock diets.

| Pigs | Layers |
|---|---|
| Increased pig growth | Good chicken growth |
| Reduced feed costs | Yellow yolk |
| | Reduced feed costs |

formulation. These feed ingredients are also subject to competition from humans, thus aggravating the situation during periods of scarcity. In the end, the livestock sector suffers the most; this is further exacerbated by feed dealers who subject most feed ingredients to adulteration. This has not only left livestock farmers to a double loss but also exposed them to substantial livelihood risk. The competition for inputs drastically affects farmer's profit margins which consequently hinders their expansion programs (Brandnock, 2012).

## Alternative feeding strategies used by farmers

To curb the problem of the ever increasing feed prices, farmers reportedly used mostly peelings from bananas, sweet potatoes and cassava. These are subjected to welting and sometimes boiling so as to reduce inherent anti-nutritional factors and also to increase digestibility by the animals. Other farmers indicated that banana peelings are chopped into small pieces, dried and given to the birds, which slows birds from losing a lot of weight in case of feed scarcity. However, this needs scientific backing to give more informed guidance to farmers. Industrial by-products used by the farmers included, wheat bran, wheat pollard, brewer's waste and by products from slaughter houses. Other farmers were buying and stocking feeds during the harvest season when the prices are lower in preparation for periods of scarcity which was in line with the findings of Katongole et al. (2012). Concentrates used by the farmers include Hendrix, Intercol and Kafica, which are mostly imported into the country and their use is justified by the need to curb rampant feed adulteration by the local feed manufactures.

## Use of rumen contents in livestock diets

Rumen content was mostly used in pig and layers diets by 23 and 13% of the respondents respectively. The inclusion of rumen contents in pig diets was reportedly easier than in layer rations because it does not involve milling which also reduces on the cost. This is because most farmers perceive pigs as animals that eat almost everything offered to them. However, despite this notion, pigs too need well formulated feeds for better performance (Mwesigwa et al., 2013). A few of the respondents reported giving flesh rumen contents to pigs without any further processing, with the fresh rumen content usually mixed with a little maize bran and given to the pigs. Despite this being an innovative survival strategy, the nutritional adequacy of this approach to pig feeding remains questionable (Kasule, 2012) and may in fact even affect production efficiency of the pigs. Rumen contents were not used in broiler diets because farmers did not envisage its usefulness to broilers.

## Degree of use of rumen contents in the study area

Kampala had the highest level of rumen content use in livestock diets among the study areas. According to discussion with key informants, the idea of rumen content use in livestock diets started at Nalukolongo Abattoir in Kampala as a pilot project over five years way back. This has been spreading to other areas; since then, however, the pace has been low due to lack of knowledge in efficient utilization of rumen contents in livestock diets.

**Proportion of rumen content use in different livestock**

The study found that rumen contents were mostly used in pigs and layer diets with varying inclusion levels. Despite rumen contents being reported as having no anti-nutritional factors (Agbabiaka et al., 2012), there is an optimum inclusion level in livestock diets that must not be surpassed, beyond which animal performance becomes compromised. In this study, 85.8% of the respondents incorporated rumen contents in layer diets at 20% inclusion level. Despite achieving their objective of egg yolk color change, the 20% rumen content inclusion level is quite high for proper layer growth performance (Odunsi, 2003). Available literature shows a reduction in average daily feed intake (AFI), hen daily egg production (HDEP), egg weight and shell thickness with increasing levels of rumen contents in layer diets (Odunsi, 2003; Efrem et al., 2016).

In pig diets, a high percentage of the respondents (77.4%) could not quantify the amount of rumen contents they use. Despite the fact that numerous feed ingredients provide nutrients that pigs require to grow, pigs too require balanced feed ration that provide optimum energy, proteins, and vitamins for better growth performances (Adesehinwa, 2008; Mwesigwa et al., 2013). Moreover, rumen contents are high in fiber that can limit feed intake and lead to poor growth due to insufficient feed utilization. Thus, the notion by most farmers that pigs can eat everything offered to them without catering for optimum nutritional needs requires mindset change for improved pig performance. No respondent indicated use of rumen contents in broiler diets as they envisaged no beneficial effects in these types of birds. However, there seems to be a knowledge gap, since use of rumen contents has been reported to improve broiler performance (Said et al., 2015; Inci et al., 2013).

**Problems encountered with use of rumen contents**

Optimum inclusion rates of rumen contents in livestock rations was the greatest challenge encountered, followed by the drying process and bad smell while contaminants in the rumen contents was the least challenge encountered by livestock farmers. In general, farmers lacked proper guidance with regard to use of rumen content in livestock diets. This has also been reported by other researchers (Kasule et al., 2014; Tadesse et al., 2017). It could be one of the reasons why the use of rumen content in livestock diets is not widely spread among farmers. In relation to drying of rumen content, some farmers reported being burnt by the heat generated from rumen contents with some getting itches and skin rushes. Bad smell from rumen content was also encountered by several farmers. Among the contaminants reported in rumen contents included, polythene bags, metallic objects and tree thorns. The sharp objects usually pierce hands during sun drying of rumen contents. In general, most feedstuffs contain contaminants from diverse sources (Lange et al., 2018). The contaminants are ingested during feeding by livestock, and polythene bags are most prevalent in livestock reared in peri-urban areas than those from rangeland areas. This is due to enormous use of plastic bags in urban and peri-urban areas. Uganda is currently yet to implement the law on barning the used plastic bags. Unrestricted disposal of plastic bags not only lead to environmental pollution but limit the sustainability of life support systems, social harmony and human health (Aurah, 2013). It is therefore imperative to limit exposure of livestock to such contaminants as it leads to depression, reduced milk outlet, bloat and eventually economic loss (Nandwa, 2014) and in extreme cases, death of livestock.

**Advice farmers needed for efficient utilization of rumen contents in livestock diets**

Farmers stated several areas where they needed advice in relation to efficient use of rumen contents for improved livestock production. Overall, most farmers indicated that they need advice in pig and poultry feed formulation. The kind of advice needed include levels of inclusion of rumen content in the diets, rumen content processing methods and its storage in order of importance. This revelation is in line with the findings of Kasule et al. (2014) who reported farmer’s own feed rations being nutritionally lacking and therefore needed advice on how to formulate nutritional quality feed and to ensure profitable and sustainable livestock production. However, this still seems a daunting challenge that calls for collective efforts and political will.

**Conclusion**

The study established that use of rumen contents in livestock diets was still not widely spread among farmers despite scientific strives showing its potential for livestock production. Rumen content was mostly used in pigs and layers diets. Generally, farmers lacked knowledge on effective inclusion levels of rumen content in livestock diets to optimize animal production performance, therefore necessitating detailed information on this potential feed resource for livestock production.

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## CONFLICT OF INTERESTS

The authors have no conflict of interest

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# Effects of *Faidherbia albida* on some important soil fertility indicators on agroforestry parklands in the semi-arid zone of Ghana

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**A study was conducted in the Guinea and Sudan Savannah zones in the Upper East Region of Ghana to investigate the effects of *Faidherbia albida* on some important soil fertility indicators. Soil sampling and analysis, litter trap, and litter bag techniques were employed to determine the soil's content of major nutrients, the rate of litterfall production and litter decomposition, respectively. Analysis of variance (ANOVA) was performed to determine differences among treatment means, while Tukey's highest significant difference (HSD) was used to perform post hoc tests among means within the same sample set. Soils under *F. albida* tree canopies were found to contain significantly higher organic carbon and total nitrogen than those outside the canopies. Peak leaf litter production occurred during the first three months of the onset of the rainy season. Annual leaf litterfall was 340 g m$^{-2}$ year$^{-1}$ in the Guinea Savannah zone and 264 g m$^{-2}$ year$^{-1}$ in the Sudan Savannah zone. The high leaf litterfall, followed by high decomposition and mineralization at the beginning of the cropping season, the high nutrient content of its leaves, coupled with its nitrogen fixing ability, make *F. albida* a potential candidate for soil improvement and improved productivity of major crops in smallholder farming systems. About 37 and 59 adult *F. albida* trees will be required to supply significant amounts of nitrogen in the Guinea and Sudan Savannah zones, respectively.**

**Key words:** Litter bag technique, litter decomposition, litter fall, litter trap technique, soil organic carbon, soil fertility.

## INTRODUCTION

Soil fertility loss is considered as one of the major biophysical causes of declining per capita food production. Soils in the semi-arid zone are typically characterized by their susceptibility to erosion and compaction, low water retention, nutrient mining and multiple nutrient deficiencies (Buresh and Tian, 1997). The low fertility and susceptibility of semi-arid soils has necessitated the need to find sustainable agricultural production methods. One of such sustainable methods has been the intentional integration of trees with field

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crops on farmlands.

One of the main purposes of agroforestry is to maintain or improve upon soil fertility and hence sustain crop productivity. However, due to the importance of subsistence agriculture in most tropical regions, agroforestry is faced with the task of combining the aims of increasing agricultural production and reducing crop yield losses that result from the competition with associated trees (Broadhead, 2015). The higher crop yields obtained nearer to trees in parklands in the semi-arid zones (Vandenbeldt, 1992) or where trees have been recently removed as in the case of conservation and tree fallows (Nye and Greenland, 1960) is a proof of the contribution of trees to soil fertility. Campbell et al. (1994) show that trees have a positive effect on soil fertility. They suggested that the primary mechanism by which trees improve soil fertility is through increased litter and soil organic matter build-up. Nkyi and Acheampong (2013) also identified plant litter decomposition as the major source of nutrients for trees and crops in the tropics. However, excessive demographic pressure and its consequent modern high input, mechanized cultivation practices have collectively threatened the population of trees on parklands. Tree population and diversity on these parklands are therefore reducing at a rate that requires immediate attention (Tom-Dery et al., 2014; Akpalu et al., 2017). The semi-arid zone of Ghana, where this study was carried out, is the area with the most endemic poverty in the country (Cooke et al., 2016), making it difficult for farmers to afford inorganic fertilizers (Morris, 2007).

A parkland is a traditional land-use system in semi-arid zones, that involves the retention and/or introduction of woody perennials, especially trees, in agricultural fields and managing them in combination with crops and livestock (Boffa, 1999), with the main aim of benefitting from the positive ecological and economic interactions that take place between the components (Bayala et al., 2015). Litter accumulation and decomposition under *Faidherbia albida* canopies have been proven by many researchers to be one of the major sources of nutrients and soil organic carbon (SOC) through which soils are improved (Dunham, 1989; Traore et al., 2004; Adamu, 2012). *F. albida* is known for the so-called “albida effect” which refers to better growth of crops or herbaceous plants under its canopy than in an open field. This phenomenon is attributable to a combination of factors including (i) increased nutrient input through nitrogen fixation and manure from litter, and excreta and urine from livestock grazing or ‘camping’ under the tree (Kang and Akinnifesi, 2000), (ii) increased nutrient availability through enhanced soil biological activity and rates of nutrient build-up (Jung, 1970; Felker, 1981), (iii) improved microclimate and soil physical properties (Akpo et al., 2005), and (iv) ability to capture nutrients and moisture from deeper horizons (Broadhead, 2015).

*F. albida* is widely distributed in Africa, occurring in the tropical and sub-tropical regions of the continent, from Senegal and Gambia in the west to Egypt in the north-east and southwards to South Africa (Barnes and Fagg, 2003). It has a long history of being incorporated into agriculture as a result of population pressures that have necessitated the sedentary use of land in the face of very short or no fallow periods. The leaves and matured fruits (pods) of *F. albida* serve as a source of fodder for livestock in the dry season when herbage is scarce. This has led to the emergence of an agro-pastoral system based on *F. albida*, millet/sorghum, maize and livestock (Barnes and Fagg, 2003). Many authors have demonstrated increased crop yields under *F. albida* (Saka et al., 1994; Payne et al., 1998; Kho et al., 2001; Manjur et al., 2014; Yengwe et al., 2018a). However, *F. albida*-crop-livestock farming system is fast disappearing, leading to deleterious effects on the sustainability of agriculture in the semi-arid zone of Ghana.

A distinct characteristic of *F. albida* is that it sheds its leaves at the onset of the rainy season and is in full leaf during the dry season, a phenomenon referred to as ‘reverse phenology’ (Wickens, 1969). This trait makes it physiologically dormant during the cropping season and therefore, considerably less competitive with field crops. This, coupled with its inability to shade crops, has made it compatible with field crops and acceptable to farmers.

Some studies have however suggested that the anticipated desirable attributes of *F. albida* such as reverse leafing phenology, the deep rooting habit, and extent of nitrogen fixation are sometimes not likely to be attained due to ecological, age and genotypic variations (Wickens, 1969; Chamshama et al., 1994). Furthermore, Sanchez (1995) suggested that the soil improving ability of *F. albida* could diminish and even disappear as one gets closer to the sub humid tropics. Moreover, due to the wide variety of factors that influence the distribution, population and diversity of trees in agroforestry systems, there is the need for farm level research as a follow up to research station, regional or global findings, as a pre-requisite for the introduction of agroforestry technologies (Zomer et al., 2009).

Recent reviews and studies on soil improvement effects of trees have largely concentrated on forest soils while not much attention was given to the relationships between tree-induced soil changes in crop production systems. For instance, only a few studies have attempted to quantify the amount of litterfall in *Faidherbia* agroforestry systems in sub-Saharan Africa and link it with nutrient release for the use of associated crops (Dunham, 1989; Yengwe et al., 2018a).

The aim of this study was to quantify the nutrient yield of *F. albida* and its effects on organic carbon and pH of parkland soils in the semi-arid zone of Ghana. The specific objectives were:

(1) To determine the effects of *F. albida* on the organic carbon, total nitrogen, available phosphorus and

exchangeable potassium content and pH of soils.
(2) To determine the trend in leaf litter production from mature *F. albida* trees in the semi-arid zone of Ghana,
(3) To determine the rate at which *F. albida* leaf litter decomposes and releases nutrients to the soil,
(4) To estimate the potential nutrient contribution from *F. albida* leaf litter.

## METHODOLOGY

### Study area

This study was conducted in the semi-arid zone, Upper East Region of Ghana, West Africa, where both the Sudan and Guinea Savannah vegetation are found. The region is located in the northeastern corner of the country between 00° and 01° West and latitudes 10° 30' North and 11°15' North (EPA, 2002). The land is generally flat with a few hills to the east and southeast. The total land area is about 8,842 $km^2$, equivalent to about 2.7% of the total land area of the country (GSS, 2012).

The Sudan Savannah zone occupies an area of about 1,900 $km^2$, and consists of short, drought and fire-resistant deciduous trees interspersed with open savannah grassland. Grass cover is very sparse and in most areas the land is bare and severely eroded especially at the peak of the dry season. Common grasses include *Andropogon, Heteropogon, Aristidia*, and *Loudetia* species (EPA, 2002). Tree cover is very low, with the commonest ones being *Anogeissus leiocarpus, Acacia* species, *Terminalia macroptera, Vitellaria paradoxa, Adansonia digitata, Ceiba pentandra, Faidherbia albida* and *Parkia filicoidea.* These are found in densely settled and cultivated areas (EPA, 2002). The area experiences a unimodal rainfall regime lasting from mid-May to October (5 to 6 months), followed by a long dry season lasting from October to May (6 to 7 months) in a year. Average annual rainfall and temperature are 885 mm and 28.6°C, respectively (EPA, 2002). Most of Northern Ghana falls within the Guinea Savannah ecological zone, covering about 147,900 $km^2$. Annual rainfall is between 1,000 and 1,300 mm per year. The peak rainfall period is usually in late August or early September with about 60% of the rainfall occurring within 3 months from July to September (Siaw, 2001).

Agroforestry systems developed from the existing vegetation in the Guinea Savannah zone comprise primarily of fruit trees like *A. digitata*, *Ficus* species, *Lannea microcarpa, Parkia biglobosa, Sclerocarya birrea* and *V. paradoxa*, and multipurpose trees such as *Azadirachta indica, Borassus aethiopum, Acacia sieberiana, Ceiba pentandra, F. albida, Senna siamea,* etc., (Michel, 2004).

### Litterfall pattern and quantity determination

The litter trap technique (Ssebulime et al., 2018) was used in determining the amounts of litter deposited by a mature *F. albida* tree. The litter traps were made with woven polypropylene sheeting. The mouth which was strengthened with flexible wire measured 30 cm in diameter, 60 cm deep and oval in shape. Ten *F. albida* trees were selected in each of the two ecological zones for litterfall collection. Care was taken to ensure that each selected tree was isolated enough from other trees to avoid litter being mixed up. Heights, diameter at breast height and average canopy width were measured for each selected tree. The canopy area of each sampled *F. albida* tree was determined by measuring crown diameters in two directions at right angles to each other and assuming that the canopy is a perfect circle. The polypropylene litter traps were placed midway under the canopies and supported by three wooden poles at a height of about 1 m from the ground to prevent livestock from tampering with the content of the bags. The traps were emptied monthly but fortnightly during the peak of the rainy season to avoid the rotting of the litter. All collected litter were composited at the end of the month, then subsequently separated into leaves, flowers, pods, branches and bark components and oven dried at 65°C for 4 days to determine their dry weights. The respective components were composited at the end of the study, sub-sampled and analyzed for organic carbon, total nitrogen, phosphorus and potassium content. Annual litter fall was calculated as the total litter collected over the twelve-month period (June 2017 to May 2018). The average amount of litter collected per litter trap was extrapolated to determine litter production on a per hectare basis.

### Litter decomposition rate

Litter bag technique was used to determine the decomposition rate of *F. albida* leaf litter. Litter bags were made from 1 mm mesh nylon nets, 25 cm × 20 cm in size and stapled at the edges.

Fifteen grams of leaf litter were gathered from underneath *F. albida* trees, weighed and filled into each of 108 bags, spread evenly in the bags to ensure that the litter had good contact with the soil (Conn and Dighton, 2000). Nine (9) bags were placed at 6 different locations in each of the two ecological zones. Six bags were randomly collected in each ecological zone at each sampling time. Bags were collected on 3, 7, 14, 21, 30, 45, 60, 75 and 105 days after placement. Litter bags were carefully rinsed with tap water to remove any adhering soil particles, dried at 70°C for 48 h and weighed to determine weight loss.

### Soil sampling

In each of the two ecological zones, 6 mature *F. albida* trees were selected (2 at each of three sites), for soil sampling. Care was taken to ensure that each selected tree was isolated enough from other trees. Measures of GPS locations, topography, soil texture where the trees were located, as well as height, diameter at breast height (DBH), and average canopy diameter were made. With the sampled tree trunk as the central point, the downslope of the topography was determined and a transect laid in that direction (labeled T1), from which 2 other transects each at 120° from T1 were laid (Takoutsing et al., 2017). With the canopy width determined, soil samples were taken from the center, and edge of the canopy, as well as 5 m from the edge of the canopy along the 3 transects, using a closed soil auger. At each point, soil samples were taken at 0 to 15 cm and 15 to 30 cm depths. The three soil samples collected at a given distance and depth under a tree was composited and sub-sampled for analysis. Soil samples were analyzed for total nitrogen, available phosphorus, exchangeable potassium, organic carbon and soil pH.

#### ***Determination of soil total nitrogen, available phosphorus, exchangeable potassium, organic carbon and soil pH***

The Kjeldahl's digestion procedure (Nelson and Sommers, 1972) was used for the determination of soil Total Nitrogen (TN) while available phosphorus was determined using Bray's 1 method (Bray and Kurtz, 1945). Ammonium acetate method (Shuman and Duncan, 1990) was used to analyze the exchangeable K in soil samples. Soil pH in $CaCl_2$ solution was determined by a pH meter after calibrating the instrument with a buffer solution and soil organic carbon content was determined using the Walkley & Black method (Walkley and Black, 1934). All samples were analyzed in 6 replicates in the laboratories of the Soil Research Institute of Ghana.

#### Data analysis

SPSS was used to perform the Shapiro-Wilk test for the normality of all data collected. Where a data set was not normally distributed, the appropriate transformation was used to ensure its normality. Analysis of variance (ANOVA) was then used to analyze the differences in leaf litter produced in the respective months, using GenStat (12th edition) whereas Turkey's Honestly Significant Difference (HSD) test was used to separate means at a significance level of 0.05. Independent t-Test was used to test the hypothesis that difference between the means of litter produced in the two ecological zones was not significantly different from zero.

Leaf litter decomposition rates were determined by finding the difference between the initial mass of litter (15 g) and that at the respective litter bag collection time periods. The annual decay constant (k) was determined using the relation $X/X_0 = e^{-kt}$, where $X_0$ is the initial dry weight of leaf litter, X is the dry weight remaining at the end of the experiment, and t is the time period (Olson, 1963). ANOVA was used to test for differences among the respective litter collection time periods, while independent t-Test was used to test the null hypothesis that the differences between means in the two ecological zones is equal to zero. Tukey's HSD was employed for post hoc tests among means within the same sample set. ANOVA was used to test for the difference in mass remaining between days of decomposition.

## RESULTS

### Litterfall pattern and quantity

The pattern of litterfall was similar in both Guinea and Sudan Savannah ecological zones. No litterfall was recorded in the months of September and October. Litterfall comprised leaves, branches and twigs, flowers, and pods (Figures 1 and 2). Matured pods fell between December and April and flowers fell (aborted) in November. The falling of branches did not follow any definite pattern (Table 1). In the Guinea Savannah zone, leaves made up 51.9%, pods 32.3%, dead branches 4.1%, and flowers 11.7% of dry matter produced, whereas in the Sudan Savannah zone leaves 40.3%, pods 34.8%, dead branches 14.2%, and flowers 10.7% of litter produced by the *F. albida* trees.

Leaf litterfall occurred throughout the year, except in September and October, reaching its peaks in June and July in the Guinea Savannah zone and in May to July in the Sudan Savannah zone. The first three months of the raining season (May - July) coincided with high deposition of leaf litter (Figure 3). There were significant differences in the quantity of leaf litter falling from one month to the other (Table 1). Annual leaf fall in the Guinea and Sudan savannah zones were 340.14 and 264 g $m^{-2}$ $year^{-1}$, respectively, while total litter production was 555.86 g $m^{-2}$ $year^{-1}$ in the Guinea Savannah zone and 404.29 g $m^{-2}$ $year^{-1}$ in the Sudan Savannah zone. Pod deposition occurred from December to April in both ecological zones (Table 1).

### Leaf litter decomposition

Biomass of buried *F. albida* leaf litter decreased with time in the two ecological zones. Mass loss rates were faster in the earlier stages. From an initial mass of 15 g applied, the biomass remaining were 3.67 g (24.5%) in the Guinea and 2.63 g (17.54%) in the Sudan Savannah zones of Ghana in 105 days after application. Significant differences were observed for leaf litter that decomposed within each ecological zone over the 105 days (Table 2).

The rate of biomass loss followed a similar trend in both ecological zones. Mass loss was faster in the first sixty days, and slowed down afterwards in both ecological zones (Figure 4).

Decomposition constants for leaf litter in the Guinea and Sudan Savannah zones were 0.012 and 0.017, respectively, indicating a higher rate of decomposition in the Sudan Savannah zone than in the Guinea Savannah zone (Figure 5).

### *Pattern of nutrient release from F. albida leaf litter*

Results from the laboratory analysis of leaf litter for nitrogen, phosphorus, and organic carbon contents for various days of decomposition indicate that there were increasing rates of loss/release of organic carbon, total nitrogen and available phosphorus in the first 60 days of decomposition in litter bags (Figure 6a and b). It was estimated that by the 105th day after deposition, with a mean leaf litter deposition of 303 g $m^{-2}$ per year, 9.31, 0.32, 0.43 and 157.30 g $m^{-2}$ of N, P, K, and OC, respectively, were released into soils under matured *F. albida* canopies year. With a mean canopy area of 291.00 and 183.00 $m^2$ in the Guinea and Sudan Savannah zones, respectively, a mature tree was estimated to release 2.71 kg N, 0.09 kg P, 0.13 kg K and 45.77 kg OC in the Guinea Savannah zone while 1.70 kg N, 0.06 kg P, 0.08 kg K and 28.79 kg OC will be released in the Sudan Savannah zone. Based on these estimates, 37 and 59 trees will be required in the Guinea and Sudan Savannah zones, respectively to release about 100 kg of N into the soil per hectare per year. In addition, 3.45 kg P, 4.63 kg K and 1,698.37 kg OC will also be added per hectare per year.

### Effects of *F. albida* on soil NPK, organic matter content and pH

#### *Soil organic carbon content*

Soil organic carbon content decreased with increasing distance from the trunk of mature *F. albida* trees and soil depth. Soil organic carbon content in mid-canopy was 0.55%, but decreased to 0.41% at the edge of the canopy, and further decreased to 0.34% 5 m from the edge of the canopy. Soil organic carbon contents did not vary significantly between the two ecological zones under study. In terms of soil depth, soil organic carbon contents reduced sharply with depth. It decreased from 0.51% in

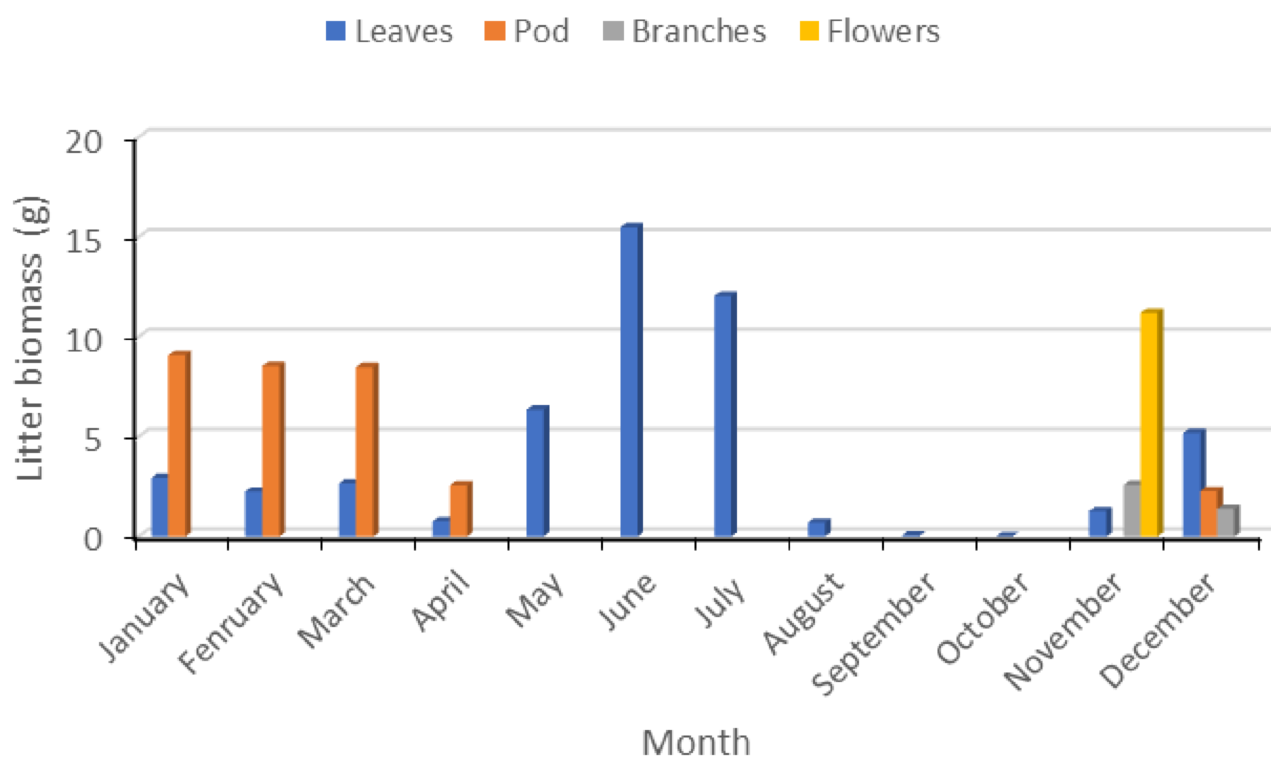


**Figure 1.** Monthly litterfall in the Guinea Savannah zone of Ghana.

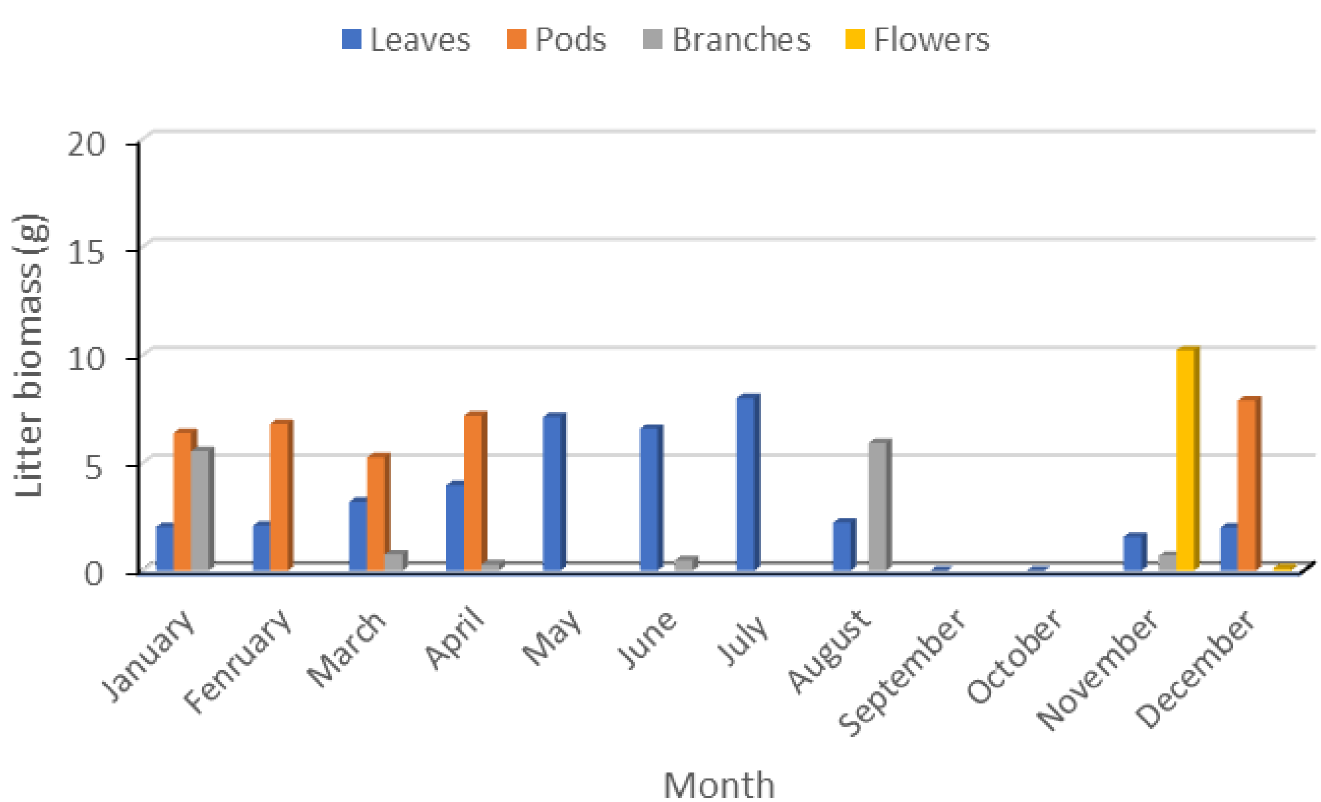


**Figure 2.** Monthly litterfall in the Sudan Savannah zone of Ghana.

**Table 1.** Mean total litterfall (leaves, flowers, pods and branches/twigs) and leaf litterfall from *F. albida* trees per litter trap in the Guinea and Sudan Savannah zones of Ghana.

| Month | Leaf litterfall (g) | | Total litterfall | |
|---|---|---|---|---|
| | Guinea Savannah | Sudan Savannah | Guinea Savannah | Sudan Savannah |
| January | 1.41[d] ±0.14 | 0.98[cd] ±0.11 | 3.59[bcd] ±1.07 | 2.12[abc] ±0.64 |
| February | 1.08[de] ±0.14 | 1.00[cd] ±0.12 | 5.17[abc] ±0.76 | 1.99[abc] ±0.49 |
| March | 1.27[de] ±0.12 | 1.37[cd] ±0.36 | 2.90[cdef] ±0.75 | 2.71[abc] ±0.80 |
| April | 0.29[ef] ±0.09 | 1.91[bc] ±0.34 | 0.88[abcd] ±0.24 | 2.28[abc] ±0.39 |
| May | 3.05[c] ±0.20 | 3.43[a] ±0.26 | 3.05[cde] ±0.20 | 3.43[ab] ±0.26 |
| June | 7.43[a] ±0.49 | 3.15[ab] ±0.19 | 7.43[a] ±0.49 | 3.22[ab] ±0.20 |
| July | 5.78[b] ±0.37 | 3.84[a] ±0.81 | 5.78[abc] ±0.37 | 3.84[ab]±0.81 |
| August | 0.34[ef] ±0.08 | 1.08[cd] ±0.11 | 0.34[ef] ±0.08 | 1.36[bc] ±0.22 |
| September | 0.02[f] ±0.01 | 0.00[d] ±0.00 | 0.18[f] ±0.01 | 0.00[c] ±0.00 |
| October | 0.00[f] ±0.00 | 0.00[d] ±0.00 | 0.00[f] ±0.00 | 0.00[c] ±0.00 |
| November | 0.61[def] ±0.11 | 0.76[cd] ±0.25 | 6.61[ab] ±1.42 | 4.82[a] ±1.45 |
| December | 2.49[c] ±0.23 | 0.96[cd] ±0.16 | 2.98[cdef] ±0.38 | 1.73[bc] ±0.67 |
| Total | 23.81 | 18.48 | 38.91 | 28.30 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 |

*Leaf and total litterfall values (± standard error of mean) for the various ecological zones followed by the same superscript alphabets do not differ significantly between the months they were collected.

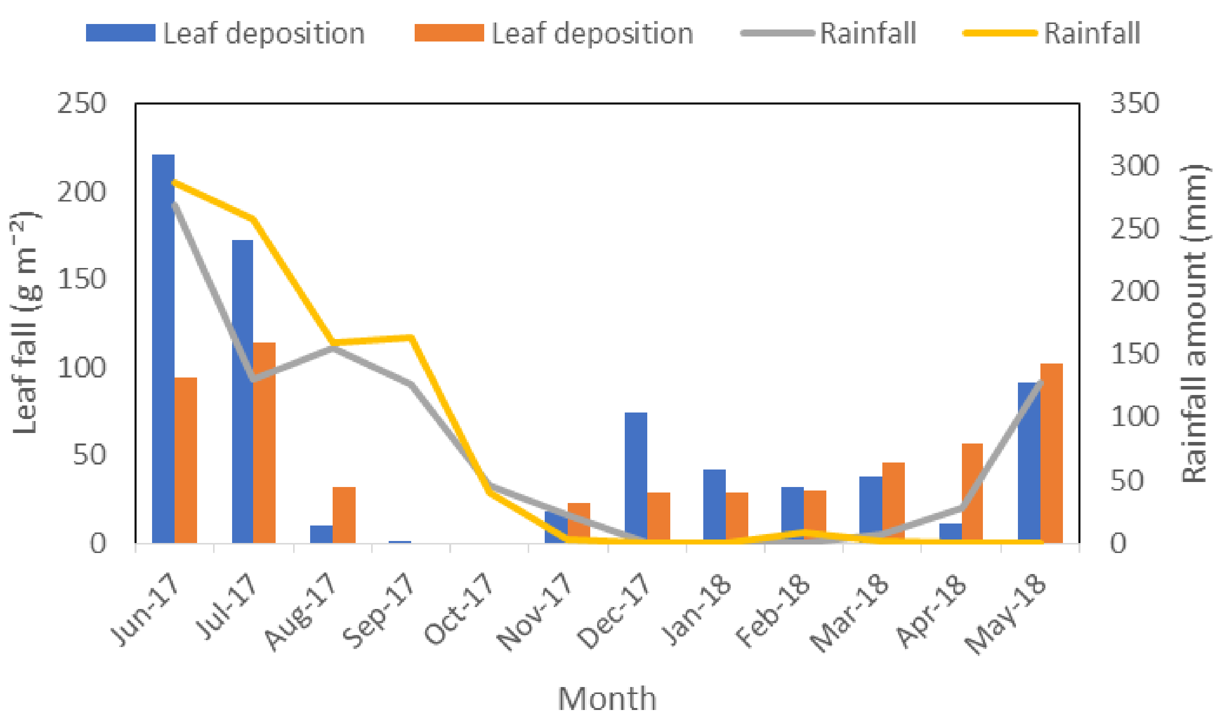


**Figure 3.** Leaf-fall pattern and rainfall in the Guinea and Sudan Savannah zones.

the topsoil to 0.36% in the subsoil (Table 3).

### *Soil total nitrogen content*

Like organic matter and carbon content, total nitrogen content decreased significantly as one moved from mid-canopy to open field. Total N content was 0.058% in mid-canopy while it decreased to 0.046% at the edge of the canopy, and 0.039% in the open field. Total N content was also significantly higher in the topsoil (0.054%) than in the subsoil (0.041%). No significant differences were

**Table 2.** Mass remaining from an initial leaf litter 15 g in two ecological zones.

| Day | Mass remaining (g) | |
|---|---|---|
| | Guinea Savannah | Sudan Savannah |
| 3 | 12.73[a] ±0.15 | 11.51[a] ±0.12 |
| 7 | 11.53[a] ±0.18 | 10.91[ab] ±0.18 |
| 14 | 9.91[b] ±0.30 | 8.91[bc] ±0.51 |
| 21 | 7.79[c] ±0.27 | 7.94[c] ±0.32 |
| 30 | 7.15[cd] ±0.23 | 7.64[c] ±0.23 |
| 45 | 6.54[cd] ±0.28 | 6.84[cd] ±0.47 |
| 60 | 6.10[d] ±0.22 | 4.87[de] ±0.68 |
| 75 | 4.73[e] ±0.40 | 3.87[ef] ±0.60 |
| 105 | 3.67[e] ±0.34 | 2.63[f] ±0.56 |
| P-value | <0.001 | <0.001 |

Differences between collection period means in the same column bearing different superscripts are significant according to Tukey's HSD post hoc test (± standard errors of the means).

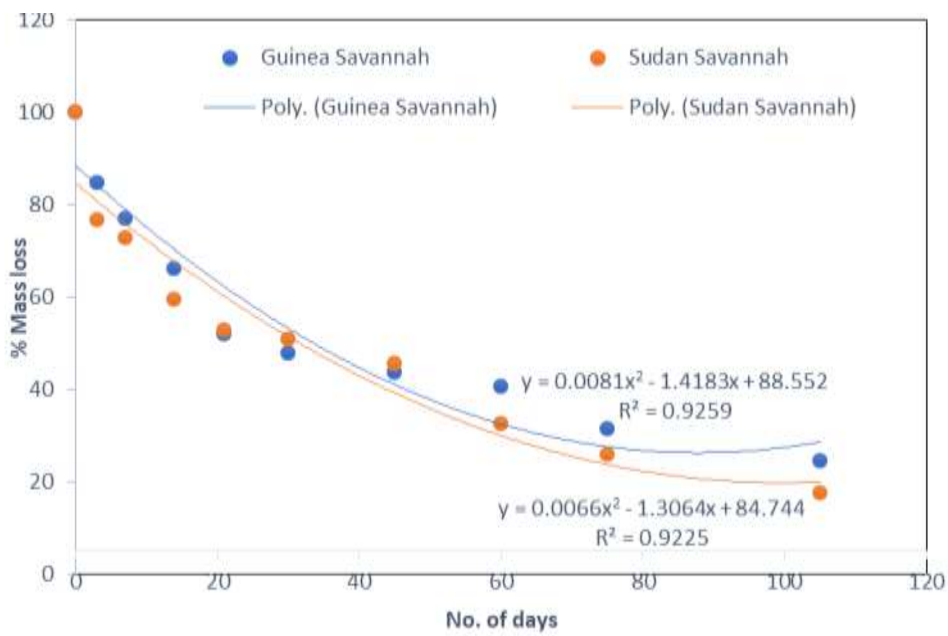


**Figure 4.** Percentage mass of litter remaining (decomposition) in litter bags with time in Guinea and Sudan Savannah zones of Ghana.

observed between the two ecological zones with respect to total nitrogen (Table 3).

### *Phosphorus and potassium*

There were no significant differences in available phosphorous between soils under *F. albida* canopy and open field. However, phosphorous content in the topsoil (15.11 ppm) was significantly higher than that in the subsoil (9.40 ppm). Soils in the Guinea Savannah zone were richer in phosphorous than those in the Sudan Savannah zone (Table 3). No significant differences were observed in potassium content with respect to all the factors (distance from *F. albida* trunk, soil depth and ecological zone) considered in this study (Table 3).

### *Soil pH*

Soil pH was unaffected by the presence of *F. albida* and therefore did not exhibit any significant difference under

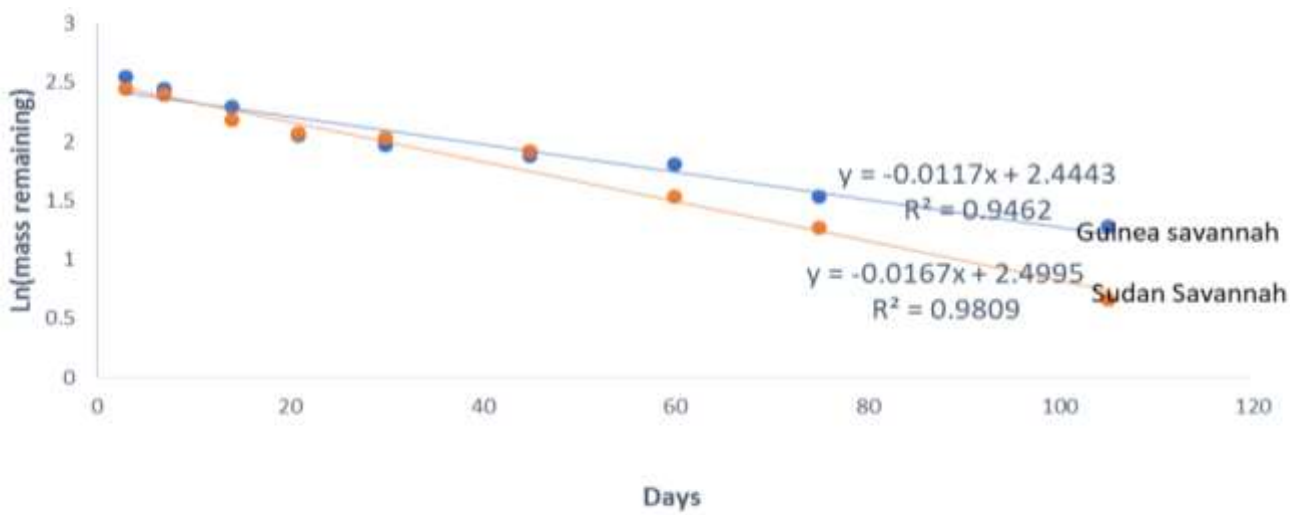


**Figure 5.** Model for the determination of decomposition rate constant in the Guinea and Sudan Savannah zones of Ghana.

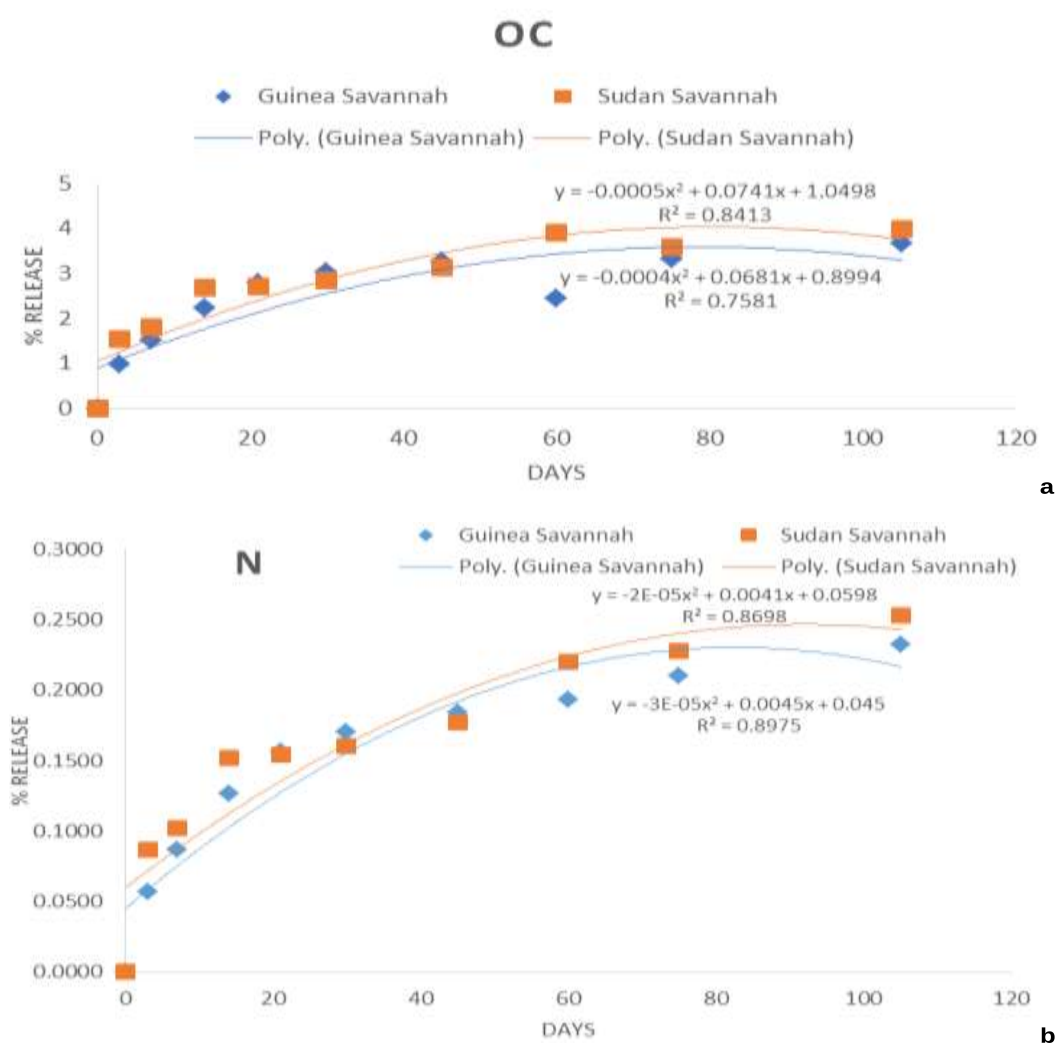


**Figure 6.** (a) Rate of Organic Carbon loss from *F. albida* leaf litter in the Guinea and Sudan Savannah Zones of Ghana. (b) Rate of Total Nitrogen loss from *F. albida* leaf litter in the Guinea and Sudan Savannah zones of Ghana.

**Table 3.** Soil major nutrients, organic carbon content and pH of soils under *F. albida* trees in the Guinea and Sudan savannah zones of Ghana.

| Factors/treatments | SOC (%) | TN (%) | P (mg/kg) | K (Cmol/kg) | pH |
|---|---|---|---|---|---|
| | | | **Distance from tree trunk** | | |
| Mid canopy | 0.554[a] (0.274) | 0.058[a] (0.235) | 13.110[a] (11.210) | 0.337[a] (0.169) | 6.10[a] (0.53) |
| Canopy edge | 0.412[ab] (0.270) | 0.046[ab] (0.232) | 12.640[a] (12.130) | 0.352[a] (0.683) | 6.03[a] (0.64) |
| Out of canopy | 0.339[b] (0.231) | 0.039[b] (0.200) | 11.070[a] (9.550) | 0.177[a] (0.141) | 5.93[a] (0.63) |
| LSD | 0.145 | 0.013 | 6.230 | 0.240 | 0.351 |
| Fpr | 0.014 | 0.014 | 0.792 | 0.278 | 0.615 |
| | | | **Soil depth** | | |
| 0 - 15 cm | 0.509[a] (0.285) | 0.054[a] (0.025) | 15.110[a] (11.54) | 0.265[a] (0.199) | 6.06[a] (0.53) |
| 15 - 30 cm | 0.361[b] (0.236) | 0.041[b] (0.020) | 9.400[b] (9.44) | 0.313[a] (0.557) | 5.98[a] (0.66) |
| LSD | 0.119 | 0.010 | 5.090 | 0.200 | 0.287 |
| P-value | 0.015 | 0.015 | 0.030 | 0.626 | 0.609 |
| | | | **Ecological zone** | | |
| Guinea savannah | 0.456[a] (0.32) | 0.049[a] (0.03) | 18.00[a] (12.34) | 0.342[a] (0.15) | 6.370[a] (0.41) |
| Sudan savannah | 0.414[a] (0.23) | 0.046[a] (0.02) | 6.00[b] (4.73) | 0.236[a] (0.57) | 5.670[b] (0.54) |
| LSD | 0.128 | 0.011 | 4.39 | 0.195 | 0.227 |
| P-value | 0.516 | 0.516 | <0.001 | 0.280 | <0.001 |

Mean values in the same column for the same factor with the same superscript are not significantly different at P < 0.05 level using Tukey's HSD. Standard deviations are in parenthesis.

and outside canopies, and in the different soil depths. However, soils in the Sudan Savannah zone were found to be significantly more acidic (5.7) than those in the Guinea Savannah zone (6.4) (Table 3).

## DISCUSSION

### Litterfall pattern and litter quantity

The litter deposition pattern found in this study corroborated the findings of several authors (Fagg and Roshetko, 1995; Fagg, 1995; Roupsard et al., 1999; Barnes and Fagg, 2003; Hadgu et al., 2009; Adamu, 2012; Wahl and Bland, 2013; Broadhead, 2015; Yengwe et al., 2018a) that, *F. albida* exhibits reverse leafing phenology. Leaf litter deposition occurred mainly between May and July, which coincides with the onset of rains and hence the beginning of the cropping season. The beginning of leaf-fall about a month into the raining season agrees with what Roupsard et al. (1999), found in other West African arid zones. About 60% of the total annual leaf litter deposited by the tree produced during this period is likely to contribute to the improved nutrient levels, as leaves are considered to contain most of the litter bound nutrients (Yang et al., 2004). Leaves falling almost throughout the year in this study corroborates the report by Dunham (1989) in Zimbabwe, but conflicted that of Jung (1970) in Bombay Senegal, who observed that 94% of leaves fell during four months of the rainy season. This could be due to the fact that the study area in Senegal experiences two rainy seasons in a year while this study area and that of Dunham (1989) experiences a single rainy season in a year.

In Chisama, Zambia, the peak of leaf litter deposition occurs between August and December (Yengwe et al., 2018a), while occurring between May and July in the semi-arid zone of Ghana suggests that leaf fall is influenced by climatic conditions, especially rainfall.

The maximum daily leaf litter deposition of 7.4 g $m^{-2}$ $day^{-1}$ (in June) in the Guinea Savannah (with annual rainfall of 911.5 mm) and 3.8 g $m^{-2}$ $day^{-1}$ in the Sudan Savannah zone (in July), with annual rainfall of 921.1 mm, was generally higher than the 1.6 g $m^{-2}$ $day^{-1}$ determined by Dunham (1989) in the woodlands of Mona, Zimbabwe, where mean annual rainfall was 757 mm.

Flower deposition occurring in only one month (November) and pod deposition following immediately from December is an indication that reproductive growth leading to pod production and maturity occurs within a short period.

Pod production occurs between December and April of the succeeding year. This period coincides with the dry season when fodder is scarce in the communities, compelling farmers to sell off their animals at cheap prices (Adzitey, 2013). The pollarding of *F. albida* branches and leaves to feed livestock however reduces the amount of litter added to the soil directly by livestock, which continuously camp under the tree to feed on falling pods from the tree. Moreover, in areas where livestock cannot have access to the trees, farmers gather the pods for sale in the local market while leaf litter, the main source of above ground organic matter are left under the tree to enrich the soil.

### Leaf litter decomposition

Leaf litter is considered to be the main source of nitrogen and organic carbon in soils in *Faidherbia*-farming systems in the semi-arid zones (Barnes and Fagg, 2003). Decomposition rate (mass loss) was faster in the initial days (first 60 days) of placement in both ecological zones than in the latter days. This might be due to the higher nitrogen content (lower C-N ratio) of leaves which could serve as substrate for microbes (Gnankambary et al., 2008), and slowing down later as the lignin and polyphenol contents increased relative to nitrogen (Couteaux et al., 1995). A similar result was also observed by Swift et al. (1979) who pointed out that some of the labile litter, upon being deposited on the soil, decompose very rapidly in a matter of days or weeks whereas the more recalcitrant components could remain in the soil for several months or even years, leaving more and more resistant compounds to build up with time. Estimated decomposition constants for the Guinea (0.012) and Sudan (0.017) Savannah zones were however lower than what Gnankambary et al. (2008) obtained at Boni village in Burkina Faso (0.077). It is probably due to differences in, and interaction of the various regulatory factors such as physical environmental conditions (especially annual mean temperature and moisture), organisms (made up of fauna and micro-organisms), and litter quality, usually defined by lignin, nitrogen, and condensed and soluble polyphenol concentrations (Zhang et al., 2008). However, according to Silver and Miya (2001), leaf litter decomposition is more strongly influenced by climate (particularly temperature) than substrate quality.

### Pattern of *F. albida* leaf litter nutrient release with time

The result from the laboratory analysis of leaf litter from *F. albida* agrees with the observation that leaf litter is a major source of nutrients and organic carbon in soils in agroforestry parklands (Vitousek, 1982; Aerts, 1996). This suggests that substantial amounts of nutrients from *F. albida* leaf litter could be released on time for use by field crops. The reduced rates of nitrogen and phosphorus released after the initial 60 days was also observed by Ribeiro et al. (2002). They noted that the higher the nitrogen and phosphorus concentration in the litter, the more rapidly those nutrients were released during decomposition. The amounts of N estimated to be released from *F. albida* leaf litter, were about 6.20 kg ha$^{-1}$ year$^{-1}$ in the Guinea Savannah zone and 1.86 kg ha$^{-1}$year$^{-1}$ in the Sudan Savannah zone of Ghana, is less than the 25 kg N ha$^{-1}$ as determined by Raghubanshi et al. (1990) in the dry tropical region of India, as a result of the relatively lower *F. albida* population densities on farmlands in this study. Retaining or planting about 37 to 59 *F. albida* trees per hectare to supply significant quantities of nitrogen (about 100 kg ha$^{-1}$) for field crops would drastically reduce the cost of procuring inorganic fertilizer as is being experienced in most of the semi-arid regions of Africa including Niger and Zambia (Garrity et al., 2010; Garrity and Bayala, 2019).

### Effect of *F. albida's* effects on soil organic carbon, N, P, K content and pH

#### *Effects of distance from tree trunk on nutrient content*

The significant differences observed in the soil organic carbon and total nitrogen contents between soils under *F. albida* canopies and those in open field is an indication that *F. albida* has the potential for improving the fertility status of parkland soils as reported by several other authors (Alexander, 1989; Brouwer et al., 1992; Ayuba and Murya, 2000; Kho et al., 2001; Adamu, 2012; Wahl and Bland, 2013; Yengwe et al., 2018b). The relatively improved soil fertility status of soils under *F. albida* canopies could not be attributed mainly to deposition of dung and urine by livestock, as suggested by some authors. This suggestion was disproved by Charreau and Vidal (1965) cited in Wickens (1969), when they worked on sites where livestock were absent for such a long time that the effects of animal dung and urine on the soil could be discounted.

Values for organic carbon and nutrient elements obtained from this study were generally lower than those found elsewhere. For instance, whereas this study found soil organic carbon (SOC) to be 0.554%, total N, 0.058%, available P, 13.11 mg/kg, exchangeable K, 0.337 cmol. kg$^{-1}$ and pH to be 6.1 under *F. albida* canopies, Adamu (2012) obtained 1.84% for SOC, 0.19% total N, 43.75 mg kg$^{-1}$ for available P, 0.32 cmol.kg$^{-1}$ for exchangeable K, and soil pH of 6.6 in the Gezewa region of Nigeria. In the woodlands of Mana Pools National Park in Zambia, Dunham (1991) obtained 1.16% for SOC, 0.148% for total N, 51 mg kg$^{-1}$ for available P, 0.84 cmol.kg$^{-1}$ for exchangeable K, and soil pH of 5.3. Sileshi (2016), in a review of studies to quantify the effects of *F. albida* on some soil nutrients observed consistent and significantly higher N, P, and K values under canopies than outside the canopies. The relatively lower nutrient levels observed in this study could be due to the low leaf litter deposition rates due to site and management practice differences, as suggested by Marriott and Wander (2006). Moreover, in this study, soil samples were collected from continuously cropped farms as compared to these other areas where the lands were relatively under fallow. Cultivated soils tend to contain less total N and other nutrients than undisturbed soils (Urioste et al., 2006). Anthropogenic factors like frequent thrash burning, pollarding for feeding livestock and direct livestock browsing could also account for these relatively lower values. Kho et al. (2001), also asserted that since the fertility improvement and competitive effects of the same agroforestry technology would differ under different conditions, it will be unrealistic to attempt to extrapolate from one location or condition to another. These results also seem to corroborate the suggestion made by Sanchez (1995) that, the soil improving ability of *F. albida* could diminish and even tend to disappear as one gets closer to the sub humid tropics.

### *Changes in some soil fertility indicators with soil depth under F. albida canopies*

This study found that SOC, total N, and available P contents significantly decreased at lower soil depth (15 - 30 cm) as compared with the topsoil (0 - 15 cm). This trend affirms the observation of Kho et al. (2001), who determined the effects of *F. albida* on soils in a millet production system in Niger. They observed that the levels SOC, total N and exchangeable K were higher in the 0 - 15 cm depth of the soil but decreased in lower depths. A similar trend was observed by Pandey et al. (2000), who recorded maximum levels of total N and SOC in the 0 - 10 cm depths, but declined sharply with depth under *Acacia nilotica* canopies in a predominantly rice-based cropping system in Madhya Pradesh, India. Decreasing levels of SOC, total N and available P with increasing soil depth most probably due to the relative higher abundance of litter deposited on the top soil, coupled with the higher microbial/biological activity in the upper horizon than in the lower horizons.

### *Effects of F. albida on some soil properties in different ecological zones.*

Significant differences were observed in available P content and soil pH in the two ecological zones studied. Whereas the other parameters such as SOC, total N and exchangeable P did not exhibit any significant differences across these two ecological zones, available P levels were three times higher in the Guinea Savannah zone than in the Sudan Savannah zone. This could be due to geological differences (Manning, 2010), and/or the management of crop residues, and other cultural practices (Hedley et al., 1982).

Soil pH is one of the most important indicators of key chemical properties of the soil (McLean, 1982) as it directly or indirectly influences soil nutrient availability, cation solubility, SOC characteristics and soil moisture regime (Lauber et al., 2009). This study found that soils in the drier Sudan Savannah zone were more acidic than those in the Guinea Savannah zone. It was noted by McLean (1982) that salt accumulation as a result of limited rainfall or inadequate drainage conditions could increase soil acidity.

## CONCLUSION AND RECOMMENDATIONS

Though leaf litter was deposited almost throughout the year (except in October and November) about 60% of the total annual leaf litter was deposited during the first three months after the onset of the raining season.

The rate of leaf litter decomposition increased steadily up to about 60 days after deposition. These high leaf litter decomposition and nutrient release rates during the first 60 days of the cropping season could contribute to the supply of nutrients for use by major field crops like sorghum, millet, maize, etc. Since leaf biomass of trees usually contains higher N/P ratio than that required by crops, P could become deficient in an attempt to supply N through *F. albida* leaf litter application. It would, therefore, be economically prudent to integrate an inorganic phosphorus source with the organic materials (Jama et al., 1997). A possible source of P could be soft rock phosphate which is a colloidal phosphate containing 18% P, and is easier for plants to assimilate (Rajan, 1987; Szilas et al., 2007).

At the current population densities of 2.3 and 1.1 trees per hectare, *F. albida* leaf litter can add only about 6.20, 0.21 0.29 and 104.82 kg of N, P, K, and OC per hectare per year in the Guinea Savannah Zone. In the Sudan Savannah zone, *F. albida* leaf litter will add about 1.86, 0.06, 0.09 and 31.38 kg of N, P, K, and OC per hectare per year. These amounts are insignificant in relation to the requirements for the production of major annual crops like maize, millet, sorghum, vegetables etc.

For the full potentials of *F. albida* in soil fertility improvement to be realized among these resource-poor farming communities, there is the need for efforts to increase its current population density of 2.29 and 1.09 trees per hectare to on parklands in the Guinea and Sudan Savannah zones, respectively to 37 and 59 trees per hectare. At this population density, mature *F. albida* has the potential of adding about 100 kg N, 3.45 kg P, 4.63 kg K and 1,698.37 kg OC to the soil annually. The shortfall in the amounts of P and K supplied by *F. albida* could be supplemented through inorganic sources. This could be achieved through awareness creation among farmers on the enrichment planting and management of *F. albida* seedlings, with a better understanding of its ecological requirements and growth habits.

The agroforestry significance of *F. albida* was partly demonstrated by its significant influence on two very important soil macro nutrients, carbon and nitrogen. Nitrogen is the most important element especially for economic reasons especially among resource-poor, smallholder farming communities found in the semi-arid zone of Ghana. It is the nutrient that is required in the largest amounts and its availability in soils is directly proportional to crop yields and is the most likely to be deficient. Soil organic carbon (or organic matter) has direct and indirect effects on nutrient use efficiency and crop production systems.

Increasing the population density of *F. albida* from one or two trees to 37 or 59 trees per hectare on parklands requires very pragmatic action among all stakeholders including policy makers, agricultural extension agents and farmers, since its success has effects on the cost of importing and distributing inorganic fertilizers.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Evaluation of morphological and quality characteristics of introduced grape cultivars produced under greenhouse conditions in Kenya

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**Grape production in Kenya is low and the country imports approximately 4,000 metric tons of wine every year. Three Chinese table grape cultivars (Jingyan, Jingxiangyu and Jingcui) and two Chinese wine grape cultivars (Beihong and Beifeng) as well as three French hybrid wine grape cultivars (Chenin Blanc, Sauvignon Blanc and Cabernet Sauvignon) were evaluated for fruit morphology (berries and bunches) and quality characteristics (Total Soluble Solids, Titratable Acidity, pH and sensory parameters) in 2018/2019 using International Organization for Vine and Wine descriptors and Economic Co-operation and Development procedure for fruit and vegetables respectively. All vines within the row were planted at a spacing of 0.9 and 1.6 m between the rows in a completely randomized design with three vines per replication and four replications for each cultivar. Collected data were subjected to ANOVA. Jingyan and Jingxiangyu had significantly bigger berries and higher bunch weight than all the other cultivars. The TSS of the grapes cultivars ranged from 16.3 to 25.2 °Brix. Beihong and Beifeng had higher TTA levels of 25.7 and 21.2 g/L respectively. Sensory data showed that Jingyan and Jingxiangyu were the most preferred cultivars. All the cultivars had ideal TSS and pH for winemaking and elaboration.**

**Key words:** Grapes, cultivar, morphology, quality, greenhouse.

## INTRODUCTION

Grapes belongs to the genus *Vitis* and family *Vitaceae* and they are believed to have originated from the Caucasian and Caspian regions (FAO, 2017). On a large scale, the genus Vitis is widely used wine and dessert due to their health benefits (Ivanova-Petropulos et al., 2015). Grapes have resveratrol (stilbenes belonging to a non- flavonoid group of phenolic compounds) which has antiviral, anticancer, antiaging, life-prolonging, anti-inflammatory and neuroprotective effects (Kundu and Surh, 2008; Stojanović et al., 2001). Grape also is a rich source of potassium and fibre which improves cardio-vascular health and blood pressure. High potassium

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intake is associated with the preservation of bone mineral density, protection against loss of muscle mass and reduced risk of stroke (Ware, 2017). Global consumption of fresh table grapes has been on the rise since 2009/2010 with consumption rising from 15.6 to 20.9 million tons in 2015 (FAS, 2015). In Kenya, grapes are utilized in winemaking, table grapes as well as raisins in the confectionary industry (HCD, 2014). The country spends millions of dollars on importing both fresh and dried grapes as well as wine brands (HCD, 2014). In 2017, Kenya imported 3,000 metric tons of grapes and 4,000 metric tons of wine (KNBS, 2017).

Grapes production in Kenya has been low due to insufficient locally adapted varieties and farmers technical know-how. Additionally, no research has been carried out in this country to understand the crop morphology or breed for locally adapted varieties. Grapes display an array of morphological characteristics which include pigmentation, growth habits, seed shape and flower colour. Morphological characterization is vital for conservation, commercialisation and breeding of new cultivars (Laurentin, 2009; Ocampo et al., 2006; Collard et al., 2005). Table grape quality is determined by the interplay of several metabolites including organic acids (tartaric and malic), total soluble sugars (glucose and fructose) and titratable acidity (Pereira et al., 2006; Dokoozlian, 2000). The composition and content of organic acids and sugars in grape berries determine the wine quality, stability and flavour, as well as the organoleptic quality of table grapes (Shiraishi et al., 2010; Rusjan et al., 2008). Organic acids are responsible for the tart taste in grapes and they influence wine colour, stability and pH. Malic and tartaric acids account for more than 90% of the total acids (Ninio et al., 2003). Sugars (glucose and fructose) in the berry are responsible for the sweetness of table grapes and raisins (Jackson, 2014). Sugars and organic acids are also important in the selection and breeding of new cultivars (Liu et al., 2007). The objective of this study was to evaluate the morphological and quality characteristics of three Chinese table grape cultivars, two Chinese wine grape cultivars and three French hybrid wine grape cultivars as a prerequisite for grapes breeding in Kenya.

## MATERIALS AND METHODS

### Experimental site

The study was carried out in a greenhouse at JKUAT main campus situated in Juja Sub-County (1°5'35.93"S, 37°0'46.31"E and 1525 m above sea level), 36 km Northeast of Nairobi, Kenya in 2018/2019.

### Grape cultivars used for this study

Three Chinese table grape cultivars (Jingyan, Jingxiangyu and Jingcui) and two Chinese wine grapes cultivars (Beihong and Beifeng) introduced from the Institute of Botany, Chinese Academy of Science as well as three French hybrids (Chenin Blanc, Sauvignon Blanc and Cabernet Sauvignon) collected from Yatta Complex Centre were evaluated for morphological and fruit quality characteristics. The Chinese grape cultivars were introduced and grown for adaptation in Kenya in 2015 while the French hybrids were introduced in 1995.

### Experimental design

All vines within the row were planted at a spacing of 0.9 and 1.6 m between the rows in May 2018. The design was completely randomized with three vines per replication and four replications for each cultivar. Training, pruning, watering, fertilization, pest and disease control were carried out as described by Strik (2011).

### Fruit morphological analysis

Morphological characteristics that were analysed included the type of flower, berries, bunches and yield. The characteristics were observed and described using the International Organization of Vine and Wine (OIV) descriptors (Table 1) from November 2018 to March 2019. Each characteristics had an OIV code and a number representing their reading. The berries and bunches were morphologically evaluated when the berries attained full veraison (change of skin colour from green to purple for Beihong and Beifeng, red for Jingyan and golden yellow for Jingxiangyu, Chenin Blanc and Sauvignon Blanc. For bunch morphological evaluation, ten bunches per cultivar were selected. For berry morphological evaluation, ten berries per bunch were selected randomly from the ten selected bunches. For yield evaluation, average bunch weight of 10 largest bunches per cultivar was recorded at the time of harvest with the help of a weighing balance and used to estimate the yield (kg) $ha^{-1}$ (OIV, 2009).

### Fruit quality analysis

#### *Total soluble solids (TSS)*

The TSS was determined as described by OECD (2005). The % brix was determined using handheld refractometer (N1, Atago CO.LTD Tokyo, Japan). Three drops of homogenized grape juice were placed on the prism of the refractometer which had been calibrated and the lid closed. The TSS content was then read on the scale to one decimal place a 20 ± 2°C while held close to the eye. This test was replicated three times. After each reading, the refractometer prism was cleaned with distilled water and dried with soft tissue paper (serviette).

#### *Total titratable acidity (TTA)*

The TTA was determined as described by OECD (2005). Thirty berries of each cultivar were crushed using a mortar and pestle. The pulp was then squeezed using a muslin cloth to extract the juice into a beaker after which it was filtered to obtain a homogenized extract using a filter paper. A pipette was used to draw 10 ml of the extract and discharged in a 250 ml beaker. Another clean pipette was used to draw 50 ml of distilled water and added to the juice in the beaker. Three drops of 1% phenolphthalein indicator were then added. The solution was titrated against 0.1N NaOH until a permanent pink colour was achieved. This procedure was replicated three times. The results were expressed as g/L of tartaric acid which is the organic acid in grapes. The following formula was used:

$$\text{TA g/L} = (\text{ml NaOH} \times \text{N (NaOH)} \times \text{acid meq. factor} \times 100)/\ \text{ml juice titrated} \quad (1)$$

**Table 1.** List of morphological characters used in this study.

| Characters | OIV Code No. | Unit of measure |
|---|---|---|
| Flower sexual organ | 151 | N/A |
| Bunch: length (peduncle excluded) | 202 | Mm |
| Bunch width | 203 | mm |
| Bunch density | 204 | N/A |
| Bunch shape | 208 | N/A |
| Bunch: number of wings of the primary bunch | 209 | N/A |
| Berry: length | 220 | Mm |
| Berry: width | 221 | Mm |
| Berry: uniformity of size | 222 | N/A |
| Berry: shape | 223 | N/A |
| Berry: colour of skin | 225 | N/A |
| Berry: firmness of flesh | 235 | N/A |
| Berry: ease of detachment from pedicel | 240 | N/A |
| Single bunch weight | 502 | grams |
| Single Berry weight | 503 | grams |

Source: OIV (2009).

***pH***

The pH was measured using a pH meter (PHM-2000, TOKYO RIKAKIKAI CO. LTD Tokyo, Japan) at room temperature (23 ± 2°C). The standardization of pH-meter was done with pH buffer solution 4.0, the electrode rinsed in distilled water and then standardized using an alkaline buffer of 7.0. The pH of the grape juice was then measured and the procedure was replicated three times.

***Sensory parameters***

Sensory evaluation was carried out by 35 untrained panellists (17 males and 18 female) aged 24 to 60 years from the Department of Horticulture and Food Security. Un-deformed mature berries of each of the harvested cultivars were presented to the panellists to rate their preference for sweetness, sourness, crispness, flavour, colour and skin toughness on a 9-point hedonic scale (Jayasena and Cameron, 2008; Lawless and Heymann, 2010). Water was provided to the panellists to rinse their mouth after each sample evaluation under a well-lit room (Santillo et al., 2014).

**Statistical analysis**

Morphological and fruit quality data were subjected to SPSS Version 25 for windows to assess the analysis of variance (ANOVA) between the cultivars. The difference among the treatments was tested by a multiple mean comparison test (HSD Tukey) at a significance level of $p < 0.05$ (IBM, 2018). Each value of the mean and standard error in the tables represented three replicates of each treatment.

## RESULTS AND DISCUSSION

### Morphological characteristics

Among the eight selected cultivars for evaluation, six cultivars (Jinyang, Jingxiangyu, Beihong, Beifeng, Chenin Blanc and Sauvignon Blanc) yielded berries (Figure 1). The results of morphological characteristics of Jinyang, Jingxiangyu, Beihong, Beifeng, Chenin Blanc and Sauvignon Blanc are presented in Table 2.

Jingyan and Jingxiangyu had longer bunches compared to the other cultivars. This is an important characteristic since the berries have room for expansion thereby increasing in size and weight. Short bunches tend to have compacted berries which reduces room for berry expansion thereby leading to a smaller/narrow berries as exhibited by Sauvignon Blanc, Chenin Blanc and Beihong. Jingyan and Jingxiangyu showed broad ellipsoid berry shape while the other four cultivars showed globose berry shape. All the cultivars were conical in shape and their berries were uniform in size. These two characteristics make the grapes suitable for commercialization. Salimov et al. (2017), states that berry uniformity is among the most important factors that influence the trade appearance of grapes. Jingyan had red rose colour, Jingxiangyu, Chenin Blanc and Sauvignon Blanc had green-yellow colour while Beihong and Beifeng had blue-black colour. This is as a result of different phenolic compounds within the grape skins. Grapes skin colour is controlled by anthocyanin influences the quality of juice, wine and the market value of table grapes (Liang et al., 2008).

### Yield estimates

The estimated yields of the six cultivars are presented in Table 3. Beifeng had three clusters, Beihong had two clusters and the other four cultivars had only one cluster


**Figure 1.** Grapes cultivars used in this study. (A) Jingyan, (B) Jingxiangyu, (C) Beihong, (D) Beifeng, € Chenin Blanc and (F) Sauvignon Blanc.

per vine (Table 3). Berry weight was highest in Jingxiangyu (7.64 g) followed by Jingyan (6.82 g) and lowest in Beifeng (1.41 g). Cluster weight was significantly different with Jingxiangyu having the highest cluster weight (440.8 g) and Beihong, Sauvignon Blanc and Chenin Blanc had the lowest cluster weights (181.3, 154.8 and 145.6 g respectively). Yield (g) per vine was highest in Beifeng (788 g) followed by Jingxiangyu (441 g). When the yield per plant was extrapolated to represent yield per hectare, Beifeng had the highest yield per hectare while Chenin Blanc had the lowest yield hectare. Similarly, Walker et al. (2005), reported that cluster and berry weight influences the overall yield of grapevines.

#### Principal component analysis

From the results of morphological characteristics, Principal Component Analysis resulted in two principal components (Figure 2) that had Eigenvalues greater than 1 (Table 4). These principal components explained more than 85% of the morphological variability for both subsets. The first principal component comprised characteristics associated with bunch length (OIV, 202), bunch density (OIV, 204), bunch shape (OIV, 208), number of wings of the primary bunch (OIV, 209), berry uniformity of size (OIV, 222), berry shape (OIV, 223), berry firmness of flesh (OIV, 235) and berry ease of detachment from the pedicel (OIV, 240) which accounted

**Table 2.** Morphological characters of the six grape cultivars used in this study.

| Characters/Cultivar | Jingyan | Jingxiangyu | Beifeng | Beihong | Chenin Blanc | Sauvignon Blanc |
|---|---|---|---|---|---|---|
| Flower sexual organ | 3:fully developed stamens and gynoecium | 3: fully developed stamens and gynoecium | 3: fully developed stamens and gynoecium | 3: fully developed stamens and gynoecium | 3: fully developed stamens and gynoecium | 3:fully developed stamens and gynoecium |
| Bunch length | 7: long about 200 mm | 7: long about 200 mm | 5: medium about 160 mm | 5: medium about 160 mm | 3: short about 120mm | 3: short about 120mm |
| Bunch width | 3: narrow about 80 mm | 3: narrow about 80 mm | 5: medium about 120 mm | 3: narrow about 80 mm | 3: narrow about 80 mm | 3: narrow about 80 mm |
| Bunch density | 5: medium | 5: medium | 1: very loose | 7: dense | 7: dense | 7: dense |
| Bunch shape | 2: conical | 2: conical | 2: conical | 2: conical | 2: conical | 2: conical |
| Bunch number of wings of the primary bunch | 1: absent | 1: absent | 3: 3 – 4 wings | 1: absent | 1: absent | 1: absent |
| Berry length | 5: medium | 5: medium | 3: narrow | 3: narrow | 3: narrow | 3: narrow |
| Berry width | 5: medium | 5: medium | 3: narrow | 3: narrow | 3: narrow | 3: narrow |
| Berry uniformity of size | 2: uniform | 2: uniform | 2: uniform | 2: uniform | 2: uniform | 2: uniform |
| Berry shape | 3: broad ellipsoid | 3: broad ellipsoid | 2: globose | 2: globose | 2: globose | 2: globose |
| Berry colour of skin | 2: rose | 1: green yellow | 6: blue black | 6: blue black | 1: green yellow | 1: green yellow |
| Berry firmness of flesh | 2: slightly firm | 2: slightly firm | 1: soft | 1: soft | 1: soft | 1: soft |
| Berry ease of detachment from pedicel | 2: easy | 3: difficult | 2: easy | 3: difficult | 2: easy | 2: easy |
| Single bunch weight | 5: medium: about 500 g | 5: medium: about 500 g | 3: low: about 300 g | 3: low: about 300 g | 3: low: about 300 g | 3: low: about 300 g |
| Single berry weight | 7: high | 7: high | 3: low | 3: low | 1: very low | 1: very low |

for 66.10% of the variation. The second principal component comprised characteristics associated with bunch width (OIV, 203), single bunch weight (OIV, 502), berry length (OIV, 220), berry width (OIV, 221) and single berry width (OIV, 503) which accounted for 19.87% of the variation.

### Fruit qualities

Fruit quality results are presented in Table 5.

#### *Total soluble solids (TSS)*

TSS of all the grape cultivars ranged from 16.3 to 25.2% (Table 5) which is greater than the recommended TSS level of 16% for grapes to be considered ripe (FAO, 2007). Jingyan and Jingxiangyu had higher TSS levels (18.4 and 22.0% respectively) as compared to their counterparts produced under open field environment in China which had TSS of 15 to 17% as reported by Jiazi, (2014). The TSS level is a quality trait for grapes that directly affect consumer preference for table grapes. Additionally, wine grapes with high TSS levels are preferred, as it is the TSS level that determines the alcohol content of most wines (Liu et al., 2006). Grapes sugar levels also affect wine quality as it is a substrate for yeast fermentation (Xin et al., 2013). Therefore, Jingyan and Jingxiangyu are considered suitable for fresh consumption while Beihong, Beifeng, Chenin Blanc and Sauvignon Blanc are considered suitable for wine processing.

#### *Total titratable acidity (TTA)*

Based on the results obtained (Table 5), Jingxiangyu, Jingyan and Sauvignon Blanc had ideal TTA levels of 6.32, 7.33 and 7.25 g/L respectively suitable for winemaking. The ideal TTA range for the production of well-balanced wine is 2- 10 g/L (Puckette, 2015). At TTA of 2 g/L, the wine tastes flat and at TTA of 10 g/L, the wine tastes tart. Organic acids are responsible

**Table 3.** Yield estimates of six grapes cultivars, Jingyan, Jingxiangyu, Beifeng, Beihong, Chenin Blanc and Sauvignon Blanc raised in a greenhouse in Kenya.

| Treatment | Cluster no vine$^{-1}$ | Cluster wt. (g) | Berry wt. (g) | Yield (g vine$^{-1}$) | kg ha$^{-1}$* |
|---|---|---|---|---|---|
| Jingyan | 1$^{c}$ | 395.6$^{b}$ | 6.82$^{b}$ | 396$^{c}$ | 2,750 |
| Jingxiangyu | 1$^{c}$ | 440.8$^{a}$ | 7.64$^{a}$ | 441$^{b}$ | 3,062 |
| Beihong | 2$^{b}$ | 181.3$^{d}$ | 2.45$^{c}$ | 363$^{c}$ | 2,518 |
| Beifeng | 3$^{a}$ | 262.8$^{c}$ | 1.46$^{d}$ | 788$^{a}$ | 5,475 |
| Chenin Blanc | 1$^{c}$ | 145.6$^{e}$ | 2.51$^{c}$ | 146$^{d}$ | 1,014 |
| Sauvignon Blanc | 1$^{c}$ | 154.8$^{e}$ | 2.58$^{c}$ | 155$^{d}$ | 1,076 |

The data are expressed as means and the treatments mean followed by the same letters in the same column are not significantly different ($p \leq 0.05$) (Zare et al., 2015).
*Assuming 6944 plants per hectare at 1.6 in row spacing and 0.9 m between rows. Data were not statistically analysed.

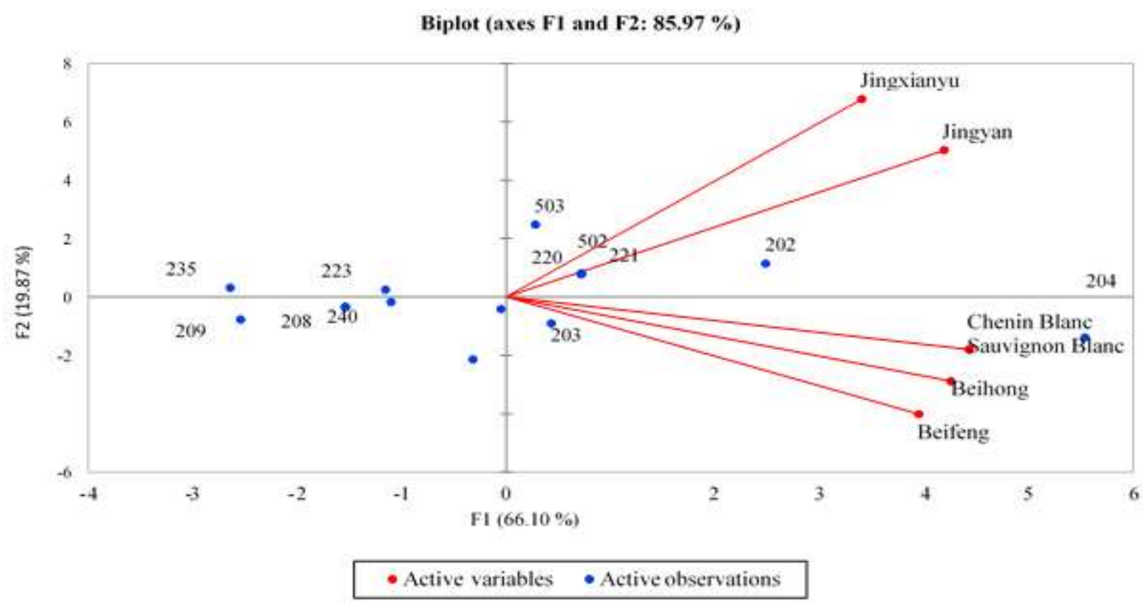


**Figure 2.** Principal component analyses of the morphological characters evaluated for the six cultivars used in this study.

for the tart taste in grapes and they influence wine colour, stability and pH (Rusjan et al., 2008). Beihong and Beifeng had higher TTA values of 25.7 and 21.2 g/L, respectively. These values are higher than their counterparts cultivated in the field in China with a TTA value range of 6.5- 9.2 g/L (Jiazi, 2014). Therefore, TTA adjustments would be required in order to enhance the stability of the wine made from the two cultivars. Debolt et

**Table 4.** Estimate of eigenvalues and cumulative variances of the first two principal components (f1, f2) of morphological characters used in this study.

| | Eigenvalue | Variability (%) | Cumulative % |
|---|---|---|---|
| F1 | 3.97 | 66.10 | 66.10 |
| F2 | 1.19 | 19.87 | 85.97 |

**Table 5.** Fruit quality characteristics of the six grapes cultivars; Jingyan, Jingxiangyu, Beifeng, Beihong, Chenin Blanc and Sauvignon Blanc used in this study.

| Treatment | TSS (°Brix) | TTA (g/L) | pH | Sweetness | Sourness | Crispness | Flavour | Skin toughness | Colour | Overall acceptability |
|---|---|---|---|---|---|---|---|---|---|---|
| Jingyan | 18.4±0.18$^{c}$ | 7.33±0.08$^{d}$ | 3.15±0.03$^{d}$ | 6.63±0.37$^{a}$ | 5.85±0.45$^{a}$ | 6.74±0.24$^{a}$ | 6.63±0.31$^{a}$ | 6.37±0.33$^{a}$ | 7.15±0.29$^{a}$ | 6.93±0.33$^{a}$ |
| Jingxiangyu | 22.0±0.17$^{b}$ | 6.32±0.06$^{e}$ | 3.42±0.02$^{b}$ | 7.30±0.29$^{a}$ | 5.59±0.46$^{ab}$ | 6.63±0.31$^{ab}$ | 6.04±0.36$^{a}$ | 6.59±0.32$^{a}$ | 5.37±0.44$^{b}$ | 6.81±0.35$^{a}$ |
| Beihong | 16.3±0.18$^{d}$ | 25.7±0.17$^{a}$ | 3.07±0.02$^{d}$ | 3.41±0.41$^{b}$ | 3.89±0.48$^{b}$ | 4.85±0.37$^{c}$ | 4.56±0.36$^{b}$ | 3.89±0.43$^{bc}$ | 6.33±0.41$^{ab}$ | 4.04±0.47$^{c}$ |
| Beifeng | 18.3±0.09$^{c}$ | 21.2±0.09$^{b}$ | 3.28±0.01$^{c}$ | 3.96±0.39$^{b}$ | 4.04±0.49$^{ab}$ | 4.96±0.28$^{c}$ | 4.22±0.29$^{b}$ | 4.41±0.32$^{c}$ | 6.15±0.40$^{ab}$ | 4.37±0.45$^{bc}$ |
| Chenin Blanc | 21.2±0.29$^{b}$ | 10.50±0.13$^{c}$ | 3.15±0.01$^{d}$ | 6.26±0.33$^{a}$ | 5.26±0.42$^{ab}$ | 5.41±0.34$^{bc}$ | 5.96±0.29$^{a}$ | 5.41±0.35$^{ab}$ | 4.85±0.41$^{b}$ | 5.59±0.43$^{abc}$ |
| Sauvignon Blanc | 25.23±0.21$^{a}$ | 7.25±0.03$^{d}$ | 3.55±0.02$^{a}$ | 7.33±0.21$^{a}$ | 4.59±0.49$^{ab}$ | 5.78±0.25$^{abc}$ | 6.44±0.29$^{a}$ | 4.74±0.37$^{bc}$ | 5.04±0.46$^{b}$ | 5.93±0.47$^{ab}$ |

The data are expressed as means ± standard error of the mean and the treatments means followed by the same letters in the same column are not significantly different ($p \leq 0.05$).

al. (2007), reported that grapes suitability for wine making is dependent on a sufficient and harmonic content of organic acids.

### *pH*

The pH range of all the cultivars ranged from 3.07 to 3.55 (Table 5) which is ideal for winemaking. White wines require a pH range of 3.1 to 3.4 and red wine a pH of 3.5 to 3.6 for quality wine elaboration (MoreFlavor Inc, 2012; Jackson, 2008). A pH value higher than 3.6 is usually undesirable as it causes a low intensity of colour, impairs microbial stability, increases susceptibility to oxidation and raises the spoilage potential of the wine produced (Grapevines, 2010). Thus, the fruits of all wine cultivars that we evaluated under greenhouse conditions in this study namely, Beifeng, Beihong, Chenin Blanc and Sauvignon Blanc had ideal pH for processing into wine.

### *Sensory properties*

Introduced French hybrid wine grapes (Chenin Blanc and Cabernet Sauvignon) were highly preferred in regards to sweetness compared to the introduced Chinese wine grape cultivars (Beihong and Beifeng). This can be attributed to their high TSS value of more than 20 °Brix. The introduced Chinese table grapes (Jingyan and Jingxiangyu) had no significant difference in regards to sweetness and therefore, their preference was equal. In regards to sourness, Beihong was the sourest while Jingyan was the least sour. The results concur with the overall acceptability where Beihong was the least acceptable cultivar and Jingyan was most acceptable among all the cultivars evaluated. The sensory quality of grapes greatly depend on the composition and content of acids and sugars and these properties are important factors when selecting new cultivars (Liu et al., 2007). Jingyan was the most preferred cultivar in regards to crispness while Beihong and Beifeng were the least preferred. The results concur with the evaluated morphological traits of berry firmness of fresh for this study where Jingyan was characterized as slightly firm while Beifeng and Beihong as soft (Table 2). Crispness is a major sensory quality characteristic of table grapes according to consumer preference and cultivars with crisp flesh texture are highly considered for table grape breeding (Sato et al., 2006; Sato and Yamada, 2003). The flavour of French hybrid wine grape cultivars was most preferred as compared to the introduced Chinese wine grape cultivars. The introduced Chinese table grape cultivars rated equally with the French hybrid wine grape cultivars in reference to the flavour. Flavour is one of the most distinct qualities for maintaining a continuous consumer preference in the fresh fruit market of which table grapes must possess (Muñoz-Robredo et al., 2012; Baldwin, 2002). Therefore, French hybrid wine grapes can be considered for the fresh fruit market together with the introduced Chinese table grape cultivars. In reference to the toughness of skin, Jingyan and Jingxiangyu were the most preferred cultivars and Beifeng was the least preferred. Jingyan stood out to be the most preferred cultivar for fruit colour.

## Conclusion

This study revealed that Jingyan, Jingxiangyu, Beifeng, Beihong, Chenin Blanc and Sauvignon Blanc had adapted well to greenhouse conditions in Kenya. This is because they were productive under greenhouse conditions, unlike Jingcui and Cabernet Sauvignon which remained vegetative over the entire season. Based on the findings of this study, Jingyan, Jingxiangyu, Beifeng Beihong, Chenin Blanc and Sauvignon Blanc grape cultivars had superior morphological and fruit quality characteristics. All the introduced wine cultivars had ideal quality characteristics for winemaking while the quality characteristics of Jingyan, Jingxiangyu, Chenin Blanc and Sauvignon Blanc revealed that these cultivars are suitable as desserts. These findings will be useful for breeders in the selection of best-performing cultivars for commercialization and/or further research based on high yields and fruit qualities. More research is needed to evaluate the factors hindering Jingcui and Cabernet Sauvignon productivity under greenhouse conditions in Kenya.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Multi environment and spatial analysis of early maturing sorghum [*Sorghum bicolor* (L.) Moench] genotypes in dry lowland areas of Ethiopia

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**In Ethiopia, drought usually occur due to delay in onset, dry spell after sowing, drought during critical crop stage (flowering and grain filling stage) and too early cessation of rainfall. These situations can be addressed by developing improved sorghum varieties which are resistance to drought. Developments of sorghum varieties resistant to drought and producing better grain yield while addressing the plant biomass requirement is one of the strategies in the sorghum breeding program in dry lowland environment. A total of 90 early maturing sorghum genotypes were evaluated along with two standard check varieties to estimate the grain yield, plant height, days to flowering, days to maturity and overall agronomic aspects and stability of performance across the test environments. The trial was conducted using Randomized Complete Block Design (RCBD) in row and column arrangement. Linear mixed model has been used to predict and identify stable and superior varieties across the test environment. Correlations of the trials range from positive +1 to -1 where positive correlation is an indication of similarity among the testing environments while negative correlation is an indication of non-similarity among testing environments. Moreover, using the biplot it was observed that the stability and correlation among testing site where the angle between the two lines measure the strength of correlation. Improvement in heritability has been obtained due to spatial variation using advanced statistical analysis methods without any additional cost. Three genotypes exhibited better yield advantage, higher plant biomass and overall plant aspect including drought tolerance. In addition, these genotypes were preferred by farmers in their overall agronomic desirability (drought tolerance, earliness, head exertion and compactness, grain size and shape and threshability. Also, the national variety releasing committed has evaluated the variety verification trial both on station and farmers' field condition in 2018/2019 and they decided the release of the candidate variety 14MWLSDT7114 (2005MI5060/E-36-1) for commercial production in dry lowland environment.**

**Key words:** Genotype, heritability, mixed model, Spatial analysis, GEI, correlated environment.

## INTRODUCTION

Sorghum is the second most widely cultivated cereal in sub-Saharan Africa following maize (FAO, 2012). Over 23 million hectares of land in the continent is allocated for sorghum production annually with the total annual grain volume of about 26 million tones. It is believed to have originated in Ethiopia as evidenced by the early history of domestication of the sorghum crop there. Ethiopia is the third largest producer of sorghum in Africa after Nigeria and Sudan. Sorghum is the most important cereal crop worldwide used for food, feed, production of alcoholic beverages, and biofuel. Sorghum is primarily grown as a food grain crop in Ethiopia and preferred next to Teff for its *injera* (leaven bread) making quality. However, the biomass produced from sorghum is equally important for sorghum growing farmers in Ethiopia in order to address the feed demand (Amare et al., 2019).

It is the third most important cereal crop area coverage, which share 18% of the area covered by cereals and 14.6% of the area covered by grain crops. The total production of sorghum is 5.1 million tons produced from 1.9 million ha of land and with the national average productivity of 2.71 tons per hectare (CSA, 2018). The overall production and productivity of sorghum have showed an increasing trend over the past decade. A small improvement in productivity of sorghum has the potential to transform rural livelihoods and also boost the national economy. Most of the sorghum acreages in Africa including Ethiopia are located in areas that are prone to high temperature and frequent drought stress. Drought stress caused by low and erratic rainfall and exasperated by high temperature common in most sorghum growing regions of the world, is the most important abiotic factor limiting sorghum productivity.

In Ethiopia, drought is usually occurring due to delay in onset, dry spell after sowing, drought during critical crop stage (flowering and grain filling stage) and too early cessation of rain. These situations can be overcome by developing improved sorghum varieties which are tolerant to drought. Since the inception of sorghum research in Ethiopia concerted, efforts have been made to realize a strong research program that could be able to develop varieties with high drought tolerance, widely adapted, high yielding, early maturing and striga resistance with multiple resistance traits to address major biotic and abiotic factors.

However, until recently the breeding program relied on exotic germplasm which had a high harvest index compared to local landraces that led to a low adoption rate because of a number of factors such as poor grain market, farmers' interest in multi-purpose and high biomass cultivars. Hence, the notion of client-oriented breeding to increase adoption of improved technologies and enhancing genetic gain through breeding is timely agenda for sorghum breeders. Taking these into account modification of the breeding program is undergoing to increase efficiency and bring sustainable impact on the research and development endeavors.

Development and deployment of high yielding, early maturing, drought tolerant and striga resistant varieties with improved nutrition has been the major strategies for the national sorghum breeding program in Ethiopia. The recent breeding pipelines will produce varieties that are more acceptable for farmers due to higher grain yield, good grain quality and acceptable biomass production while providing much greater stability of performance than currently cultivated landraces and improved varieties. Ethiopian sorghum is a great source of novel genes and valuable traits for improving the sorghum crop worldwide.

Exploitation of genetic diversity is the most important strategies for plant breeding, and this must be inferred by field performance expression of the phenotype. The consequences of the phenotypic variation depend largely on the environment. This variation is further complicated by the fact that not all genotypes react similar ways to the change in environment. Genotype by environment interactions (GEI) happens when two or more genotypes perform differently in more than two environments. The different response of genotypes across the testing environment is considered as a hindrance in identifying, selecting and recommending of crops (Taye et al., 2016). Use of appropriate design and analysis model could be very vital either to identify high performing genotypes for target environments or stable genotypes across a given set of environments. The stage of plant development in which drought stress is most severe determines the associated yield reduction in the crop. Yield reduction is the most severe among the stress damages when the plant sink capacity is being set, and pollination is disrupted and embryos are aborted (Westgate and Boyer, 1985). Breeding for drought tolerance requires careful selection of the target environment; the choice of selection environment is important to achieve high genetic gain from selection (Cooper et al., 2006).

Different statistical methods for the analysis of multi-environment trial (MET) data have been used for crop improvement programs. The aim of crop improvement is most often to select either high performing genotypes for target environments or stable genotypes across a given set of environments. MET is usually analyzed using a two-stage approach, in which variety means are first estimated separately for each trial and then combined to

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form the data for an overall analysis. The latter methods include mixed effect models (Talbot, 1984) and the fixed effects additive main effects and multiplicative interactions (AMMI) model (Welham et al., 2010). The two-stage approach is an approximation of the combined analysis of the raw plot data from all trials. If there is error variance heterogeneity between trials and spatial variation or unequal replication within trials, the approximation may be poor in estimation by classical ANOVA. Smith et al. (2001a) presented a weighted mixed model for the second-stage analysis that aimed at accommodating these sources of error variation, thereby reducing efficiency losses. The superior approach, however, is the spatial MET analysis of Kelly et al. (2007), in which individual plot data are analyzed and a separate spatial covariance structure and error variance allowed for each trial. Therefore, the objective of this study was to know and quantify the magnitude of genotype by environment interaction (GEI), heritability and identify high yielding, early maturing, with high biomass and stable sorghum variety for commercial release.

## MATERIALS AND METHODS

A total of 90 sorghum genotypes including two standard checks Melkam and Dekeba which developed by pedigree breeding method were used in this study to evaluate their performance. The experiment was conducted at six locations which represented the dry lowland sorghum growing agro-ecology, namely: Miesso, Kobo, Shiraro, Humera, Erer and Mehoni Agricultural Research Centers in 2014, 2015 and 2016 main cropping seasons (Table 1).

### Description of genotypes evaluated in this variety trial

The genotypes were developed via pedigree breeding method at Melkassa Agricultural Research Center. The genotypes involved in this variety trial were developed through crossing and have pedigree selection have been done up to F5 and F6 generations based on grain yield component traits and plant height. Multi environment evaluation has been conducted from 2014 to 2016 targeting the dry lowland environments of Ethiopia. Based on grain yield performance, plant height and flowering time, three candidate varieties were proposed to be verified and released for growers (Table 2).

### Experimental design and field managements

Randomized Complete Block Design (RCBD) was used to laid out these variety trial with two replications in a row column arrangement to minimize the special variability (trends) in estimating the genetic value. Each plot contained two rows of 5 m length separated by 0.75 m. At all locations sowing was done in between last week of June to first week of July when enough rain was received. Plantation was done manually by drilling along the farrow, and population was adjusted by thinning considering 0.20 m as spacing between plants. DAP fertilizer was applied at planting time with the rate of 100 kg $ha^{-1}$ and urea was side dressed when the plant reached at knee height at 50 kg $ha^{-1}$ basis. Days to 50% flowering, plant height (cm), grain yield per plot (GY), days to 90% physiological maturity (DTM), plant aspect (PAS) data were collected and analyzed to identify stable and superior varieties compared with the standard check variety.

### Statistical analysis

Mixed effect models have been well developed over the past three decades, first with applications to animal breeding (Henderson, 1984) and then to other disciplines. Data analyses based on mixed models are readily done with the use of modern statistical software. Mixed-effects model contains experimental factors of both fixed and random-effect types, with appropriately different interpretations and analysis for the two types. So, the data was subjected to Linear Mixed Model (LMM) analysis to estimate the prediction (BLUPs) and Heritability based on different methods (RCBD, Spatial and Spatial + MET).

The estimation of variance components in mixed model assume Gaussian random terms by restricting maximum likelihood (REML); where REML procedure maximizes the joint likelihood of all error contrasts rather than of all contrasts as in ordinary maximum likelihood. In the original description of REML, Patterson and Thompson (1971) suggest that the score equation for the variance components may be solved iteratively using the Fisher scoring (FS) algorithm. For many applications, this strategy presents computational difficulties due to the large size of the matrices to be inverted and multiplied. Thompson (1977) presented an overview of the methodology with particular reference to animal breeding applications and showed how some of the computational burdens of the FS algorithm may be overcome.

### Spatial mixed model for MET trials

The experiment laid was down in a rectangular array of $j^{th}$ trials j= 1. . . p, consists of $N_j$, plots with $r_j$, rows and $c_j$ columns ($N_j = r_j \times c_j$) (Smith et al., 2001b). The vector of data $y_j^{(NjX1)}$ is ordered correspondingly as rows with in column. The model for the combined vector of data across environment (trials) $y^{(nx1)}$= {$y_j$}, n = $\sum_{j=1}^{p} N_j$ is given by:

$$y = X\tau + Zu + e \quad (1)$$

where $\tau^{(tx1)}$ and $u^{(bx1)}$ are vector of fixed and random effect respectively. $X^{(nxt)}$ and $U^{(bx1)}$ are the associated design matrices with the former assumed to be of full column ranks. The vector of residuals is given by e. Therefore, the distribution of the vector of data y is Gaussian with mean X$\tau$ and variance matrix H= $ZGZ' + R$.

Error e term also consists of a vector of sub error {$e_j$}, where $e_j^{(Njx1)}$ is vector of plot errors for $j^{th}$ trial and decomposed into a spatially dependent process $\xi_j$ and an independent white noise process $\eta_j$. The error variance matrix for trial j is given by $R_j = \sigma_j^2 \sum_j(\alpha j) + \sigma_{\eta j}^2 I_{Nj}$ , where $\Sigma_j$ is a spatial correlation matric that is a function of $\alpha_j$ with associated variance $\sigma_j^2$. The parameter $\sigma_{\eta j}^2$ is variance of the white noise process. The assumption is that spatial process for $\xi_j$ is second order stationary so that the correlation between plots depends only on the distance between them. It also further assumed that the two dimensional process is separable so one can write $\Sigma_j = \Sigma_{cj} \otimes \Sigma_{rj}$, where $\Sigma_{cj}$ and $\Sigma_{rj}$ are the correlation matrices for column and rows respectively. However, many researches (Gilmour et al., 1997) show that separable autoregressive process of order one which is denoted by AR1xAR1 most of often provide an adequate variance structure for local spatial trend. In addition, errors from different trials are assumed to be independent.

The random effect u consists of sub vectors {$u_i$} , where $u_i^{(bix1)}$ is the vector of effect for the $i^{th}$ random term, i=1. . .q. the matrix Z is

**Table 1.** Description of testing locations.

| Site | Longitude | Latitude | Altitude in m.a.s.l | Soil type | Rain fall in mm | Minimum T°C | Maximum T°C |
|---|---|---|---|---|---|---|---|
| Miesso | 39°21'E | 8°30'N | 1470 | Vertisol | 571 | 16 | 31 |
| Shiraro | 39°9'E | 14°6'N | 1179 | Vertisol | 615 | 20.4 | 34 |
| Kobo | 39°38'E | 12°09'N | 1513 | Vertisol | 678 | 14.8 | 32 |
| Mehoni (Fachagama) | 39°70'E | 12°70'N | 1578 | Clay | 539 | 12.81 | 23.24 |
| Humera | $40^0$9'E | $9^0$16'N | 750 | Vertisol and fluvic soil | 590 | 26.7 | 40.8 |
| Erer | $42^0$15'E | $9^0$10'N | 1297 | Vertisol | 778 | 17 | 37 |

**Source:** National Metrology data of 2016/17 cropping season, m.a.s.l=meters above sea level, T°=Temperature.

**Table 2.** List of genotypes evaluated in this variety trial.

| Genotype | Pedigree | Background |
|---|---|---|
| 14MWLSDT7026 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7029 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7031 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7033 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7034 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7035 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7036 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7040 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7042 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7060 | Macia/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7073 | SDSL2690-2/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7074 | SDSL2690-2/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7098 | MR812/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7100 | MR812/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7114 | 2005MI5060/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7115 | ICSR24010/B_35 | Crossed lines at Melkassa |
| 14MWLSDT7129 | ICSR24010/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7138 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7145 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7157 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7176 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7177 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7191 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7193 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7196 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7201 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7207 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7209 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7234 | Macia/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7238 | Macia/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7241 | Macia/E-36-1 | Crossed lines at Melkassa |

**Table 2.** Contd.

| | | |
|---|---|---|
| 14MWLSDT7251 | Macia/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7253 | Macia/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7278 | Macia/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7279 | Macia/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7308 | Teshale/B-35 | Crossed lines at Melkassa |
| 14MWLSDT7310 | Teshale/B-35 | Crossed lines at Melkassa |
| 14MWLSDT7311 | Teshale/B-35 | Crossed lines at Melkassa |
| 14MWLSDT7322 | SDSL2690-2/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7324 | SDSL2690-2/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7325 | SDSL2690-2/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7329 | SDSL2690-2/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7332 | SDSL2690-2/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7354 | MR812/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7356 | MR812/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7362 | 2005MI5060/B-35 | Crossed lines at Melkassa |
| 14MWLSDT7364 | 2005MI5060/B-35 | Crossed lines at Melkassa |
| 14MWLSDT7388 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7395 | MR812/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7400 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7401 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7402 | WSV387/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7405 | Macia/76T1#23 | Crossed lines at Melkassa |
| 14MWLSDT7410 | ICSR24010/B-35 | Crossed lines at Melkassa |
| 14MWLSDT7413 | WSV387/E-36-1 | Crossed lines at Melkassa |
| 14MWLSDT7425 | MR812/B-35 | Crossed lines at Melkassa |
| Dekeba | ICSR24004 | Standard Check |
| Melkam | WSV387 | Standard Check |

partitioned conformably as [Z1 . . .Zq]. It assumed that the sub vector of u is mutually independent. Variance matrix $G_i$ for the $i^{th}$ random term has many possible forms including the standard variance component structure $G_i = \sigma_i{}^2 I_{bi}$. Let $u_g$ be the mpx1 vector of genetic effect for m varieties for each p environments ordered as varieties with in environments. It represents two-dimensional (varieties by environment) array of effect, namely $U_g{}^{(mxp)}$, where $u_g = vec(U_g)$. It is assumed that the associated variance structure has a separable form with $var(u_g) = G_e \otimes G_v$, where $G_e$ and $G_v$ are the symmetric *p x p* and *m x m* component matrices for environment and varieties, respectively. When $G_v = I_m$, just for simplicity, therefore $var(u_g) = G_e \otimes I_m$, and the matrix $G_e = \{\sigma_{jj'}\}$ is the so called genetic variance matrix. The diagonal elements are genetic variance for individual environments and the off –diagonal elements are genetic covariance between pairs of environments. The spatial mixed model for the above model 1 of MET data can then be written as:

$$y = X_\tau + Z_u + e = X_\tau + Z_0 u_0 + Z_a u_a + e, \quad (2)$$

the fixed effect $\tau$ includes environmental main effects and trial specific effects for extraneous field variation (Gilmour et al., 1997), $u_g$ is variety effects at each environment with associated design matrix $Z_g{}^{(nxmp)}$ and $u_0$ comprise and additional random effect with design matrix $Z_0$, and variance matrix $G_0$.

In breeding program, there are many possible forms of genetic variance matrix structures. In mixed model of MET data, the standard structure is given by $G_e = \sigma_v{}^2 J_p + \sigma_{ve}{}^2 I_p$, where $\sigma_v{}^2$ and $\sigma_{ve}{}^2$ are the variance components for variety main effects and *V x E* interactions respectively, where as $J_p$ is a *p x p* matric of one. This implies that all environments have the same genetic variance and all pair of environments have the same genetic covariance. Due to inefficient estimation or unstable even for moderately large values of *p*. Smith et al. (2001b) proposes an alternative variance structure model which is called factor analysis that is analogous of AMMI of Gauch (1988, 1992). This model captures the nature of heterogeneous variances and covariance that occur in most MET data. The factor analytic (FA) model is a regression-type model (*y=ax+b*), which can be fitted to an increasing number of dimensions k.

**Table 3.** Summary of early maturing variety trials.

| Trials | Row | Column | genotype | Mean grain yield (t ha$^{-1}$) | Genetic variance | Error variance |
|---|---|---|---|---|---|---|
| KB14PYTLSL | 14 | 40 | 433 | 3.17 | 2.87 | 0.34 |
| MS14PYTLSL | 14 | 40 | 433 | 2 | 0.32 | 0.181 |
| HM15SG2N02 | 30 | 6 | 90 | 1.27 | 1.69 | 0.346 |
| MS15SG2N02 | 30 | 6 | 90 | 1.48 | 0.44 | 0.131 |
| SH15SG2N02 | 30 | 6 | 90 | 2.46 | 1.09 | 0.361 |
| MH16SG2N02 | 30 | 6 | 90 | 2.46 | 0.98 | 1.133 |
| MS16SG2N02 | 30 | 6 | 90 | 2.56 | 0.28 | 0.232 |
| SH16SG2N02 | 30 | 6 | 90 | 2.19 | 0.61 | 0.628 |
| ER15SG2N02 | 30 | 6 | 90 | 1.13 | 0.15 | 0.029 |

Therefore, the model with variety main effects and k-factor analytic model for genotypes by environment interaction (GEI) is special case of (k+1) factor analytic model for variety effects at each environment. The concurrence of genotypes and populations between testing site was used to allow the trial series to be analyzed as a single MET as of each trial consisting similar hybrids, which is the current best practice method to analyze field trials for plant breeding programs.

## RESULTS AND DISCUSSION

Based on the linear mixed model analysis stable and superior varieties across the test environment has been predicted and identified (Tables 3 to 6 and Figures 1 to 4). Three genotypes exhibited better yield advantage, higher plant biomass and overall plant aspects including drought tolerance. Hence, the variety 14MWLSDT7114 (2005MI5060/E-36-1) had 21.2%, the variety 14MWLSDT7196 (WSV387/76T1#23) had 17.7% and 14MWLSDT7329 (SDSL2690-2/76T1#23) had 27.2% superiority in plant height. Mean grain yield performance of genotype 14MWLSDT7114 displayed 12.2%, genotype 14MWLSDT7196 and genotype 14MWLSDT7329 varieties had also 13.7 and 20.2% grain yield advantage compared to the standard check variety Dekeba and Melkam (Table 4). In addition to the grain yield advantage in comparison with the recently nationally released standard check variety, these three genotypes also showed stable grain yield performance across years and environments (Figure 3).

In this study three genotypes showed stable grain yield performance across years and across environments. In addition, these genotypes were preferred by farmers by their overall agronomic desirability (drought tolerance, earliness, head exertion and compactness, grain size and shape, threshability). The national variety releasing committed has been evaluated the variety verification trial (VVT) both on stations and farmers' field conditions in 2018/2019 and finally verified the release of 14MWLSDT7114 (2005MI5060/E-36-1) variety for production. The results of the summary statistics (Table 3) indicate that Kobo is the most yielder for overall genotypes in terms of grain yield and other yield parameters. In another way, Erer is less yielder when compared with all other environments in terms of yield trait.

Figure 1 indicates how early maturing sorghum lines are correlated over the environments. Correlation among the trials ranged from positive +1 to -1 where positive correlation is an indication of similarity among the testing environments while negative correlation indicates dissimilarity among testing environments. This implicates the selection for promising materials. When the correlations among environments are positive, the selection for the best material based on a given environment is similar to the selection for the best materials in the other environments, for example, KB14PYTLSL, MS15SG2N02, MS16SG2N02 and SH16SG2N02. On the other hand, when environments are negatively correlated, there is a rank change among the genotypes so that the best material selected based on a given environment shows less performance in other environments like SH16SG2N02, HM15SG2N02 and BB15SG2N02.

One can briefly observe the stability and correlation among testing sites in Figure 2 where the angle between the two lines measures the strength of correlation. Less angle between the two lines indicates existence of correlation most often if less than 90' but if the angle is just around 90' it indicates the independency among the environments. Furthermore, when the angle between the two lines is more than 90', this indicates the negative correlation between the two environments. The distance of the line from the center measures the stability of the line. Less distance from the center indicate stability while far distance from the center indicates instability of the environment. Similarly, the length of the arrows indicates that the discrimination of the trials. So, MS16SG2N02 is the most discriminating trials fallowed by KB14PYTSL and SH16SG2N02.

**Table 4.** Predictions of genotypes using linear mixed model for grain yield (t ha$^{-1}$).

| Genotype | ER15SG2N02 | HR15SG2N02 | KB14PYTLSL | MH16SG2N02 | MS14PYTLSL | MS15SG2N02 | MS16SG2N02 | SH15SG2N02 | SH16SG2N02 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| 14MWLSDT7026 | 1.32 | 1.71 | 3.98 | 3.49 | 2.54 | 1.85 | 2.99 | 2.45 | 3.33 | 2.63 |
| 14MWLSDT7029 | 1.19 | 1.45 | 3.61 | 3.23 | 2.24 | 1.64 | 2.83 | 2.56 | 2.18 | 2.33 |
| 14MWLSDT7031 | 1.15 | 1.49 | 3.85 | 3.64 | 1.99 | 1.84 | 2.96 | 3.40 | 2.92 | 2.58 |
| 14MWLSDT7033 | 1.14 | 1.38 | 3.42 | 2.89 | 2.16 | 1.53 | 2.61 | 2.62 | 2.35 | 2.23 |
| 14MWLSDT7034 | 1.27 | 2.36 | 3.89 | 4.06 | 2.39 | 1.81 | 2.99 | 2.75 | 2.19 | 2.64 |
| 14MWLSDT7035 | 1.24 | 1.34 | 3.79 | 3.72 | 2.32 | 1.75 | 2.73 | 2.73 | 3.03 | 2.52 |
| 14MWLSDT7036 | 1.29 | 1.66 | 4.04 | 4.07 | 2.39 | 1.91 | 3.07 | 2.81 | 2.80 | 2.67 |
| 14MWLSDT7040 | 1.18 | 1.27 | 3.46 | 2.96 | 2.26 | 1.54 | 2.63 | 2.29 | 2.25 | 2.20 |
| 14MWLSDT7042 | 1.22 | 1.32 | 3.60 | 3.09 | 2.37 | 1.62 | 2.76 | 2.31 | 3.12 | 2.38 |
| 14MWLSDT7060 | 1.09 | 1.30 | 3.36 | 2.15 | 1.99 | 1.51 | 2.86 | 2.62 | 2.13 | 2.11 |
| 14MWLSDT7073 | 1.06 | 1.07 | 2.97 | 3.31 | 2.07 | 1.24 | 2.12 | 2.19 | 1.49 | 1.95 |
| 14MWLSDT7074 | 1.18 | 1.46 | 3.47 | 2.31 | 2.26 | 1.54 | 2.72 | 2.48 | 2.48 | 2.21 |
| 14MWLSDT7098 | 1.30 | 1.39 | 3.50 | 1.81 | 2.71 | 1.50 | 2.45 | 1.87 | 2.54 | 2.12 |
| 14MWLSDT7100 | 1.20 | 1.64 | 3.77 | 2.67 | 2.19 | 1.76 | 2.82 | 2.93 | 2.83 | 2.42 |
| 14MWLSDT7114 | 0.93 | 1.22 | 3.28 | 2.11 | 1.46 | 1.53 | 2.73 | 3.19 | 3.31 | 2.20 |
| 14MWLSDT7115 | 1.19 | 1.24 | 3.32 | 1.66 | 2.37 | 1.43 | 2.57 | 2.15 | 2.44 | 2.04 |
| 14MWLSDT7129 | 1.09 | 0.83 | 2.64 | 1.63 | 2.33 | 0.98 | 1.85 | 1.49 | 1.16 | 1.56 |
| 14MWLSDT7138 | 1.20 | 1.40 | 3.60 | 2.48 | 2.30 | 1.63 | 2.92 | 2.74 | 1.94 | 2.24 |
| 14MWLSDT7145 | 1.32 | 1.27 | 3.42 | 1.99 | 2.81 | 1.44 | 2.43 | 1.67 | 1.93 | 2.03 |
| 14MWLSDT7157 | 1.17 | 1.31 | 3.27 | 1.23 | 2.35 | 1.40 | 2.58 | 2.25 | 1.71 | 1.92 |
| 14MWLSDT7176 | 1.32 | 1.40 | 3.82 | 3.70 | 2.63 | 1.73 | 2.58 | 2.42 | 2.68 | 2.48 |
| 14MWLSDT7177 | 1.15 | 1.39 | 3.43 | 2.53 | 2.18 | 1.53 | 2.66 | 2.42 | 1.76 | 2.12 |
| 14MWLSDT7191 | 0.95 | 0.76 | 2.54 | 2.67 | 1.88 | 0.98 | 1.85 | 2.06 | 1.88 | 1.73 |
| 14MWLSDT7193 | 1.05 | 1.23 | 3.35 | 2.84 | 1.86 | 1.52 | 2.59 | 2.99 | 2.42 | 2.21 |
| 14MWLSDT7196 | 1.31 | 1.80 | 4.02 | 2.69 | 2.47 | 1.88 | 3.09 | 2.64 | 3.45 | 2.59 |
| 14MWLSDT7201 | 1.16 | 1.49 | 3.68 | 2.72 | 2.09 | 1.71 | 3.18 | 2.91 | 2.19 | 2.35 |
| 14MWLSDT7207 | 1.25 | 1.55 | 3.99 | 4.16 | 2.27 | 1.89 | 2.99 | 3.07 | 3.10 | 2.70 |
| 14MWLSDT7209 | 1.14 | 1.17 | 3.12 | 1.91 | 2.29 | 1.31 | 2.38 | 2.06 | 1.30 | 1.85 |
| 14MWLSDT7234 | 1.30 | 1.41 | 3.95 | 3.48 | 2.47 | 1.84 | 3.15 | 2.71 | 2.75 | 2.56 |
| 14MWLSDT7238 | 1.16 | 1.35 | 3.34 | 2.45 | 2.27 | 1.46 | 2.56 | 2.30 | 1.83 | 2.08 |
| 14MWLSDT7241 | 0.93 | 0.77 | 2.70 | 1.73 | 1.72 | 1.10 | 2.36 | 2.41 | 1.02 | 1.64 |
| 14MWLSDT7251 | 1.19 | 1.17 | 3.28 | 1.86 | 2.39 | 1.40 | 2.37 | 2.20 | 1.94 | 1.98 |
| 14MWLSDT7253 | 1.20 | 0.95 | 2.85 | 1.57 | 2.63 | 1.08 | 2.04 | 1.37 | 1.61 | 1.70 |
| 14MWLSDT7278 | 1.18 | 1.40 | 3.50 | 2.19 | 2.26 | 1.57 | 2.59 | 2.61 | 2.26 | 2.17 |
| 14MWLSDT7279 | 1.23 | 1.58 | 3.58 | 3.51 | 2.42 | 1.59 | 2.70 | 2.17 | 2.43 | 2.36 |
| 14MWLSDT7308 | 1.00 | 1.06 | 3.23 | 3.59 | 1.73 | 1.45 | 2.72 | 2.87 | 2.46 | 2.24 |
| 14MWLSDT7310 | 1.24 | 1.20 | 3.43 | 1.76 | 2.53 | 1.48 | 2.57 | 1.90 | 2.64 | 2.08 |
| 14MWLSDT7311 | 1.07 | 1.06 | 3.19 | 2.39 | 2.02 | 1.39 | 2.32 | 2.70 | 2.70 | 2.09 |
| 14MWLSDT7322 | 1.01 | 1.10 | 3.18 | 1.71 | 1.80 | 1.41 | 2.75 | 2.89 | 1.64 | 1.95 |
| 14MWLSDT7324 | 1.28 | 1.51 | 3.79 | 2.74 | 2.49 | 1.73 | 2.85 | 2.45 | 2.26 | 2.35 |

**Table 4.** Contd.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14MWLSDT7325 | 1.12 | 1.14 | 3.23 | 1.77 | 2.16 | 1.40 | 2.55 | 2.35 | 1.92 | 1.96 |
| 14MWLSDT7329 | 1.46 | 1.89 | 4.40 | 5.17 | 2.85 | 2.09 | 3.32 | 2.65 | 3.64 | 3.05 |
| 14MWLSDT7332 | 1.26 | 1.63 | 3.99 | 4.96 | 2.33 | 1.88 | 3.01 | 3.01 | 2.87 | 2.77 |
| 14MWLSDT7354 | 1.15 | 1.42 | 3.53 | 2.95 | 2.14 | 1.60 | 2.71 | 2.69 | 2.67 | 2.32 |
| 14MWLSDT7356 | 1.31 | 1.54 | 3.79 | 4.00 | 2.58 | 1.71 | 2.78 | 2.21 | 3.09 | 2.56 |
| 14MWLSDT7362 | 1.29 | 1.25 | 3.44 | 1.50 | 2.68 | 1.47 | 2.69 | 1.94 | 1.94 | 2.02 |
| 14MWLSDT7364 | 1.25 | 1.51 | 3.81 | 2.22 | 2.37 | 1.76 | 2.93 | 2.71 | 3.41 | 2.44 |
| 14MWLSDT7388 | 1.22 | 1.40 | 3.64 | 3.74 | 2.32 | 1.65 | 2.77 | 2.58 | 1.65 | 2.33 |
| 14MWLSDT7395 | 1.20 | 1.46 | 3.72 | 2.05 | 2.24 | 1.71 | 2.80 | 2.90 | 3.07 | 2.35 |
| 14MWLSDT7400 | 1.24 | 1.42 | 3.64 | 3.76 | 2.42 | 1.64 | 2.74 | 2.27 | 2.57 | 2.41 |
| 14MWLSDT7401 | 1.28 | 1.67 | 3.92 | 4.42 | 2.43 | 1.83 | 2.80 | 2.73 | 2.31 | 2.60 |
| 14MWLSDT7402 | 0.92 | 0.69 | 2.53 | 1.53 | 1.78 | 0.98 | 1.92 | 2.24 | 1.50 | 1.57 |
| 14MWLSDT7405 | 1.10 | 1.29 | 3.38 | 2.16 | 2.02 | 1.51 | 2.74 | 2.77 | 2.09 | 2.12 |
| 14MWLSDT7410 | 1.20 | 1.41 | 3.57 | 2.38 | 2.28 | 1.61 | 2.91 | 2.54 | 2.46 | 2.26 |
| 14MWLSDT7413 | 1.36 | 1.51 | 4.04 | 2.63 | 2.67 | 1.87 | 3.08 | 2.48 | 2.91 | 2.51 |
| 14MWLSDT7425 | 1.14 | 1.21 | 3.28 | 1.81 | 2.20 | 1.42 | 2.51 | 2.26 | 2.29 | 2.01 |
| Dekeba | 1.29 | 1.33 | 3.51 | 3.16 | 2.66 | 1.52 | 2.53 | 1.90 | 2.16 | 2.23 |
| Melkam | 1.34 | 1.59 | 3.59 | 2.51 | 2.80 | 1.55 | 2.55 | 1.95 | 1.94 | 2.20 |
| Mean | 1.19 | 1.36 | 3.50 | 2.75 | 2.29 | 1.56 | 2.68 | 2.46 | 2.36 | 2.24 |

ER15SG2N02= Erer 2015 NVT, HR15SG2N02=Humera 2015 NVT, KB14PYTLSL=Kobo 2014 PYT, MH16SG2N02=Mehoni 2016 NVT, MS14PYTLSL=Miesso 2014 PYT, MS15SG2N02=Miesso 2015 NVT, MS16SG2N02=Miesso 2016 NVT, SH15SG2N02=Shiraro 2015 NVT, SH16SG2N02= Shiraro 2016 NVT.

**Table 5.** Genetic correlation among trials using early maturing sorghum varieties.

| Trials | KB14PYTLSL | MS14PYTLSL | HM15SG2N02 | MS15SG2N02 | SH15SG2N02 | MH16SG2N02 | MS16SG2N02 | SH16SG2N02 | ER15SG2N02 |
|---|---|---|---|---|---|---|---|---|---|
| KB14PYTLSL | 1 | 0.544 | 0.639 | 0.991 | 0.442 | 0.622 | 0.817 | 0.729 | 0.818 |
| MS14PYTLSL | 0.544 | 1 | 0.365 | 0.428 | -0.387 | 0.284 | 0.231 | 0.32 | 0.928 |
| HM15SG2N02 | 0.639 | 0.365 | 1 | 0.63 | 0.266 | 0.396 | 0.516 | 0.464 | 0.535 |
| MS15SG2N02 | 0.991 | 0.428 | 0.63 | 1 | 0.537 | 0.625 | 0.843 | 0.735 | 0.734 |
| SH15SG2N02 | 0.442 | -0.387 | 0.266 | 0.537 | 1 | 0.323 | 0.55 | 0.39 | -0.068 |
| MH16SG2N02 | 0.622 | 0.284 | 0.396 | 0.625 | 0.323 | 1 | 0.524 | 0.459 | 0.472 |
| MS16SG2N02 | 0.817 | 0.231 | 0.516 | 0.843 | 0.55 | 0.524 | 1 | 0.619 | 0.522 |
| SH16SG2N02 | 0.729 | 0.32 | 0.464 | 0.735 | 0.39 | 0.459 | 0.619 | 1 | 0.544 |
| BB15SG2N02 | 0.818 | 0.928 | 0.535 | 0.734 | -0.068 | 0.472 | 0.522 | 0.544 | 1 |

ER15SG2N02= Erer 2015 NVT, HR15SG2N02=Humera 2015 NVT, KB14PYTLSL=Kobo 2014 PYT, MH16SG2N02=Mehoni 2016 NVT, MS14PYTLSL=Miesso 2014 PYT, MS15SG2N02=Miesso 2015 NVT, MS16SG2N02=Miesso 2016 NVT, SH15SG2N02=Shiraro 2015 NVT, SH16SG2N02= Shiraro 2016 NVT.

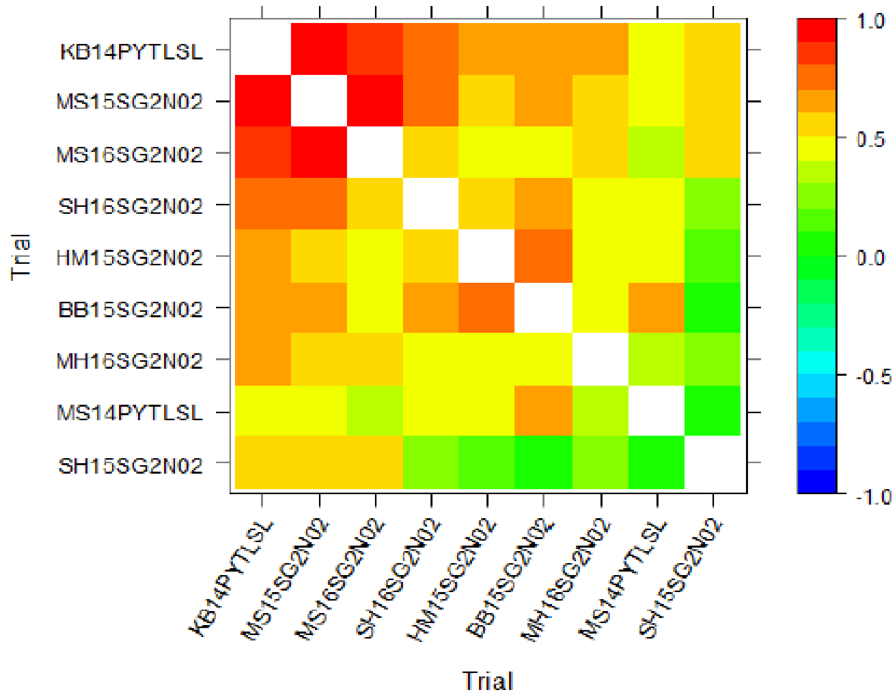


**Figure 1.** Genetic correlations amongst in early maturing sorghum testing environments.

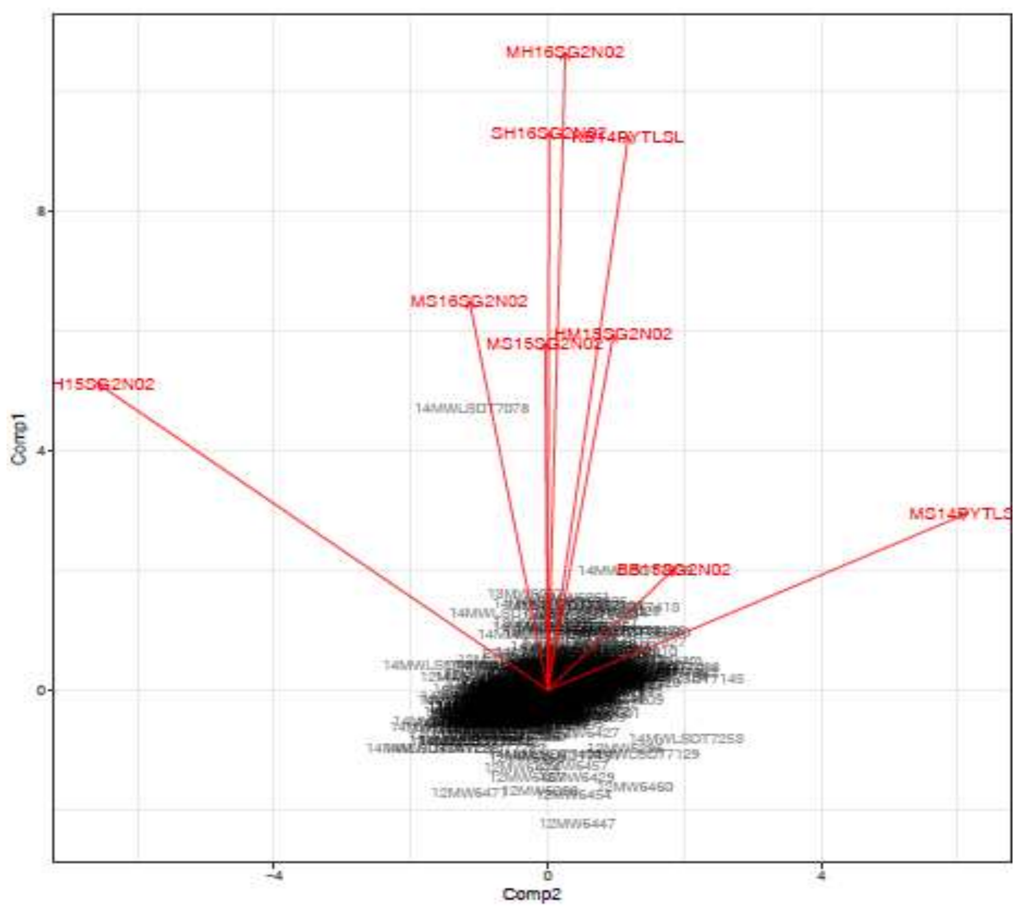


**Figure 2.** Correlation among testing environments using the angle between two lines.

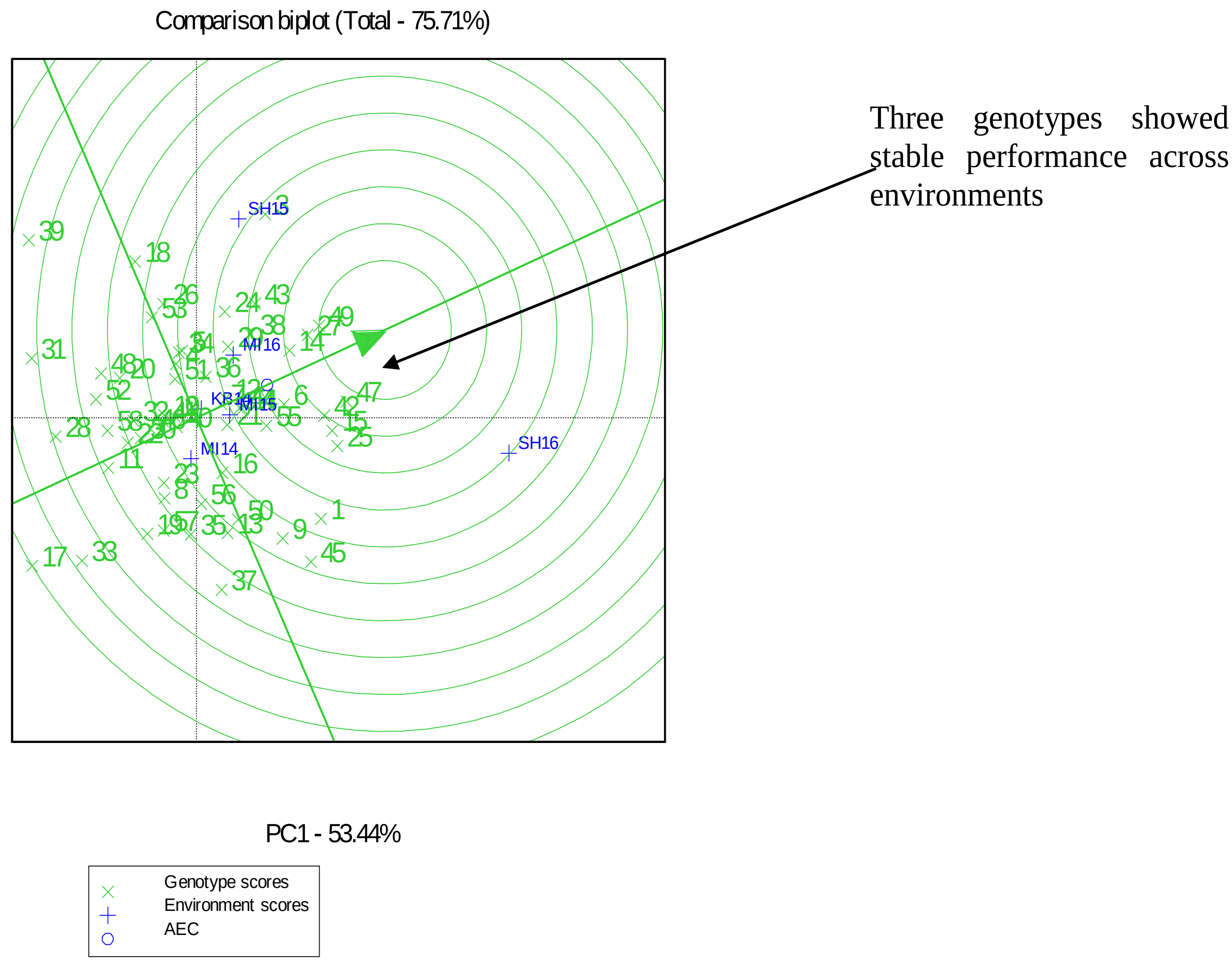


**Figure 3.** Comparison of grain yield performance among genotypes.

The comparison biplot was used to identify three superior and stable genotypes across the testing environments. Heritability is one objective in plant breeding; where high heritability indicates possible selection for targeted traits in breeding Hence, using advanced statistical models, we can increase the genetic gain. Figure 4 indicates improvements in heritability by the advanced statistical methods (Table 4).

## Conclusion

Three genotypes exhibited better yield advantage, higher plant biomass and overall plant traits including drought tolerance. The genotypes; 14MWLSDT7114 (2005MI5060/E-36-1) had 21.2%, 14MWLSDT7196 (WSV387/76T1#23) had 17.7% and 14MWLSDT7329 (SDSL2690-2/76T1#23) had 27.2% superiority in plant height. Mean grain yield performance of genotype 14MWLSDT7114 displayed 12.2%, genotype 14MWLSDT7196 and genotype 14MWLSDT7329 had also showed 13.7 and 20.2% grain yield advantage compared to the standard check variety Dekeba and Melkam. Correlation of the trial's ranges from positive + 0.928 to - 0.387 where positive correlation indicates the strength of their correlation (similarity) among the testing environments while negative correlation indicates dissimilarity among testing environments. One can briefly observe the stability and correlation among testing sites as shown in Figure 2 where the angle between the two line measures the strength of correlation and the length of the arrow showed us discriminability of the testing environments. Figure 3 indicates improvements in heritability by using advanced statistical methods (RCBD, Spatial, Spatial + MET).

From this study three genotypes were showed stable grain yield performance across years and across environments. In addition, these genotypes were preferred by farmers in their overall agronomic desirability (drought tolerance, earliness, head exertion and compactness, grain size, shape and threshability). The national variety releasing committed has been evaluated

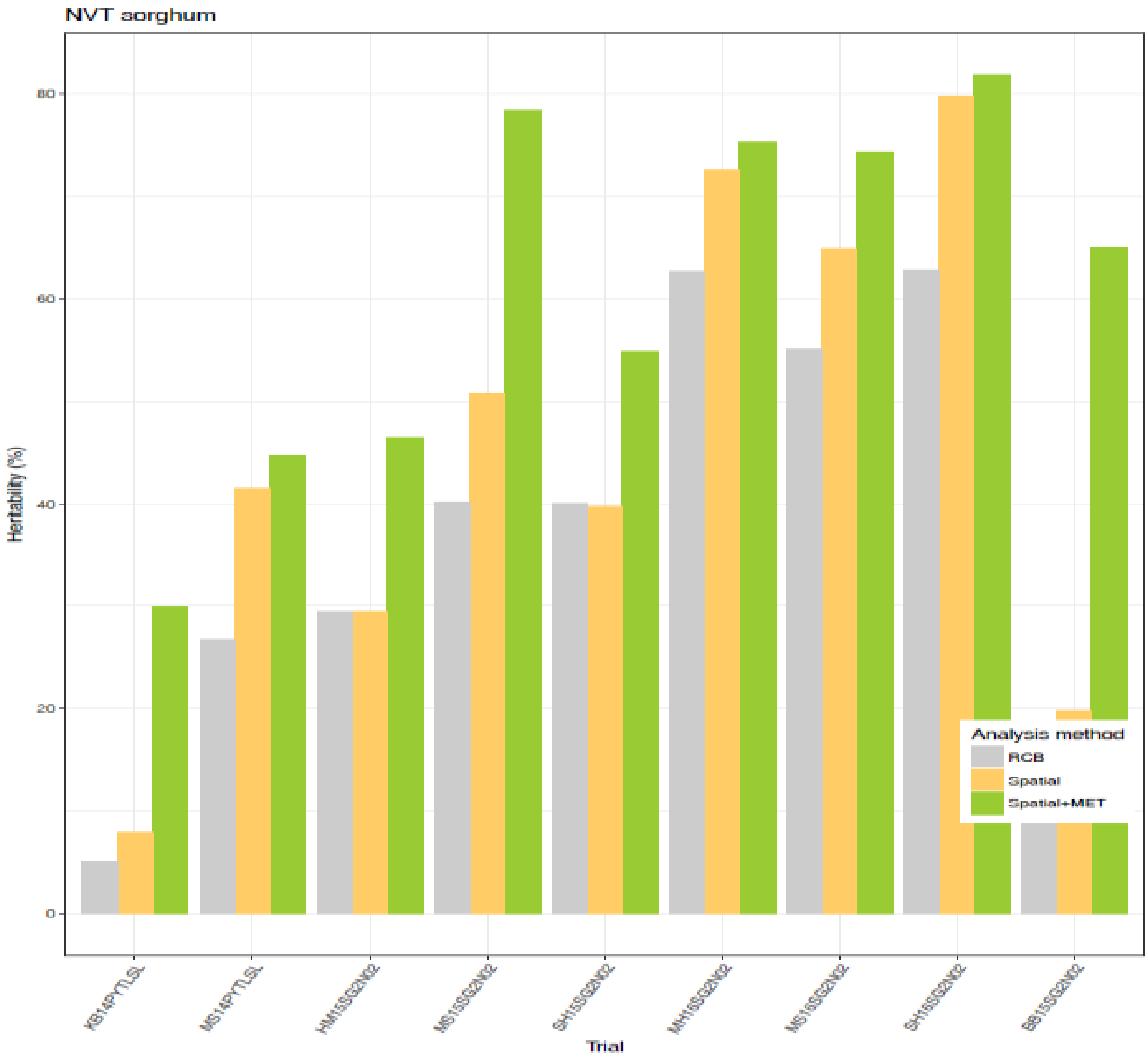


**Figure 4.** Improvements in heritability through applications of different statistical models.

**Table 6.** Predictions of top genotypes using linear mixed model for plant height (cm).

| S/N | Genotype | KB14 | MS14 | MS15 | MS16 | SH15 | SH16 | Mean |
|---|---|---|---|---|---|---|---|---|
| 1 | 14MWLSDT7026 | 195 | 195 | 161 | 169 | 213 | 222 | 192 |
| 2 | 14MWLSDT7029 | 224 | 182 | 170 | 189 | 224 | 218 | 201 |
| 3 | 14MWLSDT7031 | 190 | 187 | 167 | 182 | 242 | 217 | 198 |
| 4 | 14MWLSDT7033 | 175 | 191 | 167 | 184 | 218 | 222 | 193 |
| 5 | 14MWLSDT7034 | 187 | 192 | 159 | 184 | 239 | 215 | 196 |
| 6 | 14MWLSDT7035 | 182 | 184 | 174 | 186 | 260 | 228 | 202 |
| 7 | 14MWLSDT7036 | 191 | 187 | 166 | 193 | 229 | 235 | 200 |
| 8 | 14MWLSDT7040 | 194 | 189 | 164 | 191 | 236 | 212 | 198 |
| 9 | 14MWLSDT7042 | 191 | 199 | 171 | 195 | 241 | 227 | 204 |
| 10 | 14MWLSDT7060 | 178 | 179 | 151 | 153 | 203 | 188 | 175 |
| 11 | 14MWLSDT7073 | 198 | 176 | 160 | 166 | 234 | 217 | 192 |
| 12 | 14MWLSDT7074 | 179 | 173 | 149 | 165 | 224 | 206 | 183 |

**Table 6.** Contd.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13 | 14MWLSDT7098 | 218 | 216 | 161 | 183 | 238 | 208 | 204 |
| 14 | 14MWLSDT7100 | 247 | 203 | 174 | 186 | 251 | 234 | 216 |
| 15 | 14MWLSDT7114 | 187 | 193 | 149 | 166 | 224 | 209 | 188 |
| 16 | 14MWLSDT7115 | 189 | 192 | 152 | 175 | 252 | 220 | 197 |
| 17 | 14MWLSDT7129 | 183 | 206 | 168 | 189 | 239 | 211 | 199 |
| 18 | 14MWLSDT7138 | 198 | 194 | 172 | 177 | 239 | 216 | 199 |
| 19 | 14MWLSDT7145 | 194 | 194 | 165 | 190 | 239 | 243 | 204 |
| 20 | 14MWLSDT7157 | 221 | 198 | 161 | 174 | 235 | 234 | 204 |
| 21 | 14MWLSDT7176 | 181 | 160 | 214 | 162 | 246 | 212 | 196 |
| 22 | 14MWLSDT7177 | 195 | 168 | 200 | 164 | 198 | 198 | 187 |
| 23 | 14MWLSDT7191 | 171 | 183 | 159 | 168 | 223 | 198 | 184 |
| 24 | 14MWLSDT7193 | 186 | 182 | 147 | 155 | 210 | 196 | 179 |
| 25 | 14MWLSDT7196 | 184 | 172 | 158 | 155 | 226 | 194 | 181 |
| 26 | 14MWLSDT7201 | 192 | 181 | 154 | 188 | 246 | 213 | 196 |
| 27 | 14MWLSDT7207 | 197 | 178 | 166 | 175 | 240 | 228 | 197 |
| 28 | 14MWLSDT7209 | 188 | 182 | 164 | 179 | 224 | 220 | 193 |
| 29 | 14MWLSDT7234 | 190 | 183 | 169 | 173 | 233 | 180 | 188 |
| 30 | 14MWLSDT7238 | 51 | 183 | 166 | 180 | 228 | 228 | 173 |
| 31 | 14MWLSDT7241 | 198 | 176 | 156 | 182 | 238 | 215 | 194 |
| 32 | 14MWLSDT7251 | 204 | 182 | 178 | 181 | 255 | 237 | 206 |
| 33 | 14MWLSDT7253 | 174 | 195 | 166 | 182 | 237 | 226 | 197 |
| 34 | 14MWLSDT7278 | 197 | 181 | 155 | 161 | 214 | 204 | 185 |
| 35 | 14MWLSDT7279 | 201 | 195 | 169 | 177 | 277 | 210 | 205 |
| 36 | 14MWLSDT7308 | 275 | 199 | 179 | 205 | 278 | 272 | 235 |
| 37 | 14MWLSDT7310 | 221 | 220 | 191 | 200 | 256 | 235 | 220 |
| 38 | 14MWLSDT7311 | 219 | 198 | 166 | 214 | 279 | 253 | 222 |
| 39 | 14MWLSDT7322 | 231 | 184 | 175 | 196 | 292 | 245 | 221 |
| 40 | 14MWLSDT7324 | 234 | 223 | 192 | 215 | 277 | 259 | 233 |
| 41 | 14MWLSDT7325 | 215 | 192 | 182 | 195 | 279 | 246 | 218 |
| 42 | 14MWLSDT7329 | 197 | 183 | 163 | 169 | 257 | 209 | 196 |
| 43 | 14MWLSDT7332 | 179 | 167 | 147 | 158 | 212 | 196 | 176 |
| 44 | 14MWLSDT7354 | 173 | 154 | 153 | 163 | 190 | 192 | 171 |
| 45 | 14MWLSDT7356 | 173 | 180 | 155 | 159 | 212 | 199 | 180 |
| 46 | 14MWLSDT7362 | 192 | 175 | 157 | 173 | 218 | 214 | 188 |
| 47 | 14MWLSDT7364 | 196 | 191 | 167 | 174 | 250 | 230 | 202 |
| 48 | 14MWLSDT7388 | 196 | 182 | 163 | 167 | 223 | 214 | 191 |
| 49 | 14MWLSDT7395 | 176 | 164 | 157 | 161 | 216 | 206 | 180 |
| 50 | 14MWLSDT7400 | 200 | 179 | 164 | 170 | 221 | 208 | 190 |
| 51 | 14MWLSDT7401 | 184 | 182 | 161 | 170 | 228 | 220 | 191 |
| 52 | 14MWLSDT7402 | 256 | 219 | 175 | 181 | 290 | 276 | 233 |
| 53 | 14MWLSDT7405 | 194 | 182 | 165 | 189 | 236 | 204 | 195 |
| 54 | 14MWLSDT7410 | 203 | 205 | 170 | 181 | 236 | 217 | 202 |
| 55 | 14MWLSDT7413 | 190 | 184 | 164 | 154 | 232 | 203 | 188 |
| 56 | 14MWLSDT7425 | 188 | 182 | 156 | 190 | 244 | 225 | 198 |
| 57 | Dekeba | 135 | 149 | 136 | 141 | 186 | 177 | 154 |
| 58 | Melkam | 163 | 171 | 153 | 145 | 182 | 181 | 166 |
| | Mean | 193 | 186 | 165 | 177 | 236 | 218 | 196 |

KB14= Kobo 2014, MS14= Miesso 2014, MS15= Miesso 2015, MS16= Miesso 2016, SH15= Shiraro 2015, SH16= Shiraro 2016.

the VVT both on stations and farmers' field conditions in 2018/2019 and finally verified the release of the variety

14MWLSDT7114 (2005MI5060/E-36-1) for commercial production.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Characterization of wheat leaf rust pathogen (*Puccinia triticina*) in some parts of Ethiopia and seedling evaluation of durum wheat (*Triticum turgidum*) cultivars to the pathogen

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**Leaf rust caused by the pathogen *Puccinia triticina* is one of the important diseases of wheat in Ethiopia; and yield loss due to this disease has reached up to 75%. A study was carried out to detect physiologic races of the pathogen from some parts of Bale, Gondar and Shewa and to identify seedling resistance of Ethiopian durum cultivars. Twenty-four mono pustule isolates were made, and out of those, only three *P. triticina* races namely; BBBB, BBBN and BBBQ were identified. Race BBBN was detected for the first time in Ethiopia and was less virulent to commercial durum wheat cultivars than BBBQ and BBBB, while phenotype BBBB was relatively highly virulent to Ethiopian durum wheat and it infect 42.5% of the tested cultivars and is not virulent to Thatcher and Thatcher isoline leaf rust differential cultivars. Thirty six commercial durum wheat cultivars and three land race cultivars were evaluated against those three leaf rust pathogen races at seedling stage in the green house at Debre-Zeit Agricultural Research Center. Varieties Utuba, Lelisa, Worer, Bekelcha, Tate, Filakit, Foka, Alem tena, Hitosa, Kilinto, Denbi, Ude, LD-357, Boohai, Mukuye, Robe, Mangudo and Assasa exhibited resistance reaction (; to 2) to all the three leaf rust races, while the rest showed susceptible at least to one of the race. All the three land race cultivars (Mcd4-12, Mcd4-32 and Mcd7-42) were susceptible to all pathogen races.**

**Key words:** Durum wheat, leaf rust, *Puccinia triticina*, race, seedling resistance.

## INTRODUCTION

Wheat is a worldwide staple food crop that provides 20% of protein and calories for human consumption (FAO, 2015), and its demands are progressively growing with the elevations of human population (Wageningen FSC, 2016); however, world wheat productivity is growing at 1% rate.

In Ethiopia, wheat is an important crop, where it represents 14% of caloric intake and considered as

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second most important food crop (FAO, 2015) in areas of cultivation of crops in Ethiopia (REAP, 2015). Currently, the rapid urbanizing sub-Saharan Africa, subjected to increase wheat consumption is about 38% at 2023 (Geraled et al., 2010) and this elevation in wheat demand in Ethiopia may be due to the emergence of many food processing industries (REAP, 2015).

The use of crop diversity is a key approach to improve productivity and achieve food security (David et al, 2001). Where, Ethiopian durum wheat landraces are diverse and possess high variation that has not been exploited (Teklu and Hammer, 2008). Ethiopian landraces contributed to world wheat improvement for instance and the Ethiopian durum wheat landrace *ST464* was one of the major sources of *Sr13 that is, Ug99* resistance gene (Klindworth et al., 2007). On the other hand, more than 90 bread and 36 durum wheat varieties had been used for production since 1950s. In Ethiopia however, the national average yield is still 2.78 t/ha, which is lower than potential yields of 8 to 10 t/ha (CSA, 2017).

In general, the low productivity is mainly attributed to lack of durable resistant varieties to the prevalent wheat rusts. Hence, leaf rust caused by *Puccinia triticina* Eriks, is one of the most important diseases in Ethiopia (Ayele et al., 2008) where the production loss due to leaf rust reached 70-75% in susceptible wheat varieties at hot spot areas (Ayele et al., 2008; Mengistu et al., 1991; Draz et al., 2015; Ordonez et al., 2010; Shimelis and Pretorius, 2005). During 2007-2009 cropping seasons, incidence of 30.2% was recorded for leaf rust in Oromia, Amhara South, and Tigray regions. The average prevalence of leaf rust for the above mentioned locations was 53.3% (Tesfay et al., 2016).

Recently in Tigray region, 22 races of *P. tiriticina* were identified, among them PHTT and PHRT were dominant, but the broadest virulence spectrum was recorded from TKTT race, making all Lr genes ineffective except Lr 9. While the evaluation of these varieties, *Mekelle-3, Mekelle-4, Picaflor,* and *Dashin* were susceptible to TKTT, THTT, and PHTT races, *Mekelle-1 and Mekelle-2* were susceptible to races TKTT and THTT, but resistant to PHTT. But the *Digalu* was only susceptible to TKTT race. Unlike bread wheat varieties, the durum varieties, *Ude* and *Dembi* were resistant to these races (Tesfay et al., 2016). In general, most information on identification of *P. triticina* races were obsolete, and even the recent studies of race identification and identification of resistance wheat cultivars were limited to some parts of Tigray region and some bread wheat cultivars only.

In Ethiopia, wheat production used to be characterized by high biodiversity in crops and low input systems, and the management of wheat rusts largely relied on genetic host resistance (Sewalem et al., 2008); however, host resistance might not always be readily available in commercial cultivars. In addition, this requires regular and continuous search for finding a new source of resistant genes in the cultivated and wild forms of wheat.

Till date, most of the research works on wheat diseases focused on yellow rust and stem rust in Ethiopia, while, research on wheat leaf rust is limited. Therefore, characterization of *P. triticina* isolates and identification of resistance in durum wheat cultivars and landraces to the prevailing races would be essential for breeding and variety deployment strategies in the country.

## MATERIALS AND METHODS

### Collection of wheat leaf rust samples

A total of 24 leaf rust infected samples were collected from east Shewa (9), North Gondar (5) and Bale (10) zones in Ethiopia, respectively. The infected wheat fields were assessed along the main roads and accessible routes in each survey districts at 5-10 km interval based on vehicle odometers and each field assessed in X patterns, and all necessary information like location, variety name and GPS data were taken. Four to five freshly infected young leaves were taken using sterile scissors and placed in glassine bags; the infected leaf samples were transported to a laboratory at Debre Zeit Agricultural Research Center under dry conditions and then allowed to dry at room temperature for 24 h. Spores from each sample collected using motorized pump in a gelatin capsule and then stored at 4°C under dry conditions for a while.

### Isolation and multiplication of *P. triticina* inoculum

The bulked spores from infected dried leaf rust samples were collected in a gelatin capsule using a vacuum pump; then, the spores were suspended in light mineral oil (that is, soltrol 130) and sprayed on seven days old seedlings of susceptible durum wheat cultivar *RL6089* using motorized pump. The plants were then misted with fine droplet of distilled water and incubated in a plastic dew chamber for 24 h at 18-22°C with close to 100% RH. Then, the seedlings were transferred to a greenhouse bench of temperature 18-25°C and RH of 70%. A portion of spore samples was kept in a refrigerator at 4°C as a backup.

Seven days after inoculation leaves containing a single fleck were selected from the base of a leaf. The remaining leaves within a pot removed using sterilized scissors. A single leaf with fleck was covered with cellophane bag and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunson, 2004). About 12-14 days after inoculation when the mono pustule was well developed, each mono pustule were collected in gelatin capsule using motorized vacuum pump. Spores harvested from a mono pustule were suspended with light weight mineral oil (soltrol 130) and inoculated on to seven days old seedlings of susceptible durum cultivars that is, RL6089. About 12-14 days after inoculation, the spores from each mono pustule were collected in separate gelatin capsule as indicated above and stored at 4°C to be used for inoculation of leaf rust differential lines. These procedures were repeated until sufficient amount of single spores produced for race analysis purpose. In this way, 24 mono pustule isolates were developed for race analysis.

### Inoculation of *P. triticina* isolates to wheat leaf rust differential lines

Five seeds from each 16 differential lines and susceptible check, *RL 6089* were grown in 4 cm x 4 cm diameter pots containing soil, sand and compost in 2:1:1 (v/v/v) ratio, respectively. A single pustule derived spores(approximately 3-5 mg of spores per ml) was suspended in light weight mineral oil (soltrol 130) and sprayed on to seven days old seedlings using motorized pump and then placed in dew chamber close to 100% RH at 18-22°C for 24 h. Then, seedlings in each pot were transferred to the greenhouse within a plastic cubicle to avoid contamination. The greenhouse temperature and humidity were maintained between 18-25°C, 60-70%, respectively.

#### Leaf rust assessment on differential lines and race designation

Twelve days after inoculation, disease scores (infection types) were taken using 0-4 scoring scale employed by Long and Kolmer (1989). Infection types were grouped into low and high reaction type; where low refers to resistance or incompatibility (infection type 0-2) and high refers to susceptible or compatibility (infection type 3-4). Accordingly, a virulence and virulence of isolates were determined by low (L) and high (H) infection types, respectively.

Physiological race designations were made as described by Long and Kolmer (1989); while the sixteen differential lines were grouped into four sets in the following order: Group1; Lr1, Lr2a, Lr2c, Lr3; group 2; Lr9, Lr16, Lr24, Lr26; group 3; Lr3ka, Lr11, Lr17, Lr30; and group 4; LrB, Lr10, Lr14a and Lr18. Each isolate assigned a four letter race code based on the reaction of differential lines (Long and Kolmer, 1989). For instance, low infection type (L) on the four hosts on a set was assigned with a letter B, while high infection type (H) in the four hosts was assigned with a letter T. Hence, if an isolate produces low infection type on the 16 differential hosts, the races were assigned with a four letter race code BBBB. In the same way, an isolate which produces a high infection type on the 16 wheat leaf rust differential hosts have a race code TTTT. If an isolate produces a low infection type on Lr9 but a high infection type on the remaining 15 differential hosts, the race is designated as TKTT. In such a way, the race nomenclatures were completed for all 24 isolates.

#### Seedling tests of durum cultivars and landraces

Three different *P. triticina* races were used to inoculate 36-durum wheat cultivars, three landraces lines and susceptible check. Similar procedures were applied as indicated above for seedling growth, inoculation, incubation and disease scoring. Data on infection types were recorded 12 days after inoculation using standard disease score scale 0-4 (Long and Kolmer, 1989). Resistance and susceptible cultivars and landraces were determined by low and high infection types for each isolates.

## RESULTS AND DISCUSSION

### Identification of *P. triticina* races in Bale, Gondar and Shewa

Three different virulent phenotypes (races) of *P. triticina* were found in three zones in Ethiopia (East Shewa, Bale and North Gondar) as described in Table 1. Races BBBQ, BBBN and BBBB were detected from Bale zone, while BBBB was identified from East Shewa and North Gondar zones as shown in Figure 1. Leaf rust race BBBQ was virulent only to the Thatcher lines with genes LrB and Lr10, whereas, leaf rust race BBBN was virulent only to the Thatcher (bread wheat) lines with LrB and Lr14a genes. Out of the three identified races, BBBB is the most frequent and predominant with a frequency of 91.6%, whereas the remaining two (BBBQ and BBBN) were detected once each with a frequency of 4.1%. Race BBBB is virulent to Thatcher line (bread wheat); thus, virulence to leaf rust resistance genes in the Thatcher iso-line series could not be determined for this phenotype. Phenotype BBBB is virulent on durum wheat, but virulent on Thatcher and other susceptible bread wheat cultivars and has not been detected elsewhere except Ethiopia, and temporarily named as ETH-BBBB race.

*P. triticina* isolates from Ethiopia that was virulent to durum wheat but not virulent to Thatcher wheat was previously noted (Roelfs et al., 1992; Ordonez and Kolmer, 2007b). Before a large cultivation of bread wheat in Ethiopia, ETH-BBBB isolate may be left over from *P. triticina* population that was adopted to Ethiopian durum wheat cultivars. If this isolate had been present in Ethiopian durum land race cultivars, it may have higher genetic variability than other recently introduced *P. triticina* isolates (Kolmer and Acevedo, 2016). Isolates of *P. triticina* with high virulence to durum wheat have recently been found and characterized in Mexico (Singh et al., 2004), France (Goyeau et al., 2011), Spain (Martinez et al., 2005), Italy (Mantovani et al., 2010), Israel and Turkey (Kolmer, 2009), Argentina (Ordonez and Kolmer, 2007b) and Ethiopia (Mengistu et al., 1991; Kolmer and Acevedo, 2016). On the other hand, the *BBBQ* was identified in Ethiopia as other isolates recovered from durum wheat in other countries. This phenotype was collected from emmer wheat and had virulence only to lines with genes *LrB* and *Lr10,* while, *BBBQ* was a virulent to many bread wheat cultivars; thus, availability of this isolate may be influenced by the presence of durum wheat (Kolmer and Acevedo, 2016).

Similar findings were reported as Altar C84 durum wheat which may become susceptible to durum leaf rust (BBBQ) in Mexico (Singh et al., 2004). Other study determined that Altar C84 carried Lr72 (Herrera-Foessel et al., 2014) and many other CIMMYT cultivars and durum germplasm from around the world were susceptible to this new virulence phenotype of *P. triticina.* Additional virulence variation to durum wheat cultivars may be present in the *BBBQ* isolate from Ethiopia and other countries Kolmer and Acevedo (2016); however, a differential set of durum wheat genotype will need to be developed to properly assess such isolates, because the Thatcher differentials do not detect much virulence variation in these isolates.

The isolates with phenotype *BBBN* was virulent on *LrB and Lr14a*. These isolates may have some virulence to bread wheat cultivars, because *BBBN* was isolated from bread wheat cultivar (Sofumer) collected in Bale zone. Phenotype *BBBN* previously detected in Mexico and other parts of the world are reported as virulent to *Lr72* (Singh et al., 2004), but detected for the first time in Ethiopia, however, further works and conformations

**Table 1.** Identified *P.triticina races* in some parts of Ethiopia during 2107.

| Race | Virulent | No. of isolate | Location |
|---|---|---|---|
| **BBBB** | - | 22 | Bale East-Shewa and North Gondar zones |
| **BBBQ** | LrB and Lr10 | 1 | Bale zone |
| **BBBN** | LrB and Lr14a | 1 | Bale zone |


**Figure 1.** The distributions of the *P. triticina* races across the assessed areas.

required.

In this study, only twenty-four isolates were collected from Oromia and Amhara regions of Ethiopia because of very low prevalence of wheat leaf rust, hence, number of characterized isolates was limited, and only three phenotypes identified. Also, Kolmer and Acevedo (2016) identified small amount of phenotypes from wide geographical area and large amount of collections. Conversely, in other populations of *P. triticina* collection even from relatively small geographical area large number of phenotypes was reported in Tigray, Ethiopia (Tesfay et al., 2016).

## Seedling tests of durum wheat cultivars and landraces to identified races

Seedlings of 36 commonly grown Ethiopian durum wheat varieties and some land race cultivars including susceptible check morocco were evaluated for the seedling resistance against phenotypes (*BBBB, BBBQ,* and *BBBN*) of leaf rust identified in the current study. Based on the reaction to the leaf rust phenotypes, cultivars were categorized into seven groups.

Group 1: Cultivars with seedling resistance to all leaf rust races; Utuba, Lelisa, worer, Bekelcha, Tate, Filakit, Foka, Alem tena, Hitosa, Kilinto, Denbi, Ude, LD-357, Boohai, Mukuye, Robe, Mangudo and Assasa exhibited resistance reaction (; to 2) to all the three leaf rust races while the rest 18 were susceptible at least to one race.

Group 2: Cultivars with seedling resistance to BBBB and BBBQ only; the landrace Mcd4-32 was resistance to BBBB and BBBQ.

Group 3: Cultivars with resistance to BBBB only; the land race Mcd7-42 was resistance to BBBB race only.

Group 4: Cultivars with resistance to BBBQ only; Mossobo and landrace line Mcd4-12 were resistance to BBBQ phenotype.

Group 5: Cultivars with seedling resistance to BBBQ and BBBN races; Tesfaye, Ejersa, Bichena, Oda, Ilani, Cocorit-71, Malefia and Kokate showed resistance reaction to BBBQ and BBBN phenotypes, but susceptible to BBBB.

Group 6: Cultivars with seedling resistance to only BBBN phenotype; Arendeto, Tob-66 and Obsa were resistance to BBBN only.

Group 7: Cultivars with no seedling resistance to all the three leaf rust races; Megenagna, Toltu and Selam showed susceptible reaction to all of the three races. However, Megenagna and Selam showed high level of field resistance under field condition (Habtamu, 2019).

Phenotype BBBN was less virulent on commercial durum wheat cultivars than BBBQ and BBBB. Only 20% of tested cultivars were infected by this race; however, this less virulent to durum cultivars might be because of its detection for the first time in Ethiopia and the isolate collected from bread wheat, thus, not well adapted the environment and not withstand the durum wheat cultivars. While phenotype BBBB was relatively highly virulent to Ethiopian durum wheat and it infect 42.5% of the tested cultivars, it is not virulent to Thatcher and Thatcher isoline leaf rust differential cultivars, and this findings is in agreement with the previous report by Kolmer and Acevedo (2016). In addition so far, this phenotype was not reported elsewhere except Ethiopia. Phenotype BBBQ have intermediate virulence, and it infect about 27.5% of the tested durum cultivars.

Megenagna and Selam showed resistance reaction under natural infections while susceptible reaction to all the three races at seedling stage in the greenhouse. Similarly, Mossobo and three landrace lines (Mcd4-12, Mcd4-32 and Mcd7-42) showed resistance reaction under field condition (Habtamu, 2019), but, susceptible reaction to at least one of the races at their seedling stage. This variation might be due to the varieties that may have genes responsible for adult plant resistance at field condition, but poorly expressed at their seedling stage in the green house. In addition, varieties in the green house were evaluated through inoculation of virulence races of leaf rust. However, varieties in the field have a chance to be infected with weak population of leaf rust. Hence, this circumstance might be the cause for the resistance variation of varieties in their adult and seedling stages. Such adult plant and seedling resistance variations within single variety were also previously reported (Tesfaye et al., 2016). According to Parlevliet (1988), wheat cultivars that exhibited intermediate (2+) and/or susceptible ($>3^-$ ) infection types at seedling stage may possess race non -specific or adult plant resistance, thus, cultivars Megenagna, Selam, Mossobo, Mcd 4-12, Mcd4-32 and Mcd7-42 may provide durable resistance because field assessment results confirmed their slow rusting characters (Habtamu, 2019). Bekelcha and Utuba showed high level of resistance in both seedling and adult plant stages, whereas, Boohai, Tate, Denbi, Worer, Mukuye and Mangudo demonstrated moderate level of resistance in both seedling and adult plant stages.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Food transition in the Gulf Cooperation Council Region

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**This study investigates (and forecasts) food consumption patterns in the Gulf Cooperation Council (GCC) region from 1961 to 2023. A vector auto-regressive model (VAR) was used to estimate systems of equations of per capita food consumption of major food items, and their trends over time, for the purpose of highlighting changes in the region's dietary patterns. The study area had shown symptoms of diet transition marked by a shift toward consuming more sugar and animal products, and fewer vegetables and cereals. These changes have been accompanied by substantial food waste and an increase in diet-related diseases. The region needs to develop policy programs that combine government action, research, education, and mass media communication programs to raise public awareness about the benefits of diet and exercise, and to promote more healthful eating patterns.**

**Key words:** Diet transition, food consumption, waste, Gulf Cooperation Council (GCC).

## INTRODUCTION

Eating patterns worldwide have witnessed a gradual process of "westernization" and evolution, a phenomenon called "food transition." Diets across countries are converging toward one characterized by a greater proportion of added sugar, fats, and animal products, and a lesser proportion of cereals and vegetables. The food chain is becoming increasingly complex with food products that are more processed, sophisticated, and ready-to-eat. A serious related problem is that of food loss and waste.

The Food and Agriculture Organization of the United Nations (FAO, 2012) has argued that food loss represents a significant cost to the world economy, and greatly jeopardizes global food security. Food loss contributes to food price escalation because it decreases market supply. In addition, it results in environmental degradation, as scarce resources are used to produce, process, handle, transport, and dispose wasted food. Thus, decreasing food loss and waste is an important element in mitigating hunger, raising income, and improving food security.

This study investigates (and forecasts) food consumption patterns in the Gulf Cooperation Council region (GCC) for the period 1961 to 2023. Food loss and waste, and diet-related health problems will also be discussed as associated phenomena. The study's main objective is to shed light on changes in diet patterns to induce policy changes to enhance food security and

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human health in the region. A secondary objective is to determine the degree of shortage of data and information regarding a number of variables on this topic; if these gaps were filled, it could facilitate the design and implementation of policies to enhance food systems' sustainability.

This study relies on the concept of "food transition" (also called "nutrition transition"), introduced by Popkin (1993) and Guyomard et al. (2012). Food transition is an overall change in food consumption patterns consisting of two main characteristics: The first is a rise in calorie intake, with proportionally equal increases in all food products. Once caloric saturation is achieved, the second step, called "diet transition," occurs, in which cereal and vegetable consumption decreases, and consumption of sugar, fats, and animal products increases. Developed countries achieved the second step of "food transition" within a century. Most other countries are now following a similar pattern but at a considerably accelerated rate. The duration of food transition has reduced to 20 years in emerging countries and 40 years in developing countries.

## METHODOLOGY

This study used the vector Auto-Regressive (VAR) model to estimate systems of equations of per capita consumption of major food items and their trends over time. Linear and quadratic forms were used. Hannan-Quinn (HQIC), Akaike (AIC and AICC), and Schwarz's Bayesian (SBIC) information criteria were determined, and their minimum values were used to indicate the optimum model. Appendix 1 provides the theoretical model, and a summary of estimation results of the system equations. The estimated equations were used to forecast the per capita consumption of each variable for the period 2014 to 2023.

The study used time series data of per capita food consumption of major food items, food loss and waste at distribution and consumption stages, and health indicators related to food intake. FAO (2018) data have been used. Weighted averages have been calculated for the GCC region, weighted according to each country's proportion of the total population. Other sources of data and information used in the study are specified at appropriate places throughout.

### Data limitations

The GCC region lacks time series data regarding food consumption and food waste. The FAO statistics database is the only source of data available. Moreover, most of the required food consumption data are not available for Bahrain, Oman, and Qatar in the FAO database. However, the total population of the other GCC countries included in the study accounted for 86% of the region's total population (GCC, 2014). Thus, it is reasonable to generalize this study's results to the entire region.

## RESULTS AND DISCUSSION

This section discusses food consumption patterns, food waste, and diet-related health problems prevailing in the GCC states.

### Food consumption in the GCC region

Since 1961, the GCC region has experienced a steady increase in food supply and consumption. The huge oil revenues that have been earned since the mid-20th century have enabled the region to maintain high levels of food imports. Per capita food consumption of the different food items will be discussed in detail, as follows.

### Consumption of animal products

This group of items has been divided into two sub-groups. This is because one of the VAR model's underlying assumptions is that the number of parameters should not exceed the number of observations.

### Group 1: Beef, mutton, poultry, fish and seafood

Table 1 in Appendix 1 provides estimates of the coefficients of current per capita consumption of beef $(Y_{1t})$, mutton $(Y_{2t})$, poultry $(Y_{3t})$, and fish and seafood $(Y_{4t})$, plus their standard errors, t statistics, and $p\,values$ (all the roots of $|\Phi(L)|=0$). The estimated system of equations (1) is provided below:

$$\underbrace{\begin{bmatrix} Y_{1t} \\ Y_{2t} \\ Y_{3t} \\ Y_{4t} \end{bmatrix}}_{\hat{\mathbf{Y}}_t} = \underbrace{\begin{bmatrix} -1.198 & 0.083 & -0.002 \\ 0.419 & 0.089 & -0.002 \\ -12.688 & 0.375 & 0.000 \\ 1.238 & -0.009 & 0.001 \end{bmatrix} \begin{bmatrix} 1 \\ t \\ t^2 \end{bmatrix}}_{\hat{\psi}\mathbf{Z}_t} + \underbrace{\begin{bmatrix} 0.516 & -0.025 & 0.014 & 0.475 \\ 0.003 & 0.771 & -0.021 & 0.166 \\ -0.435 & 1.604 & 0.439 & 0.786 \\ 0.111 & -0.043 & 0.005 & 0.754 \end{bmatrix} \begin{bmatrix} Y_{1t-1} \\ Y_{2t-1} \\ Y_{3t-1} \\ Y_{4t-1} \end{bmatrix}}_{\hat{\Phi}\mathbf{Y}_{t-1}}, \quad t = 2,3,4,\ldots,54 \qquad (1)$$

The eigenvalues of matrix $\hat{\Phi}(L)$ are $\lambda = (0.886\ 0.601\ 0.601\ 0.404)$. All eigenvalues are less than one, which means that all the roots of $|\hat{\Phi}(L)|=0$ fall outside the unit circle. When considering that the data have a quadratic trend, the time series of per capita consumption of these food items are thus stationary. By comparing the $p\,value$ $(pr>|T^*|)$ with a 0.05 significance level, the independent variables have significant effects on the dependent variables, as follows:

The first equation represents current per capita beef consumption. The quadratic trend term ($t^2$) has a significant negative effect on current beef consumption, whereas per capita consumption of beef and fish lagged

**Table 1.** Estimation results of beef, mutton, poultry, fish, and seafood consumption in the GCC region.

| Equation | Coefficients | $\hat{\beta}$ | $S.E_{\hat{\beta}}$ | $t$ | $(pr>\|T^*\|)$ | Ind.Var. |
|---|---|---|---|---|---|---|
| | $\beta_{10}=\psi_{10}$ | -1.198 | 1.369 | -0.880 | 0.3861 | 1 |
| | $\beta_{11}=\psi_{11}$ | 0.083 | 0.059 | 1.410 | 0.1662 | $t$ |
| | $\beta_{12}=\psi_{12}$ | -0.002 | 0.001 | -2.360 | 0.0228 | $t^2$ |
| $Y_{1t}$ Beef | $\beta_{13}=\phi_{11}$ | 0.516 | 0.137 | 3.760 | 0.0005 | $Y_{1t-1}$ |
| | $\beta_{14}=\phi_{12}$ | -0.025 | 0.198 | -0.130 | 0.8998 | $Y_{2t-1}$ |
| | $\beta_{15}=\phi_{13}$ | 0.014 | 0.048 | 0.290 | 0.7721 | $Y_{3t-1}$ |
| | $\beta_{16}=\phi_{14}$ | 0.475 | 0.156 | 3.050 | 0.0038 | $Y_{4t-1}$ |
| | $\beta_{20}=\psi_{20}$ | 0.419 | 0.808 | 0.520 | 0.6069 | 1 |
| | $\beta_{21}=\psi_{21}$ | 0.089 | 0.035 | 2.550 | 0.0141 | $t$ |
| | $\beta_{22}=\psi_{22}$ | -0.002 | 0.001 | -2.810 | 0.0074 | $t^2$ |
| $Y_{2t}$ Mutton | $\beta_{23}=\phi_{21}$ | 0.003 | 0.081 | 0.040 | 0.9678 | $Y_{1t-1}$ |
| | $\beta_{24}=\phi_{22}$ | 0.771 | 0.117 | 6.600 | 0.0001 | $Y_{2t-1}$ |
| | $\beta_{25}=\phi_{23}$ | -0.021 | 0.029 | -0.730 | 0.4706 | $Y_{3t-1}$ |
| | $\beta_{26}=\phi_{24}$ | 0.166 | 0.092 | 1.810 | 0.0775 | $Y_{4t-1}$ |
| | $\beta_{30}=\psi_{30}$ | -12.688 | 3.483 | -3.640 | 0.0007 | 1 |
| | $\beta_{31}=\psi_{31}$ | 0.375 | 0.150 | 2.500 | 0.0161 | $t$ |
| | $\beta_{32}=\psi_{32}$ | 0.000 | 0.003 | 0.130 | 0.8967 | $t^2$ |
| $Y_{3t}$ Poultry | $\beta_{33}=\phi_{31}$ | -0.435 | 0.349 | -1.240 | 0.2196 | $Y_{1t-1}$ |
| | $\beta_{34}=\phi_{32}$ | 1.604 | 0.504 | 3.180 | 0.0026 | $Y_{2t-1}$ |
| | $\beta_{35}=\phi_{33}$ | 0.439 | 0.123 | 3.570 | 0.0009 | $Y_{3t-1}$ |
| | $\beta_{36}=\phi_{34}$ | 0.786 | 0.396 | 1.980 | 0.0534 | $Y_{4t-1}$ |
| | $\beta_{40}=\psi_{40}$ | 1.238 | 1.207 | 1.030 | 0.3106 | 1 |
| | $\beta_{41}=\psi_{41}$ | -0.009 | 0.052 | -0.180 | 0.8617 | $t$ |
| | $\beta_{42}=\psi_{42}$ | 0.001 | 0.001 | 0.850 | 0.3972 | $t^2$ |
| $Y_{4t}$ Fish and seafood | $\beta_{43}=\phi_{41}$ | 0.111 | 0.121 | 0.910 | 0.3654 | $Y_{1t-1}$ |
| | $\beta_{44}=\phi_{42}$ | -0.043 | 0.175 | -0.250 | 0.8070 | $Y_{2t-1}$ |
| | $\beta_{45}=\phi_{43}$ | 0.005 | 0.043 | 0.120 | 0.9057 | $Y_{3t-1}$ |
| | $\beta_{46}=\phi_{44}$ | 0.754 | 0.137 | 5.480 | 0.0001 | $Y_{4t-1}$ |

one period $(Y_{1,t-1}, Y_{4,t-1})$ has a significant positive effect on it. Per capita consumption of beef has increased considerably in the study period.

The second equation represents current per capita mutton consumption. The trend (t), and per capita consumption of both mutton and fish lagged one period $(Y_{2,t-1}, Y_{4,t-1})$ have significant positive effects on the current consumption of mutton, whereas the quadratic trend term ($t^2$) has a negative effect on it. Per capita consumption of mutton meat has increased considerably in the study period.

The third equation represents current per capita poultry

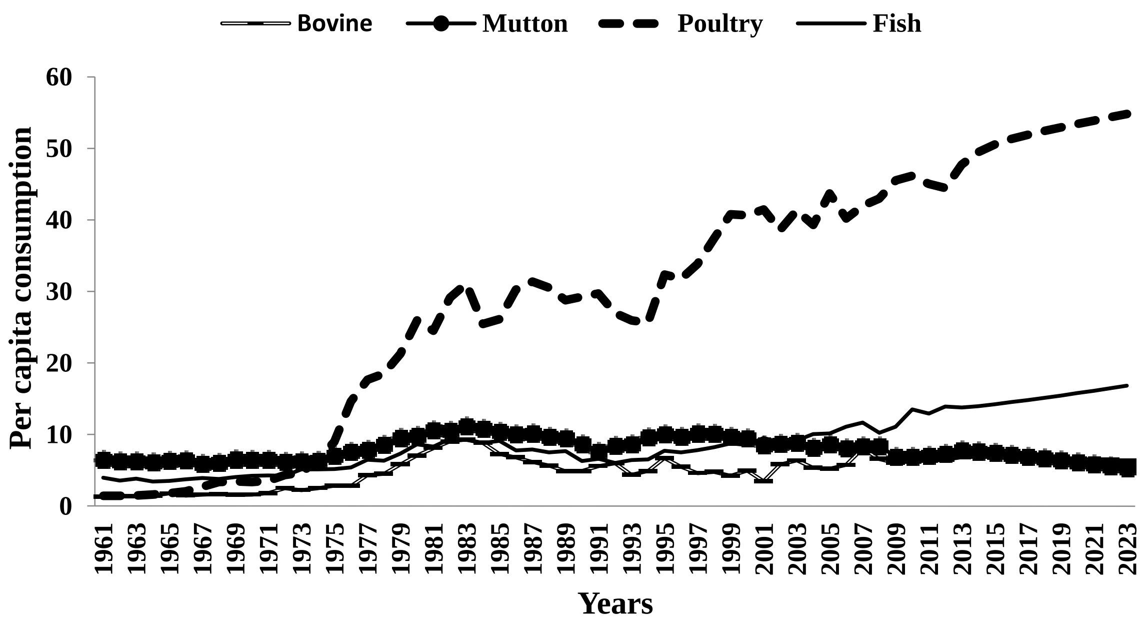


**Figure 1.** Real and predicted per capita consumption of beef, mutton, poultry, fish, and seafood in the GCC region from 1961 to 2023 (kg/capita/year).
Data source: Food and Agriculture Organization,(1b) and the predicted values: estimated model.

consumption. The constant term has a negative effect on current poultry consumption, whereas the linear trend (t) and per capita consumption of mutton, poultry, and fish lagged one period $(Y_{2,t-1}, Y_{3,t-1}, Y_{4,t-1})$ have significant positive effect on it. Per capita poultry consumption is dramatically increasing over time. From an average of 6.3 kg from 1961 to 1964, it has increased to 45.8 kg from 2009 to 2013. It is expected to reach 54.9 kg in 2023.

The fourth equation represents current per capita consumption of fish and seafood. Per capita fish consumption lagged one period $(Y_{4,t-1})$ has significant positive effect on the current consumption of fish and seafood, whereas the trend did not show a significant effect on it. Per capita consumption of fish and seafood has increased considerably during the study period, and is expected to continue increasing over time. The estimated model (1) is used for predicting the per capita consumption of this group of food items from 2014 to 2023. The predicted, as well as real per capita consumption, of these food items have been plotted in Figure 1.

## Group 2: Meat, butter, eggs, and milk

Table 2 in Appendix 1 provides the maximum likelihood estimates of system equations of current per capita food consumption of meat $(Y_{1t})$, butter $(Y_{2t})$, eggs $(Y_{3t})$, and milk $(Y_{4t})$; the standard errors of these estimates; and t statistics. Per capita meat consumption is the summation of per capita consumption of beef, mutton, poultry, and fish. The estimated system of equations (2) is provided below:

$$\underbrace{\begin{bmatrix} Y_{1t} \\ Y_{2t} \\ Y_{3t} \\ Y_{4t} \end{bmatrix}}_{\mathbf{Y}_t} = \underbrace{\begin{bmatrix} 0.052 & 0.358 \\ 0.286 & 0.003 \\ 0.026 & 0.013 \\ 14.020 & -0.364 \end{bmatrix} \begin{bmatrix} 1 \\ t \end{bmatrix}}_{\boldsymbol{\psi}\,\mathbf{Z}_t} + \underbrace{\begin{bmatrix} 0.630 & 1.424 & 0.783 & 0.024 \\ -0.009 & 0.342 & -0.004 & 0.013 \\ -0.013 & 0.402 & 0.888 & 0.003 \\ 0.419 & 10.574 & 3.868 & 0.326 \end{bmatrix} \begin{bmatrix} Y_{1t-1} \\ Y_{2t-1} \\ Y_{3t-1} \\ Y_{4t-1} \end{bmatrix}}_{\Phi \Delta \mathbf{Y}_{t-1}}, \quad t = 2,3,4,\ldots,54 \tag{2}$$

The eigenvalues of matrix $\hat{\Phi}(L)$ are $\lambda = (0.9463,\ 0.6952,\ 0.5394,\ 0.0053)$. All eigenvalues are less than one, which mean that all the roots of $|\hat{\Phi}(L)| = 0$ fall outside the unit circle, and that the time series data of per capita consumption of these items are stationary with linear trends. By comparing $p\ value\ (pr > |T^*|)$ with a 0.05 significance level, the independent variables have shown significant effects on the dependent variables, as follows:

The first equation represents the per capita consumption of meat. The linear trend and meat per capita consumption lagged one period $(Y_{1,t-1})$ has shown a significant positive effect on current meat consumption.

**Table 2.** Estimates of system equations of current per capita consumption of meat, butter, eggs, and milk in the GCC region.

| Equation | Coefficients | $\hat{\beta}$ | $S.E_{\hat{\beta}}$ | $t$ | $(pr>\|T^*\|)$ | Ind. Var. |
|---|---|---|---|---|---|---|
| | $\beta_{10}=\psi_{10}$ | 0.052 | 1.716 | 0.030 | 0.976 | 1 |
| | $\beta_{11}=\psi_{11}$ | 0.358 | 0.119 | 3.020 | 0.004 | $t$ |
| $Y_{1t}$ | $\beta_{12}=\phi_{11}$ | 0.630 | 0.119 | 5.290 | 0.000 | $Y_{1t-1}$ |
| Meat | $\beta_{13}=\phi_{12}$ | 1.424 | 1.694 | 0.840 | 0.405 | $Y_{2t-1}$ |
| | $\beta_{14}=\phi_{13}$ | 0.783 | 0.510 | 1.530 | 0.132 | $Y_{3t-1}$ |
| | $\beta_{15}=\phi_{14}$ | 0.024 | 0.055 | 0.440 | 0.659 | $Y_{4t-1}$ |
| | $\beta_{20}=\psi_{20}$ | 0.286 | 0.155 | 1.840 | 0.072 | 1 |
| | $\beta_{21}=\psi_{21}$ | 0.003 | 0.011 | 0.310 | 0.755 | $t$ |
| $Y_{2t}$ | $\beta_{22}=\phi_{21}$ | -0.009 | 0.011 | -0.790 | 0.431 | $Y_{1t-1}$ |
| Butter | $\beta_{23}=\phi_{22}$ | 0.342 | 0.154 | 2.230 | 0.031 | $Y_{2t-1}$ |
| | $\beta_{24}=\phi_{23}$ | -0.004 | 0.046 | -0.100 | 0.924 | $Y_{3t-1}$ |
| | $\beta_{25}=\phi_{24}$ | 0.013 | 0.005 | 2.530 | 0.015 | $Y_{4t-1}$ |
| | $\beta_{30}=\psi_{30}$ | 0.026 | 0.294 | 0.090 | 0.929 | 1 |
| | $\beta_{31}=\psi_{31}$ | 0.013 | 0.020 | 0.640 | 0.528 | $t$ |
| $Y_{3t}$ | $\beta_{32}=\phi_{31}$ | -0.013 | 0.020 | -0.630 | 0.530 | $Y_{1t-1}$ |
| Eggs | $\beta_{33}=\phi_{32}$ | 0.402 | 0.290 | 1.390 | 0.172 | $Y_{2t-1}$ |
| | $\beta_{34}=\phi_{33}$ | 0.888 | 0.087 | 10.180 | 0.000 | $Y_{3t-1}$ |
| | $\beta_{35}=\phi_{34}$ | 0.003 | 0.009 | 0.330 | 0.745 | $Y_{4t-1}$ |
| | $\beta_{40}=\psi_{40}$ | 14.020 | 4.664 | 3.010 | 0.004 | 1 |
| | $\beta_{41}=\psi_{41}$ | -0.364 | 0.322 | -1.130 | 0.265 | $t$ |
| $Y_{4t}$ | $\beta_{42}=\phi_{41}$ | 0.419 | 0.324 | 1.290 | 0.202 | $Y_{1t-1}$ |
| Milk | $\beta_{43}=\phi_{42}$ | 10.574 | 4.605 | 2.300 | 0.026 | $Y_{2t-1}$ |
| | $\beta_{44}=\phi_{43}$ | 3.868 | 1.386 | 2.790 | 0.008 | $Y_{3t-1}$ |
| | $\beta_{45}=\phi_{44}$ | 0.326 | 0.149 | 2.190 | 0.034 | $Y_{4t-1}$ |

Per capita meat consumption is increasing significantly over time.

The second equation represents the per capita consumption of butter. The constant, per consumption lagged one period $(Y_{2,t-1})$, and consumption of milk lagged one period, $(Y_{4,t-1})$ have significant positive effects on current butter consumption, whereas the trend has no significant effect on it. The per capita butter consumption is stable over time.

The third equation represents the per capita egg consumption. Only the lagged one period consumption $(Y_{3,t-1})$ has a significant positive effect on its current per capita consumption, whereas the trend has no significant effect on it. Consumption of eggs per capita is stable over time. The last equation represents the per capita consumption of milk. The constant, per capita consumption of butter lagged one period $(Y_{2,t-1})$, per capita egg consumption lagged one period $(Y_{3,t-1})$, and per capita milk consumption lagged one period $(Y_{4,t-1})$, have significant positive effects on the current per capita consumption of milk. The trend has no significant effect on the current milk consumption, which means that milk consumption is stable over time.

The estimated model (2) has been used to predict per capita consumption of meat, butter, eggs, and milk from 2014 to 2023. The predicted, as well as real per capita consumption of these food items, have been plotted in Figure 2.

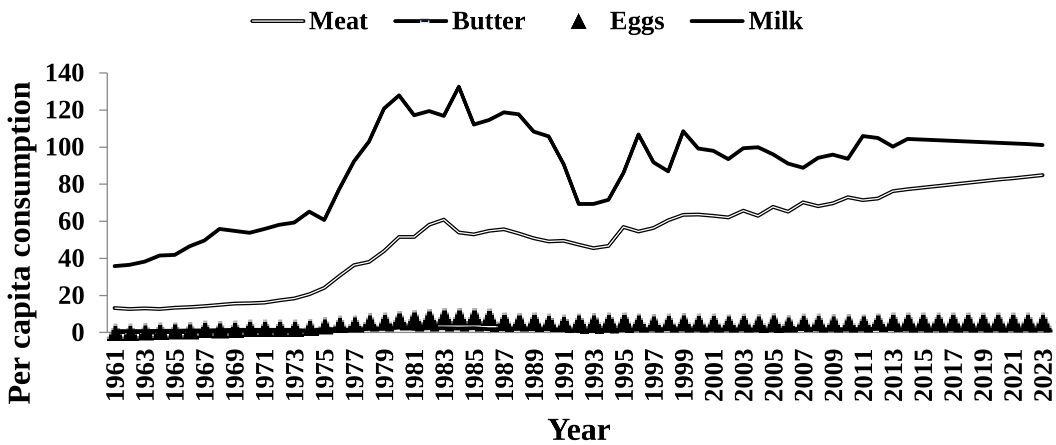


**Figure 2.** Real and predicted per capita consumption of meat, butter, eggs, and milk in the GCC region from 1961 to 2023 (kg/capita/year)
Data source: Food and Agriculture Organization,(1b) and their predicted values: estimated model.

In conclusion, the per capita consumptions of meat, poultry, fish, and seafood all show upward trends. Per capita consumption of poultry has the highest increase over this period. Seafood consumption has also shown an upward trend. The aggregate per capita consumption of all kinds of meat has shown a dramatic increase. From an average of 13.1 kg during 1961 to 1965, it has increased to 59.9 kg from 2005 to 2009, a 357.3% increase. It is expected to continue increasing over time and reach 85 kg by the year 2023. This may represent a symptom of diet transition in the GCC region.

## Consumption of food cereals and pulses

This group includes current per capita consumption of cereals $(Y_{1t})$, pulses $(Y_{2t})$, rice $(Y_{3t})$, and wheat $(Y_{4t})$. Table 3, Appendix 1, provides the estimated system of equations, their standard errors, statistics $t$, the corresponding $p values$ and all the roots of $|\Phi(L)|=0$. The estimated model (3) is shown below:

$$\underbrace{\begin{bmatrix}\hat{Y}_{1t}\\ \hat{Y}_{2t}\\ \hat{Y}_{3t}\\ \hat{Y}_{4t}\end{bmatrix}}_{\hat{\mathbf{Y}}_t}=\underbrace{\begin{bmatrix}33.01 & 0.717 & -0.004\\ 2.928 & -0.065 & 0.002\\ 9.635 & -0.296 & 0.008\\ 28.771 & 1.401 & -0.017\end{bmatrix}\begin{bmatrix}1\\ t\\ t^2\end{bmatrix}}_{\hat{\psi}\mathbf{Z}_t}+\underbrace{\begin{bmatrix}0.828 & -1.242 & -0.106 & -0.183\\ -0.02 & 0.558 & 0.032 & 0.008\\ -0.046 & -1.254 & 0.901 & 0.082\\ -0.052 & 0.597 & -0.195 & 0.519\end{bmatrix}\begin{bmatrix}\hat{Y}_{1t-1}\\ \hat{Y}_{2t-1}\\ \hat{Y}_{3t-1}\\ \hat{Y}_{4t-1}\end{bmatrix}}_{\hat{\Phi}\Delta\hat{\mathbf{Y}}_{t-1}} \quad t=2,3,4,\ldots,54 \tag{3}$$

The eigenvalues of matrix $\hat{\Phi}(L)$ are $\lambda=(0.9304, 0.6944, 0.6944, 0.4947)$. All eigenvalues are less than one, meaning that all the roots of $|\hat{\Phi}(L)|=0$ fall outside the unit circle. When considering that the data have a quadratic trend, the time series of per capita consumption of all food items are thus stationary. By comparing the $p$ $value$ $(pr>|T^*|)$ with a 0.05 significance level, the independent variables have shown significance effects on the dependent variables, as follows:

The first equation represents per capita consumption of cereals. The constant, linear trend (t) and consumption of cereals lagged one period $(Y_{1,t-1})$ have significant positive effects on cereal current consumption.

The second equation represents the per capita consumption of pulses (including beans, lentils, and peas). The constant, quadratic trend, consumption of pulses lagged one period $(Y_{2,t-1})$, and consumption of rice lagged one period $(Y_{3,t-1})$, have significant positive effects on the current consumption of pulses, whereas the linear trend and consumption of cereals lagged one period $(Y_{1,t-1})$ have significant negative effects on it.

The third equation represents the per capita consumption of rice. The quadratic trend, consumption of rice lagged one period $(Y_{3,t-1})$ and consumption of wheat lagged one period $(Y_{4,t-1})$ have significant positive effects on current consumption of rice $(Y_{3t})$, whereas the linear trend (t) and consumption of pulses lagged one period $(Y_{2,t-1})$ have significant negative effects on it. The last equation represents the current per capita

**Table 3.** Estimates of system equations of current per capita consumption of cereals, pulse, rice, and wheat in the GCC region.

| Equation | Coefficients | $\hat{\beta}$ | $S.E_{\hat{\beta}}$ | $t$ | $(pr>\lvert T^*\rvert)$ | Ind.Var. |
|---|---|---|---|---|---|---|
| $Y_{1t}$ Cereals | $\beta_{10}=\psi_{10}$ | 33.008 | 15.439 | 2.140 | 0.038 | 1 |
| | $\beta_{11}=\psi_{11}$ | 0.717 | 0.406 | 1.770 | 0.084 | $t$ |
| | $\beta_{12}=\psi_{12}$ | -0.004 | 0.008 | -0.530 | 0.600 | $t^2$ |
| | $\beta_{13}=\phi_{11}$ | 0.828 | 0.100 | 8.310 | 0.000 | $Y_{1t-1}$ |
| | $\beta_{14}=\phi_{12}$ | -1.242 | 1.574 | -0.790 | 0.434 | $Y_{2t-1}$ |
| | $\beta_{15}=\phi_{13}$ | -0.106 | 0.224 | -0.470 | 0.638 | $Y_{3t-1}$ |
| | $\beta_{16}=\phi_{14}$ | -0.183 | 0.116 | -1.570 | 0.122 | $Y_{4t-1}$ |
| $Y_{2t}$ Pulses | $\beta_{20}=\psi_{20}$ | 2.928 | 1.190 | 2.460 | 0.018 | 1 |
| | $\beta_{21}=\psi_{21}$ | -0.065 | 0.031 | -2.080 | 0.043 | $t$ |
| | $\beta_{22}=\psi_{22}$ | 0.002 | 0.001 | 3.780 | 0.001 | $t^2$ |
| | $\beta_{23}=\phi_{21}$ | -0.020 | 0.008 | -2.550 | 0.014 | $Y_{1t-1}$ |
| | $\beta_{24}=\phi_{22}$ | 0.558 | 0.121 | 4.600 | 0.000 | $Y_{2t-1}$ |
| | $\beta_{25}=\phi_{23}$ | 0.032 | 0.017 | 1.830 | 0.074 | $Y_{3t-1}$ |
| | $\beta_{26}=\phi_{24}$ | 0.008 | 0.009 | 0.940 | 0.354 | $Y_{4t-1}$ |
| $Y_{3t}$ Rice | $\beta_{30}=\psi_{30}$ | 9.635 | 5.913 | 1.630 | 0.110 | 1 |
| | $\beta_{31}=\psi_{31}$ | -0.296 | 0.156 | -1.900 | 0.063 | $t$ |
| | $\beta_{32}=\psi_{32}$ | 0.008 | 0.003 | 2.670 | 0.010 | $t^2$ |
| | $\beta_{33}=\phi_{31}$ | -0.046 | 0.038 | -1.200 | 0.238 | $Y_{1t-1}$ |
| | $\beta_{34}=\phi_{32}$ | -1.254 | 0.603 | -2.080 | 0.043 | $Y_{2t-1}$ |
| | $\beta_{35}=\phi_{33}$ | 0.901 | 0.086 | 10.510 | 0.000 | $Y_{3t-1}$ |
| | $\beta_{36}=\phi_{34}$ | 0.082 | 0.044 | 1.850 | 0.071 | $Y_{4t-1}$ |
| $Y_{4t}$ Wheat | $\beta_{40}=\psi_{40}$ | 28.771 | 17.303 | 1.660 | 0.103 | 1 |
| | $\beta_{41}=\psi_{41}$ | 1.401 | 0.455 | 3.080 | 0.004 | $t$ |
| | $\beta_{42}=\psi_{42}$ | -0.017 | 0.009 | -2.000 | 0.051 | $t^2$ |
| | $\beta_{43}=\phi_{41}$ | -0.052 | 0.112 | -0.470 | 0.641 | $Y_{1t-1}$ |
| | $\beta_{44}=\phi_{42}$ | 0.597 | 1.764 | 0.340 | 0.737 | $Y_{2t-1}$ |
| | $\beta_{45}=\phi_{43}$ | -0.195 | 0.251 | -0.780 | 0.440 | $Y_{3t-1}$ |
| | $\beta_{46}=\phi_{44}$ | 0.519 | 0.130 | 4.000 | 0.000 | $Y_{4t-1}$ |

consumption of wheat. The linear trend and consumption of wheat lagged one period $(Y_{4,t-1})$ have significant positive effects on current consumption of wheat, while the quadratic trend $(t^2)$ has a significant negative effect on it. The estimated model has been used to predict the per capita consumption of cereals, pulses, rice, and wheat from 2014 to 2023. The predicted, as well as real per capita consumption of these food items, have been plotted in Figure 3.

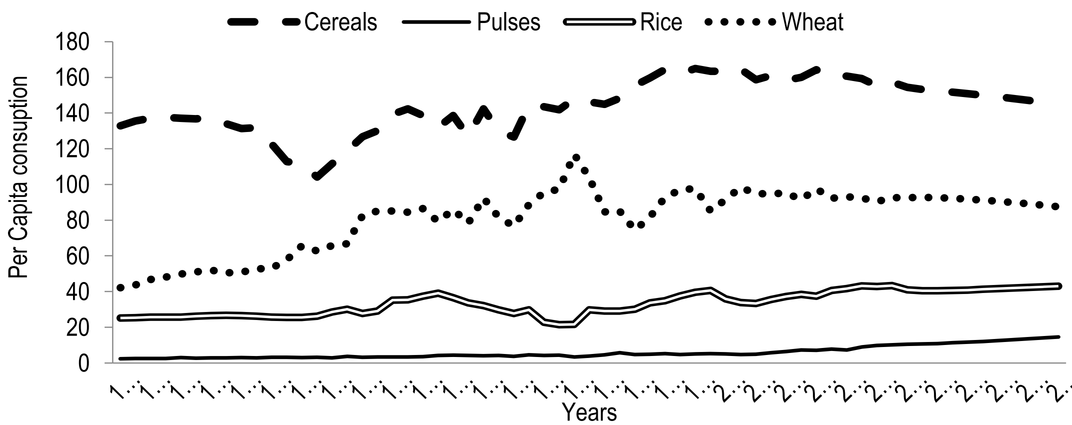


**Figure 3.** Real and predicted per capita consumption of cereals, pulses, rice, and wheat in the GCC region from 1961 to 2023 (kg/capita/year).
Data source: Food and Agriculture Organization, [1b] and the predicted values: estimated model.

Wheat is the GCC's main food grain, as depicted in Figure 3. Per capita wheat consumption was increasing at a decreasing rate from 1961 to 2002, and then starts decreasing at an increasing rate, and it will continue decreasing over time during the prediction period. This may be another symptom of diet transition in the study area.

Rice is the second-most important food cereal in the GCC region. Its per capita consumption has increased over time during the study period. The grain did not show a sign of diet transition. This may be attributed to the increasing number of expatriates, who accounted for about 40% of the GCC population in 2013, especially Asian people whose main food is rice. Furthermore, most of the expatriates stay for a limited time, whereas newcomers join the population, a matter expected to destabilize the rice diet pattern.

Per capita cereal consumption as an aggregate has been fluctuating over time. It decreased at a decreasing rate from 1961 to 1973, and then started increasing at an increasing rate. An inflection point occurred in 1992 when the growth rate of per capita cereal consumption started to decrease onwards for the rest of the study period. Per capita consumption of food cereals started to decrease in 2007 and is expected to continue decreasing in the projection period. This is another symptom of diet transition in the study area. Consumption of pulses has shown a slight increase over time. However, since 1993, it has been stabilizing at around an average of 5 kg, and is expected to be stable going forward.

## Consumption of fruits, vegetables, sugar, and sweeteners

The estimated model of current per capita consumption of fruits $(Y_{1t})$, vegetables $(Y_{2t})$, sugar, and sweeteners $(Y_{3t})$; the standard errors of these estimates; $t$ statistics; corresponding $p values$; and all the roots of $|\Phi(L)|=0$ are given in Table 4 of Appendix 1. The estimated system of equations (4) is provided below:

$$\underbrace{\begin{bmatrix} Y_{1t} \\ Y_{2t} \\ Y_{3t} \end{bmatrix}}_{\hat{\mathbf{Y}}_t} = \underbrace{\begin{bmatrix} 10.779 & 0.866 & -0.017 \\ 36.398 & 6.544 & -0.126 \\ 2.263 & 0.172 & -0.001 \end{bmatrix} \begin{bmatrix} 1 \\ t \\ t^2 \end{bmatrix}}_{\hat{\psi}\mathbf{Z}_t} + \underbrace{\begin{bmatrix} 1.243 & -0.081 & 0.873 \\ -0.043 & 0.102 & -0.173 \\ 0.064 & 0.002 & 0.814 \end{bmatrix} \begin{bmatrix} Y_{1t-1} \\ Y_{2t-1} \\ Y_{3t-1} \end{bmatrix}}_{\hat{\Phi}_1 \Delta \mathbf{Y}_{t-1}} + \underbrace{\begin{bmatrix} -0.571 & 0.054 & -0.311 \\ -0.194 & -0.200 & 2.250 \\ 0.057 & -0.032 & -0.295 \end{bmatrix} \begin{bmatrix} Y_{1t-2} \\ Y_{2t-2} \\ Y_{3t-2} \end{bmatrix}}_{\hat{\Phi}_2 \Delta \mathbf{Y}_{t-2}}, \quad t = 3,4,\ldots,54 \tag{4}$$

The eigenvalues of matrix $\hat{\Phi}(L)$ are $\lambda = (0.750, 0.750, 0.680, 0.680, 0.543, 0.543)$. All eigenvalues are less than one, which means that all the roots of $|\hat{\Phi}(L)|=0$ fall outside the unit circle. When considering that the data have a quadratic trend, the time series of per capita consumption of these food items are, thus, stationary. By comparing the $p\ value$ $(pr>|T^*|)$ with a 0.05 significance level, the following points regarding the estimated equations can be stated.

The first equation represents current per capita fruit consumption. The constant, fruits consumption lagged one period $(Y_{1,t-1})$ and sugar consumption lagged one period $(Y_{3,t-1})$, have positive significant effects on current fruit consumption, whereas consumption of vegetables lagged one period $(Y_{2,t-1})$ and fruits lagged two periods $(Y_{1,t-2})$ have negative significant effects on it.

The second equation represents current per capita

**Table 4.** Estimates of system equations of current per capita consumption of fruits, vegetables, and sugar in the GCC region.

| Equation | Coefficients | $\hat{\beta}$ | $S.E_{\hat{\beta}}$ | $t$ | $(pr>\|T^*\|)$ | Ind.Var. |
|---|---|---|---|---|---|---|
| $Y_{1t}$ Fruits | $\beta_{10}=\psi_{10}$ | 10.779 | 5.157 | 2.09 | 0.043 | 1 |
| | $\beta_{11}=\psi_{11}$ | 0.866 | 0.703 | 1.23 | 0.225 | $t$ |
| | $\beta_{12}=\psi_{12}$ | -0.017 | 0.012 | -1.44 | 0.158 | $t^2$ |
| | $\beta_{13}=\phi_{111}$ | 1.243 | 0.151 | 8.25 | 0.000 | $Y_{1t-1}$ |
| | $\beta_{14}=\phi_{112}$ | -0.081 | 0.047 | -1.75 | 0.088 | $Y_{2t-1}$ |
| | $\beta_{15}=\phi_{113}$ | 0.873 | 0.481 | 1.81 | 0.077 | $Y_{3t-1}$ |
| | $\beta_{16}=\phi_{211}$ | -0.571 | 0.170 | -3.36 | 0.002 | $Y_{1t-2}$ |
| | $\beta_{17}=\phi_{212}$ | 0.054 | 0.049 | 1.11 | 0.272 | $Y_{2t-2}$ |
| | $\beta_{18}=\phi_{213}$ | -0.311 | 0.422 | -0.74 | 0.465 | $Y_{3t-2}$ |
| $Y_{2t}$ Vegetables | $\beta_{20}=\psi_{20}$ | 36.398 | 16.597 | 2.19 | 0.034 | 1 |
| | $\beta_{21}=\psi_{21}$ | 6.544 | 2.263 | 2.89 | 0.006 | $t$ |
| | $\beta_{22}=\psi_{22}$ | -0.126 | 0.039 | -3.24 | 0.002 | $t^2$ |
| | $\beta_{23}=\phi_{121}$ | -0.043 | 0.485 | -0.09 | 0.930 | $Y_{1t-1}$ |
| | $\beta_{24}=\phi_{122}$ | 0.102 | 0.150 | 0.68 | 0.499 | $Y_{2t-1}$ |
| | $\beta_{25}=\phi_{123}$ | -0.173 | 1.549 | -0.11 | 0.912 | $Y_{3t-1}$ |
| | $\beta_{26}=\phi_{221}$ | -0.194 | 0.547 | -0.36 | 0.724 | $Y_{1t-2}$ |
| | $\beta_{27}=\phi_{222}$ | -0.200 | 0.157 | -1.28 | 0.208 | $Y_{2t-2}$ |
| | $\beta_{28}=\phi_{223}$ | 2.250 | 1.358 | 1.66 | 0.105 | $Y_{3t-2}$ |
| $Y_{3t}$ Sugar | $\beta_{30}=\psi_{30}$ | 2.263 | 1.435 | 1.58 | 0.122 | 1 |
| | $\beta_{31}=\psi_{31}$ | 0.172 | 0.196 | 0.88 | 0.384 | $t$ |
| | $\beta_{32}=\psi_{32}$ | -0.001 | 0.003 | -0.24 | 0.814 | $t^2$ |
| | $\beta_{33}=\phi_{131}$ | 0.064 | 0.042 | 1.53 | 0.133 | $Y_{1t-1}$ |
| | $\beta_{34}=\phi_{132}$ | 0.002 | 0.013 | 0.14 | 0.888 | $Y_{2t-1}$ |
| | $\beta_{35}=\phi_{133}$ | 0.814 | 0.134 | 6.08 | 0.000 | $Y_{3t-1}$ |
| | $\beta_{36}=\phi_{231}$ | 0.057 | 0.047 | 1.21 | 0.234 | $Y_{1t-2}$ |
| | $\beta_{37}=\phi_{232}$ | -0.032 | 0.014 | -2.38 | 0.022 | $Y_{2t-2}$ |
| | $\beta_{38}=\phi_{233}$ | -0.295 | 0.117 | -2.51 | 0.016 | $Y_{3t-2}$ |

vegetable consumption. The constant parameter and the linear trend $(t)$ have positive significant effects on the current consumption of vegetables $(Y_{2t})$, whereas the quadratic trend $(t^2)$ has a significant negative effect on it.

The third equation represents current per capita sugar consumption. Per capita consumption of sugar lagged one period $(Y_{3,t-1})$ has a significant positive effect on current sugar consumption, whereas per capita consumption of vegetables lagged two periods $(Y_{2,t-2})$ and per capita consumption of sugar lagged two periods $(Y_{3,t-2})$, have negative significant effects on it. The

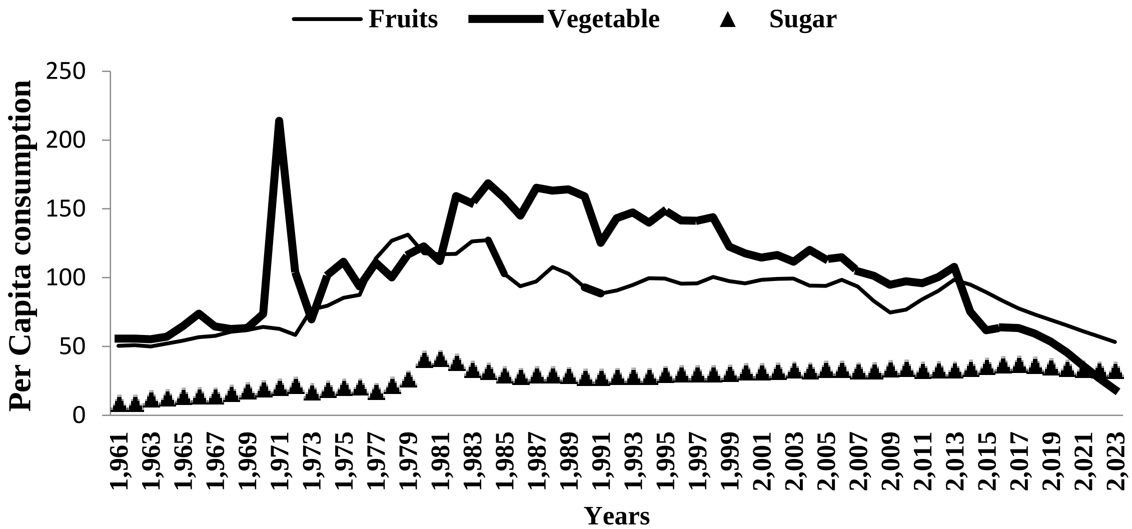


**Figure 4.** Actual and predicted per capita consumption of fruits, vegetables, sugar, and sweeteners in the GCC region from 1961 to 2023 (kg/per capita/year).
Data source: Food and Agriculture Organization,[1b] and the predicted values: estimated model.

estimated model 4 was used to predict the per capita consumption of these food items from 2014 to 2023. The predicted as well as real per capita consumption patterns since 1995 are plotted in Figure 4.

In 1971, per capita consumption of vegetables showed an unexplained exotic number for Saudi Arabia. The figure shows that the per capita consumption of vegetables and fruits decreased over time since the early 1980s and will continue decreasing in the prediction period. Per capita consumption of sugar and sweeteners has continuously increased over the study period, and are expected to continue increasing in the prediction period. All these trends are signals of a diet transition.

Overall, it is clear that the GCC states have witnessed a diet transition. However, the results of this study could not be compared with those of previous studies due to a lack of similar studies in the region.

## Food losses and waste in the GCC region

Food losses refer to a decrease in edible food mass throughout the supply chain that specifically leads to a decrease in food available for human consumption. Food losses and wastes are measured only for products directed at human consumption, excluding feed and parts of products that are inedible. Therefore, food that was originally meant for human consumption but which subsequently was removed from the human food chain is considered a loss or waste even if it is then directed to a non-food use such as feed, bio-energy, and so on (Parfitt et al., 2010).

Food can be lost or wasted in different stages of production (in-farm, post-harvest, packing, and processing), distribution, processing, and final consumption (food service including restaurants, cafeterias, fast food, caterers, and households), or during disposal (composted food wastes, and food wastes dumped in landfills). Food loss that occurs due to retailer and consumer behavior is called “food waste.”

According to the FAO (2012), wastage at the consumer level is typical of food systems in developed countries, while losses from production to the retail level characterize those of developing countries. The exact causes of food losses vary throughout the world, depending on the specific conditions of each country. These conditions include crop production choices and patterns, internal infrastructure and capacity, marketing chains and channels for distribution, as well as consumer purchase and food use practices. Developing countries can incur significant losses at harvest time or when crops are left un-harvested due to lack of effective demand. For the case of food cereals, drying, threshing, and milling can cause huge losses. Regarding perishable fruits and vegetables, approximately half the crop is usually lost due to poor handling and packaging and in transportation.

While many food products are ultimately biodegradable, their non-consumption means that the scarce resources used in their cultivation, production, marketing, and processing are also wasted. Thus, food loss and waste also imply loss of human labor, land, water, fertilizer, and other inputs as well as loss of fuel for transportation, processing, and cold storage. Furthermore, “cleaning up”

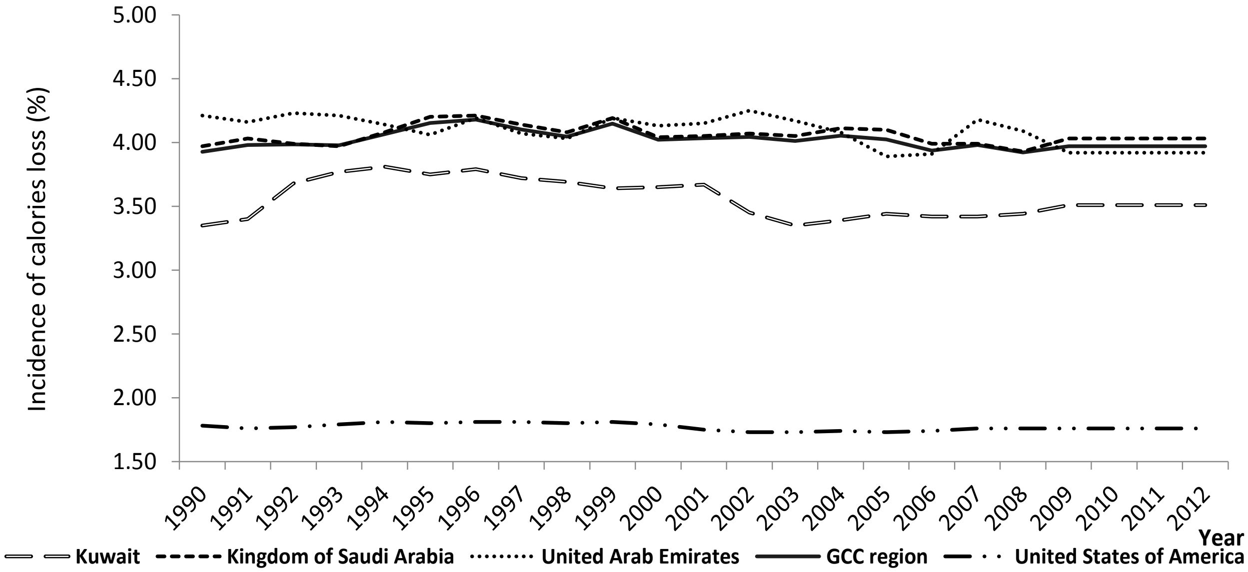


**Figure 5.** Incidence of caloric losses at retail distribution level in GCC region and USA from 1990 to 2012 (percentage). Data source: The Food and Agriculture Organization (1b)series.

food waste and food packing materials imposes further costs in terms of labor, energy, and environmental contamination.

With reference to the aforementioned literature about food loss and waste, food waste in retail distribution and consumption levels are the most relevant to the GCC region. This is because GCC countries depend mainly on international markets for their food, thus making “in-farm” as well as “post-harvest” and “in packing” losses irrelevant, except for a limited number of food items produced domestically. Thus, the discussion about food wastes in the study area has been confined to the retail distribution and consumption levels.

## Food wastes at the retail distribution level

Regarding food waste at the retail distribution level, the FAO (2013) has provided estimates of the incidence of caloric losses for the period 1990 to 2012 (Figure 5). USA food loss data have also been plotted for purposes of comparison. The figure shows a high incidence of food waste in the study area. The United Arab Emirates (UAE) has shown the highest level of food waste from 1990 to 2008, which was then surpassed by the Kingdom of Saudi Arabia (KSA) from 2008 onward. Food waste in the GCC region is very high compared with that of the USA. The average caloric loss in the region during the same period was 2.3 times that of the USA. Kuwait does relatively better in terms of food wastage, when compared with KSA and the UAE.

## Food waste at the consumption level

Food waste at the consumption level is caused mainly by consumer purchase and food usage practices, among other reasons. Regarding the study area, the major challenge is the paucity of data and information regarding the exact volume of food wasted in households and food service operations. Only estimates are found for the whole continent of Southeast Asia, which includes the study area. For instance, Gustafson and Otterdijk (2011) have estimated per capita food waste in South/Southeast Asia as falling in the range of 120 to 170 kg/year, whereas in Europe and North-America it is approximately 280 to 300 kg/year.

As a matter of culture in the GCC region, offering plenty of food for family members, guests, and to anyone who might ask for it, is an appreciated social behavior and sign of generosity. Furthermore, the volume of food purchases (and waste) dramatically increases on special occasions, such as the fasting month of Ramadan and other social and religious events. For instance, setting up lavish food tables during Ramadan and Eid festivals, weddings, parties, and informal get-togethers is common. Supermarkets, restaurants, and cafés are renowned for their excessive waste via unsold food items and damaged goods.

Minimizing food waste is a difficult challenge in a culture in which food is so readily thrown away, even though this contradicts the Islamic teaching in the holy Quran, which emphasizes moderation in food consumption; shuns extravagant, wasteful behaviors; and asks Muslims to avoid waste. For instance, one passage states, “Eat and drink, but be not excessive; indeed, He likes not those who commit excess.”

## Diet–related health concerns in the GCC region

It is well recognized in the health field that food consumption is closely related to many non-communicable diseases and health problems. These include the following:

(1) Obesity and being overweight, which are related to the quantity and quality of food intake, among other factors
(2) Diabetes: Type II diabetes, the disease’s most common form, is largely related to consuming too much sugar, especially processed and refined versions like high fructose corn syrup and refined flours, plus not enough fresh fruits, vegetables, and whole grains.
(3) Hypertension and dyslipidemia, which are highly linked to the quantity and quality of food intake.
(4) Osteoporosis: Calcium is important for healthy bones, while its deficiency can lead to osteoporosis. Highly refined foods like fast food, junk food, and sodas, deplete the body of vital minerals including calcium.
(5) Cardiovascular disease: High cholesterol levels and clogged arteries often arise from too much stress and saturated fat, and insufficient fiber, omega-fats, or exercise. Women especially are at risk for heart attacks after menopause.
(6) Cancer: A growing volume of research connects diet to many types of cancer. Nitrites in processed meat, artificial colors, sweeteners, preservatives, and pesticides used in producing non-organic crops, can all increase cancer risks.

Regarding the situation in the GCC region, all the aforementioned diseases are common. Obesity and being overweight represent major public health problems in GCC states. According to Alhyas et al. (2011a), the prevalence of overweight in the region is estimated in the range of 25-50%, and obesity is in the range of 13–50%. Importantly, these health problems are increasing over time. The most affected groups are women, children, and the elderly.

In another study, Ng. et al. (2011) have noted that the prevalence of overweight and obesity are astounding particularly in Kuwait, Qatar, KSA, and Bahrain, where between two-thirds and three-quarters of adults and 25-40% of children and adolescents are overweight or obese. The authors have argued that these levels are higher than those found in developed countries, such as the USA, Australia, and the UK.

Regarding hypertension and diabetes, their prevalence rates in the study area are among the highest in the world. According to the International Diabetes Federation (2014), KSA, Kuwait, and Qatar are among the world’s “top ten” countries for diabetes prevalence in 2013, with rates of 24, 23.1, and 22.9%, respectively. The prevalence rates of diabetes were 19% in the UAE, 21.8% in Bahrain, and 14.2% in Oman. People with diabetes are two to six times more likely to develop cardiovascular disease than people without diabetes.

Estimates of hypertension prevalence in the region vary between 6.6 and 33.6%. The UAE and Bahrain have shown the highest hypertension rates. With respect to hyperglycemia and dyslipidemia, their estimated prevalence rate among adults fall in the range of 10-20%, with an upward trend over time. This higher prevalence rate is associated with advancing age and female groups. The estimates for dyslipidemia fall in the range of 2.7-51.9%, with an increasing trend over time (Alhyas et al., 2011b). Regarding cancer prevalence, there is no known study of the prevalence of diet-related cancers among the population of the GCC states (Ng et al., 2011).

## CONCLUSION AND RECOMMENDATIONS

The GCC region has shown symptoms of a diet transition marked by a shift toward a more varied diet, with more items of animal origin and containing more added sugar and fat. This is accompanied by substantial food waste and the prevalence of health problems related to such food intake, including being overweight, obesity, diabetes, hypertension, and dyslipidemia. Food waste at the retail level is also substantial. Regarding food waste at the consumption level, the study region lacks sufficient and systematic data and information. Overall, the current situation reflects the need for proper food waste legislation, a thoughtful long-term strategy, and effective mechanisms for its implementation. The region needs to develop a policy program considering food transition, food waste, and diet-related chronic diseases. The program should include a combination of government action, and educational as well as mass media programs, to encourage commitment to dietary guidelines sensitive to traditional and religious concerns, and to raise public awareness regarding diet, exercise, and food waste. Finally, it is important for the GCC region to consider the data shortage regarding food consumption and waste, and to intensify research on the underlying causes of nutrition transition, food wastage, and prevalence of diet-related health problems. Ultimately, this will facilitate the design and implementation of policies that enhance the sustainability of food systems to improve human health in the GCC region.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

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**APPENDIX**

The *VAR*(*p*) model could be written as follows:

$$VAR(p): \quad \mathbf{Y}_t = \boldsymbol{\psi}\mathbf{Z}_t + \boldsymbol{\gamma}_1\mathbf{Y}_{t-1} + \boldsymbol{\gamma}_2\mathbf{Y}_{t-2} + \ldots + \boldsymbol{\gamma}_p\mathbf{Y}_{t-p} + \boldsymbol{\varepsilon}_t \ ,$$
$$t = p+1, p+2, \ldots, T$$

where $\mathbf{Y}_t = (Y_{1t}, Y_{2t}, \ldots, Y_{nt})'$, $t = p+1, p+2, \ldots, T$ denote a $n$-dimensional time series vector of the random variables under study, $\mathbf{Z}_t = (1, t, t^2)'$ is a $3 \times 1$ vector of determinants, $\boldsymbol{\psi}, \{\boldsymbol{\gamma}_i, i = 1, 2, \ldots, p\}$ are $n \times 3, n \times n$ coefficient matrices, and $\boldsymbol{\varepsilon}_t$ is a sequence of $n \times 1$ independent white noise vectors with zero mean and non-singular contemporaneous covariance matrix given by $\boldsymbol{\Sigma}_\varepsilon$. The model is a system of seemingly unrelated regression (*SUR*) equations with independent variables including lagged vectors $\mathbf{Y}_{t-1}, \mathbf{Y}_{t-2}, \ldots, \mathbf{Y}_{t-p}$ and vector of deterministic terms; $\mathbf{Z}_t$. The model is based on the following assumptions ((Lütkepohl ,1991) and (Pesaran and Pesaran ,1997)):

**Assumption 1**: $E(\boldsymbol{\varepsilon}_t) = \mathbf{0}$, $E(\boldsymbol{\varepsilon}_t\boldsymbol{\varepsilon}_t') = \boldsymbol{\Sigma}_{\boldsymbol{\varepsilon}}$ *for all t, where* $\boldsymbol{\Sigma}_{\boldsymbol{\varepsilon}} = \{\sigma_{ij}^2, i, j = 1, 2, \ldots, n\}$ is *a positive definite matrix*, $E(\boldsymbol{\varepsilon}_t\boldsymbol{\varepsilon}_{t'}') = \mathbf{0}$ *for all* $t \neq t'$, *and* $E(\boldsymbol{\varepsilon}_t \mid \mathbf{Z}_t) = 0$.

**Assumption 2**: *All the roots of* $|\boldsymbol{\gamma}(L)| = |I_n - \sum_{i=1}^{p} \boldsymbol{\gamma}_i L^i| = 0$ *fall outside the unit circle*, *or, all eigenvalues of the* $np \times np$ *companion matrix have modulus less than one*, *and* $\mathbf{I}_n$ is $n \times n$ *identity matrix*.

**Assumption 3**: $(\mathbf{Y}_{t-1}, \mathbf{Y}_{t-2}, \ldots, \mathbf{Y}_{t-p}, \mathbf{Z}_t)$, $t = p+1, p+2, \ldots, T$ *are not perfectly collinear*.

Given that no restrictions were imposed on parameters of the model, and that assumption 2 holds, the general form of the multivariate linear model could be represented by:

$$\underset{(m \times n)}{\mathbf{Y}} = \underset{(m \times k)}{\mathbf{X}} \ \underset{(k \times n)}{\mathbf{B}} + \underset{(m \times n)}{\mathbf{E}}$$

where $\mathbf{Y} = (\mathbf{Y}_{p+1}, \mathbf{Y}_{p+2}, \ldots, \mathbf{Y}_T)'$, $\mathbf{X} = (\mathbf{X}_{p+1}', \ldots, \mathbf{X}_T')'$, $\mathbf{X}_t = (\mathbf{Z}_t', \mathbf{Y}_{t-1}', \ldots, \mathbf{Y}_{t-p}')$, $\mathbf{B} = (\boldsymbol{\psi}, \boldsymbol{\gamma}_1, \ldots \boldsymbol{\gamma}_p)'$, $\mathbf{E} = (\boldsymbol{\varepsilon}_{p+1}, \boldsymbol{\varepsilon}_2 \ldots, \boldsymbol{\varepsilon}_T)'$, $m = T - p$, and $k = np + 3$. Then coefficient matrix $\mathbf{B}$ can be estimated by using the conditional least squares $(LS)$ method (Johnson and Wichern, 1992) which is:

$$\hat{\mathbf{B}}_{ls} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{Y}$$

and this estimation can be used for estimating the covariance matrix of error vector; $\boldsymbol{\Sigma}_\varepsilon$ which given by $\hat{\boldsymbol{\Sigma}}_\varepsilon = \sum_{t=p+1}^{T} \hat{\boldsymbol{\varepsilon}}_t \hat{\boldsymbol{\varepsilon}}_t' / m$, where $\hat{\boldsymbol{\varepsilon}}_t = (\mathbf{Y}_t - \mathbf{X}_t\hat{\mathbf{B}}_{ls})$ is the residual vector. Let $\hat{\boldsymbol{\beta}}_{ls} = vec(\hat{\mathbf{B}}_{ls})$ denotes the operator that stacks the columns of the $(k \times n)$ matrix $\hat{\mathbf{B}}_{ls}$ into a long $(nk \times 1)$ vector. The estimate $\hat{\boldsymbol{\beta}}_{ls}$ is consistent and asymptotically normally distributed with asymptotic covariance matrix; $avar(\hat{\boldsymbol{\beta}}_{ls}) = \hat{\boldsymbol{\Sigma}}_\varepsilon \otimes \left[(\mathbf{X}'\mathbf{X})^{-1}/m\right]$ (Hamilton, 1994) and (Lütkepohl, 1991). From the statistical properties of $\hat{\boldsymbol{\beta}}_{ls}$, all important statistical hypothesis tests as well the predicted values to dependent variables in *VAR*(*p*) model can be performed.

Prediction of dependent variables: with given independent variables matrix; $\tilde{\mathbf{X}}_{T+h|T} = (\mathbf{D}_{T+h}', \tilde{\mathbf{Z}}_{T+h}', \hat{\mathbf{Y}}_{T+h-1|T}', \ldots, \hat{\mathbf{Y}}_{T+h-p|T}')$, the best linear predictor with period of $h$ length; $\mathbf{Y}_{T+h}$ is given by:

$\hat{\mathbf{Y}}_{T+h|T} = \hat{\mathbf{B}}'_{ls}\tilde{\mathbf{X}}'_{T+h|T}$ , $h = 0,-1,...,$ *Furthermore*

Where $\mathbf{Z}_{T+h} = (1, T+h, (T+h)^2)$ , and $\hat{\mathbf{Y}}_{T+j|T} = \mathbf{Y}_{T+j}$ *for* $j \leq 0$ . The mean square error matrix $(MSE)$ of the $h$ -step forecast (Green, 2003) is given by $\hat{\Sigma}(h) = \Sigma(h) + MSE[(\mathbf{B} - \hat{\mathbf{B}}_{ls})'\tilde{\mathbf{X}}'_{T+h|T}]$. In practice, the second term; $MSE[(\mathbf{B} - \hat{\mathbf{B}}_{ls})'\tilde{\mathbf{X}}'_{T+h|T}$ is often ignored and $\hat{\Sigma}(h)$ is computed as:

$$\hat{\Sigma}(h) = \sum_{s=0}^{h-1} \hat{\Lambda}_s \Sigma_\varepsilon \hat{\Lambda}'_s$$

were $\hat{\Lambda}_s = \sum_{j=1}^{s-1} \hat{\Lambda}_{j-1}\hat{\Phi}_j$ , $\Lambda_0 = \mathbf{I}_n$ , $s = 1,2,...,p$ , . Asymptotic $(1-\alpha).100\%$ confidence intervals for the individual elements of $\hat{\mathbf{Y}}_{T+h|T}$ are then computed as:

$$\hat{Y}_{k,T+h|T} - Z_{(1-\alpha/2)}.\hat{\sigma}_k(h) < Y_{k,T+h} < \hat{Y}_{k,T+h|T} + Z_{(1-\alpha/2)}.\hat{\sigma}_k(h)$$

where $Z_{1-\alpha/2}$ is the $(1-\alpha/2)$ quartile of the standard normal distribution, and $\hat{\sigma}_k(h)$ denotes the square root of the $k^{th}$ diagonal element of $\hat{\Sigma}(h)$.

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*Full Length Research Paper*

# Volumetric space distribution of wood as a tool in sustainable forest

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**The objective of this work was to evaluate the distribution of forest volume identified through interpolation maps from data obtained from the forest. We sampled 36 plots with 1-ha area. Special maps were used by means of interpolation adjusted by the Spline method. The results indicate that after 20 years of traditional exploration, no significant oscillations were identified for volumes. However, the area explored showed a significant recovery in the total volume in this period. In addition, it showed the appearance of new species that contributed to the increase of biodiversity. This influenced the growth rates of the trees, the establishment of natural regeneration, the growth of seedlings and sticks. These will replace the losses occurring during the exploration and the increase in carbon sequestration. It should be emphasized that this method proved to be efficient in determining points of greater volumetric distribution and consequent forest recovery.**

**Key words:** Geotechnologies, spatial distribution, forest volumetric.

## INTRODUCTION

Brazil has the second largest forest area in the world and covers an area of approximately five million square kilometers (km²) situated in the Legal Amazon, which corresponds to approximately 65% of the national territory. Of this total, 3.3 million km² are wet tropical forests, destined to the sustainable exploitation, regulated by a cycle of cut of 30 years (Higuchi et al., 2008).

Reduced impact exploration is carried out through forest management techniques, and the elaboration of the forest management plan is necessary.

It is necessary to analyze the logging maps and the volumetric distribution as a tool to know the efficiency of

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the management plan, if wood volume recovered after logging and future logging in the area.

Muñoz Braz et al. (2005) comments that the cutting cycle, the remaining stock and the periodic annual increment (IPA) of the species are the main factors to determine the annual extraction rate in a stand and the intensity of the forest cut, but are not taken into account before their exploitation, which implies a possible increase in the cutting cycle for the next harvest.

On the other hand, there is an intense process of deforestation and over-harvesting contributing to the growing decline in the demand for forest products in the world (Bradshaw et al., 2010). This event has increased the valorization of these species and consequently the exploratory pressure on individuals below the limit allowed for cutting, as well as the deforestation in the Amazon region. Thus, it becomes necessary to implement public policies as a mechanism to reduce this degradation left by forest overexploitation, deforestation and forest fires (Fonseca et al., 2015).

In terms of sustainable exploitation Brandelero et al. (2007) argued that interventions aimed at more accurate silvicultural treatments have been sought to achieve maximum yield according to the potential of species and other local environmental factors. Those measures contribute to reducing impacts to ecosystems, since the biodiversity of tropical forests undergo several changes in its structure due to the dynamics of growth, mortality and or exploitation of these species. Knowing the spatial distribution of the forest species of their volumetry will require the adoption of new tied tools, so that the forest inventory is carried out with precision.

For Ortiz et al. (2006), the techniques of geoprocessing assume great importance in the study of the identification and correlation of variables that affect the forest productivity through operations in Geographic Information Systems (GIS) associating these variables in the form of digital maps enabling a precision forestry. Remote sensing is an important tool that can support estimating and monitoring forest resources (Turner et al., 2003; Zolkos et al., 2013).

It is necessary to apply alternative techniques in obtaining data and even providing a complementary analysis of the dynamics of forest exploitation. In this context, geotechnologies can contribute to obtaining information quickly (before, during and after forest disturbance), with relative accuracy and low cost, contributing to the monitoring, planning and decision making of the forest manager (Facco et al*.,* 2016).

Among the available options, we can highlight the vegetation indices obtained by red and infrared spectral bands close to the images obtained by orbital sensors, the most common being the normalized difference vegetation index (NDVI), which allows us to gather information about the amount of green biomass, vegetation growth and development (Martins and Silva, 2014).

Taylor et al. (2006) defined precision forestry as the planning and conduction of a particular site of the forest with the management of forestry activities and operations aimed at improving the quality and use of wood, reducing waste, increasing profits and maintaining the quality of the environment. The knowledge of the qualitative variables of a native forest, associated to geotechnologies with new tools of registries of maps with specialized information, has contributed to the sustainable planning of the use of the forest resources within the forest inventory (Souza et al*.,* 2007).

Thus, this study aimed to evaluate the distribution of forest volume identified through interpolation maps, based on data obtained from the forest inventory. It is hypothesized that monitoring a forest scenario through interpolation maps applied to the forest inventory is capable of assessing forest production capacity in a given area, thus making forest exploitation more accurate.

## MATERIALS AND METHODS

### Characterization of the experimental area

The study area is located at Morro do Felipe, in the municipality of Vitória do Jari, Amapá State, at the coordinates (0º00'55 "S and 5º20'20" W) registered from January 8 to 16, 2012. The experimental area (Figure 1) presents a forest ecosystem of the Ombrophylous Dense Forest type and type Am climate, by classification of Köppen.

The average annual precipitation reaches 2,234 mm with a rainy period from December to May. A dry season of three months occur that begins in June and that is characterized by a monthly precipitation inferior to 8% of the annual volume of rain. The average annual temperature is 25.80ºC with the thermal amplitude varying more or less 20ºC between the maximum and the minimum monthly value. The soils are of the Yellow Latosol type Dystrophic with heavy clayey texture (Azevedo et al., 2008).

### Study area history

Over 27 years of monitoring (1984, 1985, 1986, 1988, 1990, 1994, 1996, 2004 and 2011) Jari's 36 ha experimental area underwent three interventions that led to a reduction in basal area, where statistical analysis was performed to compare volumes between periods.

In 1983, a forest inventory was carried out considering all trees with Chest Height Diameter (DBH) ≥ 50 cm. In 1985, forest exploitation (1st intervention) occurred in the primary forest area removing 15, 25 and 35% of the total volume of trees with DBH ≥ 50 cm, corresponding to approximately 25, 40 and 60 m³/ha, respectively. Trees with DBH ≥ 60 cm from 26 species of commercial value in the region were explored. In 1990, there was a gale (natural phenomenon) that felled several trees (2nd intervention). Silvicultural treatment was carried out in 1994 by applying two types of thinning: systematic thinning, with two intensities of reduction of the original basal area (30 and 50%), and selective thinning (Carneiro et al., 2019).

### Experimental design and data collection

Exactly 36 plots of one-hectare area (100 m × 100 m) were sampled.

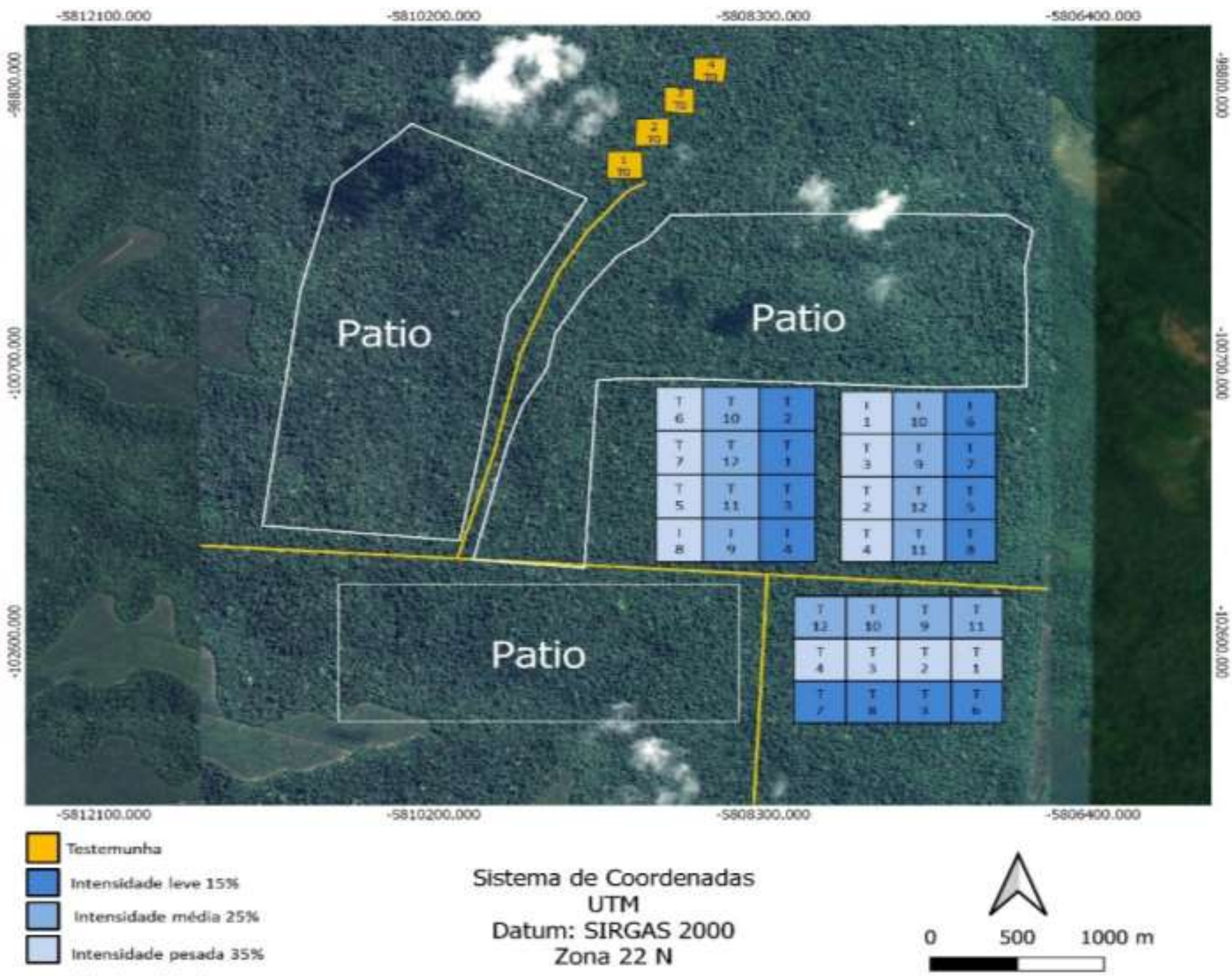


**Figure 1.** Location of the experiment and distribution of the plots and their respective repetitions of the 12 silvicultural treatments applied, presenting different combinations of intensity of exploration and thinning.

The legend classification was performed using the "natural breaks" method distributed in four color classes classified in low volumetry (blue color: 25-30 $m^3$. $ha^{-1}$), medium volumetry (green color: 30-35 $m^3$. $ha^{-1}$), moderate volumetry (yellow color: 35-40 $m^3$. $ha^{-1}$) and high volumetry (red color: 40-45 $m^3$. $ha^{-1}$).

Each plot of one hectare was divided into 10m ×10m subplots to facilitate monitoring activities. The plots and subplots were demarcated with pickets (1.2m PVC pipes with a diameter of 25mm) which were painted on top with acrylic red paint for easy visualization (Silva, 2005).

For the analysis of floristic composition, the identification of the individuals was carried out in the forest by the usual name from an on-site visit by the Parataxibotan technicians of Embrapa Amazônia Oriental. The botanical material of the less common species and the groups of species that raised doubts was collected to be identified, by comparison, in the IAN Herbarium of Embrapa Amazônia Oriental.

Data collections were carried out by Embrapa Amazônia Oriental between 1984 and 2011. Spatial coordinates were collected in the UTM system to correlate the spatial position with the total volume of each plot. Data collection was performed with Garmim 76CSX global positioning system (GPS) equipment, configured in DATUM and coordinate positioning by the SIRGAS 2000 system.

In the monitoring plots, tree species larger than 20 cm of CHC (circumference at the Height of the Chest) with subsequent transformation in DCH (Diameter at Chest Height) were selected. Only individuals with tree size required by current legislation were considered and sampled and identified in the field with specialist assistance (botanical identifier).

The diameter structure was characterized by the distributions of tree numbers, basal area, stem volume per hectare, species and diameter class.

## Data analysis

For the data processing, the following programs were used: MFT (Tropical Rainforest Monitoring) developed by Embrapa Amazônia

**Table 1.** Comparison between the evaluated periods for the total volume values of the species from rank group-1 (number of individuals in row 1) and group-2 (number of individuals in column j), "U" statistic (values on upper diagonal) and Statistical probability values (P) of the Mann-Whitney U test for differences of commercial species groups in an area of 36 ha of native forest in Jari, Amapá, Brazil.

| Correlation | 1984 | 1986 | 1988 | 1990 | 1994 | 1996 | 2004 | 2011 |
|---|---|---|---|---|---|---|---|---|
| 1984 | | G1=1583<br>G2=1045<br>U =379 | G1=1626<br>G2=1002<br>U= 336 | G1=1673<br>G2=955<br>U =289 | G1=1502<br>G2=1126<br>U= 460 | G1=1600<br>G2=1028<br>U= 362 | G1=1272<br>G2=1356<br>U= 606 | G1=1188<br>G2=1368<br>U= 522 |
| 1986 | Z=3.03<br>P=0.002** | | G1=1353<br>G2=1275<br>U=609 | G1=1406<br>G2=1222<br>U= 556 | G1=1197<br>G2=1431<br>U= 531 | G1=1333<br>G2=1295<br>U= 629 | G1=1080<br>G2=1548<br>U4= 14 | G1=1016<br>G2=1540<br>U=350 |
| 1988 | Z=3.51<br>P=0.000** | Z= 0.44<br>P= 0.661 | | G1=1352<br>G2=1276<br>U= 610 | G1=1136<br>G2=1492<br>U= 470 | G1=1283<br>G2=1345<br>U= 617 | G1=1042<br>G2=1586<br>U= 376 | G1=992<br>G2=1564<br>U=326 |
| 1990 | Z=4.04<br>P=0.000** | Z=1.04<br>P= 0.300 | Z=0.43<br>P= 0.669 | | G1=1089<br>G2=1539<br>U=423 | G1=1247<br>G2=1381<br>U= 581 | G1=1010<br>G2=1618<br>U= 344 | G1=963<br>G2=1593<br>U= 297 |
| 1994 | Z=2.12<br>P=0.034** | Z=-1.32<br>P= 0.188 | Z=-2.00<br>P =0.045* | Z=-.2.53<br>P= 0.0113 | | G1=1446<br>G2=1182<br>U= 516 | G1=1124<br>G2=1504<br>U=458 | G1=1041<br>G2=1515<br>U= 375 |
| 1996 | Z=3.22<br>P=0.001** | Z=0.21<br>P= 0.831 | Z=-0.35<br>P= 0.727 | Z=-0.75<br>P= 0.451 | Z=1.49<br>P= 0.137 | | G1=1043<br>G2=1585<br>U= 377 | G1=979<br>G2=1577<br>U=313 |
| 2004 | Z=-0.47<br>P=0.636 | Z=-2.64<br>P= 0.008** | Z=-3.06<br>P= 0.002** | Z=-3.42<br>P= 0.001** | Z=–2.14<br>P= 0.032* | Z=-3.05<br>P= 0.002** | | G1=1226<br>G2=1330<br>U= 560 |
| 2011 | Z=-1.24<br>P=0.214 | Z=-3.22<br>P=0.001** | Z=-3.50<br>P= 0.001** | Z=-3.83<br>P= 0.000** | Z=-2.93<br>P= 0.003** | Z=-3.65<br>P= 0.000** | Z=-0.81<br>P= 0.421 | |

Statistically significant values considering $\alpha$=5%, ** $\alpha$=1%.

Oriental, which will serve to analyze the parameters referring to floristic and forest structure and Excel to calculate the estimated volume in the 100%. In order to verify the sample sufficiency of the floristic composition, before the application of the treatments, the data of the number of species, according to the area, were adjusted and analyzed using the program R.

In 1984, the first forest inventory was carried out and one year later the exploitation occurred. Statistical analysis (Mann-Whitney U test) was performed to determine if there was a change in the growth dynamics of the species.

Anderson Darling's normality test was used to evaluate the normality of the total volume (m³) in the years that was considered not significant with the value of p = 0.448. The Bartlett variance homogeneity test was performed, which was significant with a value of 0.041. In view of the violation of the homoscedasticity assumption, the Krukal - Wallis test was used with seven degrees of freedom, which was significant p = 0.000 with H = 42.91. Then, the Mann-Whitney test (U) was used to test the equality of the medians in the years whose results are found in Table 1. Statistical analysis (Mann-Whitney U test) was performed to determine if there was a change in the growth dynamics of the species.

The volume of the first year of measurement (1984) was compared with all remedies (1986, 1988, 1990, 1994, 1996, 2004, 2011). There was logging in 1985, then the post-log remediation (1986) was carried out and compared with the following remediations and successively until the end of remediation 2011.

The maps elaborated to evaluate the wood volume were those of 1984 (before the logging), 1986, 1988, 1990, 1994, 1996, 2004 and 2011 (after the logging) of the plots calculated by the formulas (Equations 1 and 2), and from the value of this volume and linked to the UTM coordinates of each tree, the interpolation maps were prepared by the inverse square distance method.

The first stage of the statistical analysis consisted of the exploratory study of the data, allowing to verify the trend of the characteristics evaluated as a function of longitude (WE) and latitude (NS). The inverse distance square method (IDS) was used which is a univariate deterministic interpolator of weighted averages. The farther an observed point is from the smaller estimate its influence on the value of inference. The IDS, described by is defined by the (Equation 1)

$$\hat{Z}_{i=} \frac{\sum_{i=1}^{n}\left[\frac{1}{d_i^2}*Z_i\right]}{\sum_{i=1}^{n}\left(\frac{1}{d_i^2}\right)} \quad (1)$$

where: Ẑi is the interpolated value; Zi is the value of the sampled attribute and di is the Euclidean distance between the sampled and the estimated point.

The Spline method was used to adjust the curve defined mathematically by two or more control points. Interpolation splines pass through all control points and are considered an approximation technique that consists of dividing the intervals of interest into several subintervals and interpolating, as smoothly as possible, these subintervals with small degree polynomial.

A Spline function s (x, y) is a polynomial function in the variables x and y, more specifically a Spline of order p, with p = 2k-1 and k integer and positive. It is a polynomial of degree p in x for each fixed y, and in the same way it is a polynomial of degree p in y for each fixed x, that is (Equation 2):

$$s(x,y) = \sum_{j=0}^{p}\sum_{i=0}^{p} a_{ij}\, x^i y^j \quad (2)$$

It is a polynomial function that is adjusted to a small number of points and that are applied to sets of numbers ensuring that the joining of the various functions is continuous. They are suitable for very soft surfaces not fitting to surfaces with significant fluids.

The choice of this method was mainly due to the number of samples (n = 36), which was satisfactory for the proposed analysis. The ARCGIS 10.1 program was used for calculations of interpolation and mapping (ESRI, 2012).

## RESULTS

### Statistical analysis in the volumetric time line

Comparing 1984 with the other years, a statistical difference was observed with the 1986, 1988, 1990, 1994, and 1996 remedies, and there was no statistical difference with 2004 and 2011. In 1986, two years after exploration, compared with the other periods, statistical differences are detected over time. In the following measures, it was observed that the area began to come into equilibrium.

In 1986, there was biomass gain, but then it was gradually lost. After five years of exploitation, there was a slight increase (1986-1988-1990). But statistically, this growth showed no significant difference, a fact that may have been influenced by the loss of biomass caused by natural mortality.

Comparing the years of 1984, statistical difference was observed compared to the measurements of 1986, 1988, 1990, 1994 and 1996, and there was no statistical difference with 2004 and 2011. The 1986 remedy was compared with the other periods, and statistical differences over time were found, not unlike in the following remedies. It was observed that the area began to come into equilibrium with a small increase in biomass.

In 1986, there was biomass gain, but then it was gradually lost. After five years of exploration, there was a slight increase (1986-1988-1990). But statistically, this growth did not show a significant difference, a fact that may have been influenced by the biomass loss caused by natural mortality.

In the passage from 1990 to 1994, there was an increase in volumetry of almost 2 m³/ha. In 1994, a new intervention was carried out in the area through the application of silvicultural treatment, where it had a new reduction of basal area, being close to the period of 1986; this event was the implicator for the decrease of volumetry. In the period 1994-1996, the statistical analysis was not significant, due to the short time after the silvicultural treatment (Table 1).

The Z value refers to statistics calculated to confirm hypothesis h0 or h1 of the values of the tests performed.

The post-harvest periods from 1986 to 1996 do not differ statistically, with the exception of the period from 1988 to 1994. Likewise, the period before the 1984 harvest does not differ from the post-harvest 20 years (2004 and 2011), indicating the recovery of the density of trees in the managed forest area. The variance between 1994-1996 was the determinant for the non-significance, which explains the difference between the periods, the dendrometric values practically remained, reflecting the equal effects of the applied silvicultural treatment, inhibiting the changes on the vegetation occurred.

Soon after in 2011, the total volume exceeded the initial volume by 1.7%, from 39.19 to 39.86 m³, confirming the volume recovery (m³) in treated areas (Table 2).

The biomass growth in native forest depends on the type of disturbance that occurred in the period and the variation of the density of trees found in the plots can be explained by the intensity of exploitation and the silvicultural treatment that occurred in this area. After the exploration, the native forests enter the reconstruction phase (closing of large clearings) and can present high density of adult representatives of pioneer and secondary species, due to the high capacity of repopulation and growth in these areas.

The events (exploration plus treatment application) made a difference from 2004, becoming statistically significant. For the years 2004 and 2011, the analysis did not detect statistical differences due to the oscillation of the variance between the plots, recovering the total volume after 20 years, showing that the statistical analysis corroborated with information from the images of the volumetric maps.

### Volumetric scenary before silvicultural treatment

For the year 1984, there was a predominance of commercial tree species with a volume of 35-40 m³ ha; there is also a high volumetric value of 40-45 m³ ha. After logging in 1986, there was an increase in the volume of individuals. 35 - 40 m³ ha and 30- 35 m² and a reduction in the volume of individuals of 40-45 m³. This increase may be related to the diameter growth of the remaining individuals. Featuring W –SW oriented spatial configuration. Before and after logging, there is a predominance of individuals of 35 - 40 m³ (Figure 2).

In 1986, one year after the exploration, there was a slight reduction in the total volume of 6.4%, with no change in the scenery for moderate volumetry, but for the average volumetry explained by the process of succession of the individuals facilitated by the heavy exploration which removed the large individuals in the year 1984. For this period, we observe a spatial configuration oriented in the NE-E direction.

In 1988, there was still a decrease in total mean volume to 35.83 m³ ha$^{-1}$. The same characteristic was observed for the spatial dynamics of the previous period of 1986. However, there was an isolated increase in the mean volume (30-35 m³ ha$^{-1}$) in the N-SE sense, predominance in the moderate volumetry (35-40 m³. ha$^{-1}$). In this period, there was a decrease of individuals of the high volume class.

In 1990, there was a greater fall in total volumetry of 8.6% due to climatic phenomena in the region (Figure 3),

**Table 2.** Total volume (m³ ha-¹) of silvicultural and control treatments and percentage volume variation over the years of study at Jari, Vitória do Jari, Amapá, Brazil.

| Year | Volume (Treatment) | Volume change in % | Volume (Treatment) | Volume change in % |
|---|---|---|---|---|
| 1984 | 39.19632 | | 43.31995 | |
| 1986 | 36.68616 | - 6.4 | 44.19451 | + 2.0 |
| 1988 | 36.21706 | - 7.6 | 43.97722 | + 1.5 |
| 1990 | 35.82988 | - 8.6 | 42.66765 | - 1.5 |
| 1994 | 37.59115 | - 4.1 | 43.88412 | + 1.3 |
| 1996 | 36.16934 | - 7.7 | 44.01208 | + 1.6 |
| 2004 | 39.11322 | -0.2 | 43.50308 | +0.4 |
| 2011 | 39.86219 | + 1.7 | 44.87421 | +3.6 |

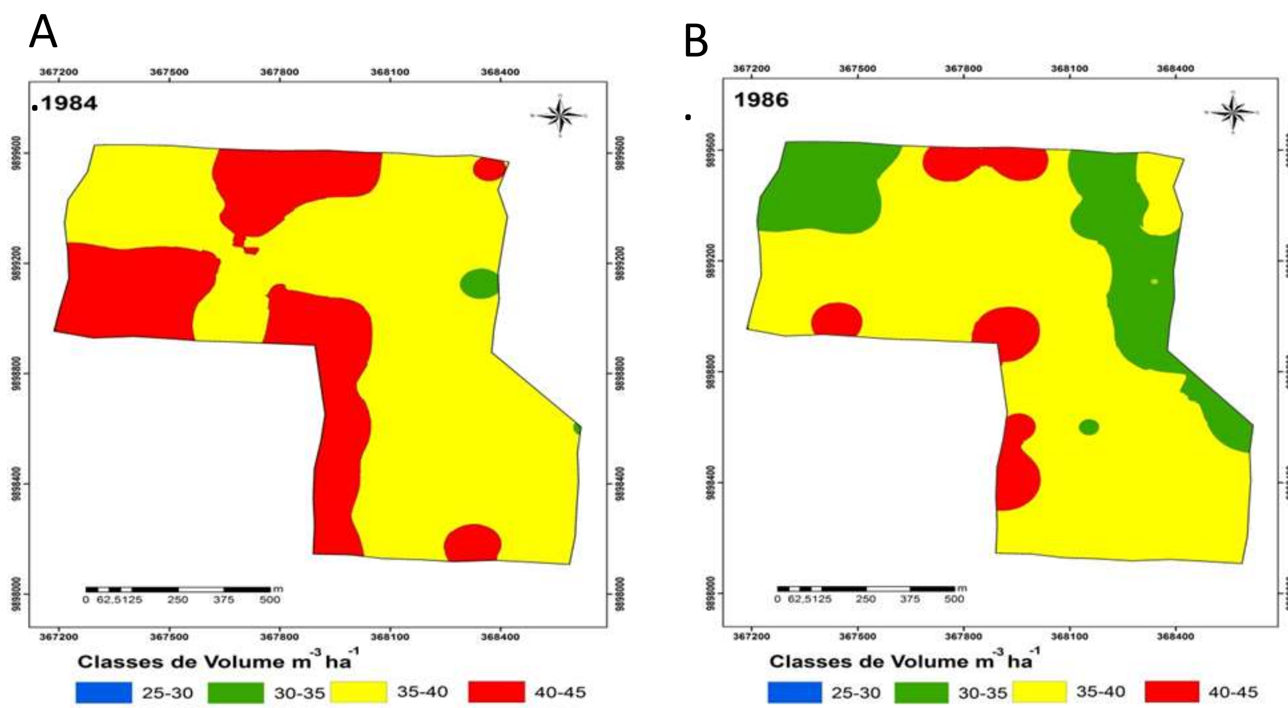


**Figure 2.** Volumetric configuration for the years (a) 1984 and (b) 1986 in a stretch of 36 ha of native forest no município de Jari in Amapá State.

which knocked down several large trees (surrounding area) and successional forest processes, significant in the occurrence of a change in the spatial configuration in the NE-E direction. It is observed that a decrease in the mean volume (30 - 35 m³. ha-¹) was explained by the successional process.

## Volumetric scenario after silvicultural treatments

The forest scenery had a major change in 1994 (Figure 4) with the predominance of the moderate volume (35-40 m³. ha-¹). This scenery occurred through the application of silvicultural treatments that reduced the basal area of non-commercial trees. The presence of isolated spots was observed in the spatial configuration oriented in the NW-SE direction, of the low volume (25-30 m³. ha-¹) and high volumes (40-45 m³. ha-¹).

It is important to note in these maps that there is the generation of points (islands) with volume value inside them for exploration and volume values below 30 m³. This is because the surface generated by the Spline method is adjusted by control points, assuring the continuous junction of the observed points, being appropriate for a phenomenon to be interpolated with gradual variations in its values. It was observed that there

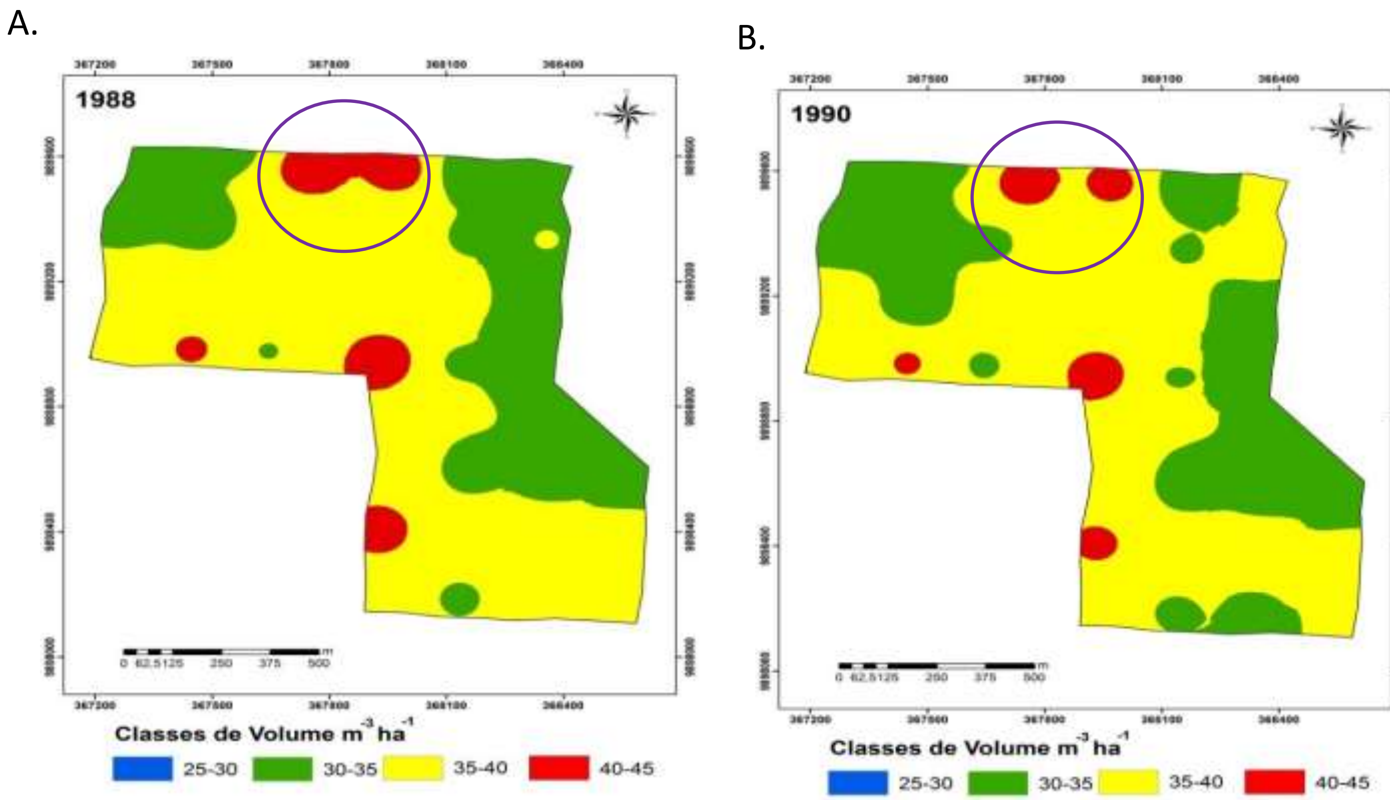


**Figure 3.** Volumetric configuration for the years (a) 1986 and (b) 1990 in a stretch of 36 ha of native forest in Amapá State.

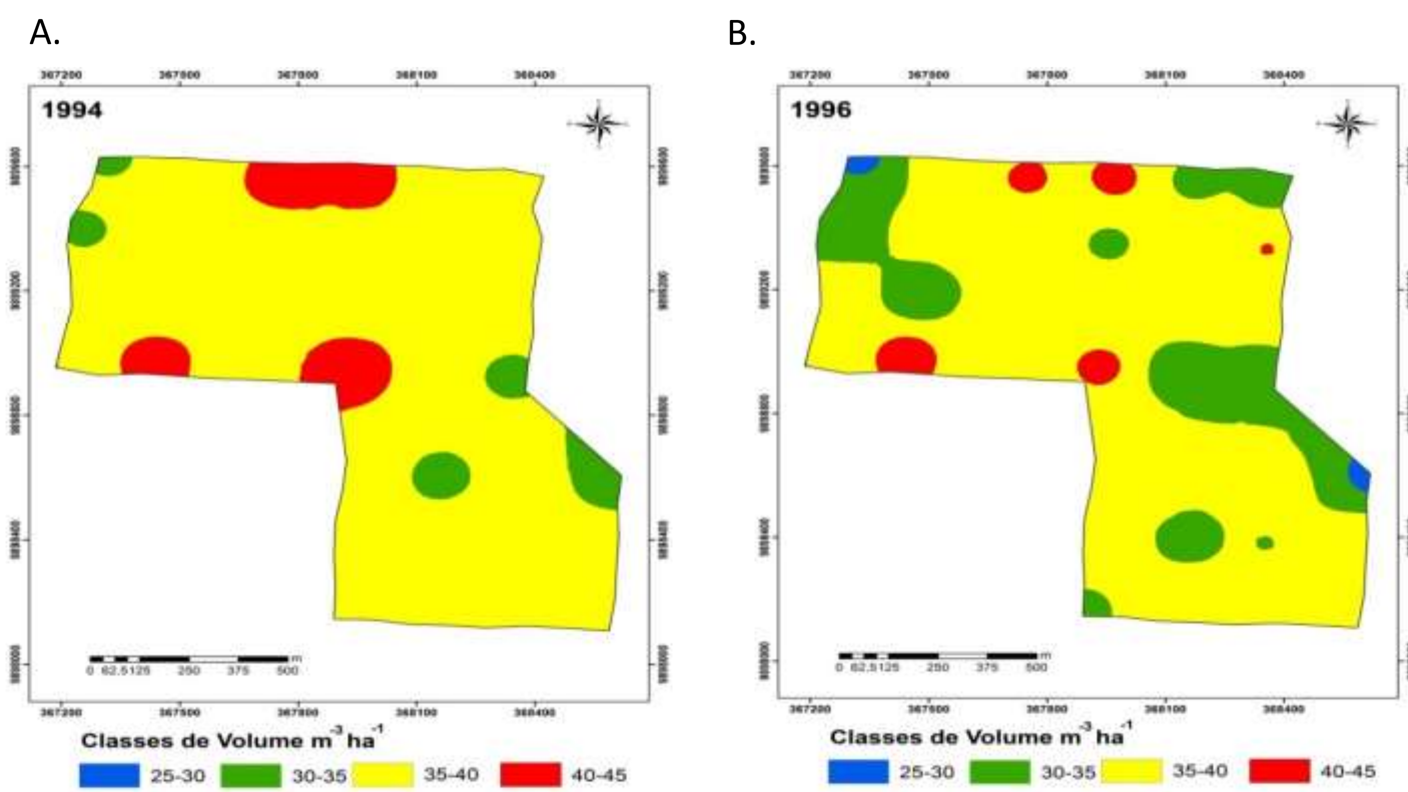


**Figure 4.** Volumetric configuration for the years (a) 1994 and (b) 1996 in a stretch of 36 ha of native forest in the Amapá State.

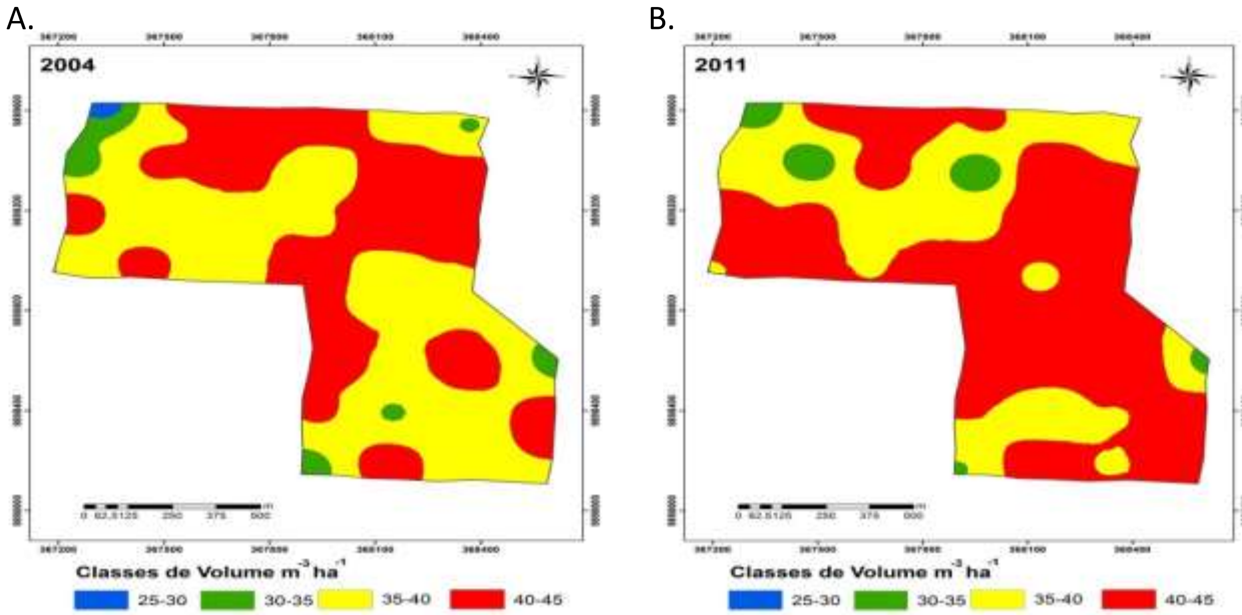


**Figure 5.** Volumetric configuration for (a) 2004 and (b) 2011 in a 36 ha stretch of native forest in the Amapá State.

was no statistical difference between the years of 1994 and 1996, for volumes above 40 m³ ha-¹. These sceneries are represented with the same scale and same caption classification. Note that there is the largest accumulation of mean volume in the NW-SE direction.

The forest scenery remained in 1996, with the predominance of the moderate volume (35-40 m³. ha-¹) with emergence of several high volume points and a slight increase in the mean volume (30-35 m³. ha-¹) concentrated in the extremities. This occurred through the application of silvicultural treatments in 1994 that reduced the basal area of the trees. Two points of the low volume (25-30 m³. ha-¹) were observed in a spatial configuration oriented in the NW-SE direction and the reduction of points in the high volumes (40-45 m³).

From 2004 onwards, this scenario begins to change with reference to the dominance of the high volume (40-45 $m^3$. $ha^{-1}$) presenting a spatial configuration oriented in the direction N-E and N-S and five other isolated islands. The moderate volume configuration (35-40 $m^3$ $ha^{-1}$) predominates over the high volume (40-45 $m^3$. $ha^{-1}$) with the appearance of several medium volume points and only a low volume point located at the extremities. 2011 shows a small increase in the high volume scenario (40-45 $m^3$. $ha^{-1}$), surpassing the moderate scenario. In this year, the spatial configuration is oriented in the sense N-E and N-S added to four other isolated islands of the average volume (Figure 5).

The forest inventory applied to the interpolation method for the production of spatial maps was efficient, determining the point of greatest volume production (m³) in the stands and the year of greatest variation in percentage (1.7%) in total volume, indicating that this precision tool can be used in future analyzes of volume in the forest inventory to 100%.

There were no significant statistical differences for volume in the first 20 years after the exploration (1986, 1988, 1990, 1994 and 1996). This occurred only from 2004, twenty years after the forest harvest. With this, the capacity of recovery of the forest around two decades was verified. Therefore, only forest biomass was considered. This cannot be understood with the biomass of the commercial species of the structure observed before the exploration.

The results obtained by the spatial maps allow identifying which are the units of samples more productive to do the initial planning of the forest exploitation. In addition, the results allow identifying less productive areas to plan interventions (silvicultural treatments) and to provide volume increase (m³).

Making the difference in commercial volume between 1984 and 2011, positive and negative values were obtained, and it was found that 76.92% of commercial species did not recover their initial volume. The species that were able to recover their volume over the 27 years were: *Carapa guianensis* (4,4769 $m^3$/ha), *Caryocar villosum* (1.2969 $m^3$/ha), *Dinizia excelsa* (0.8161 $m^3$/ha), *Licaria crassifolia* (4.4097 $m^3$/ha), *Qualea paraense* (2.4453 $m^3$) and *Ruizterania albiflora* (7.651 $m^3$). The species that came close to regaining their initial volume were: *Hymenelobium excelsum* (1.0912 $m^3$), *Hymenelobium petraeum* (0.2422 $m^3$), *Hymenelobium sericeum* (2.4872 $m^3$), *Manilkara bidentata* (17.5454 $m^3$), *Nectandra micranthera* (11.9406 $m^3$), *Platymiscium* sp.

(2.0828 $m^3$), *Pouteria oppositifolia* (19.7799 $m^3$), *Pouteria* ssp. (69.0232 $m^3$), *Vatairea* sp. (4.2838 $m^3$) and *Vouacapoua americana* (0.4692 $m^3$).

## DISCUSSION

There is a growing demand for information on appropriate forestry techniques for native forests, especially information that takes into account trees that have fallen below the permitted cutting limit, with behaviors different from commercial species, with low increment, scarcity in number and volume of species new potentials (Azevedo et al., 2008).

Precision forestry aims to survey productivity spots within a field, identify potential areas for 2nd cut, make localized decisions, minimize costs, improve resources and productive activities, minimize environmental impacts, increase productivity and maximize profits (Ortiz et al., 2006).

In this study, the growth rate of forest species was 1.7% in 2011. This figure can be explained by the number of undesirable competing individuals that were removed, whose crowns were competing for light with the tree tops of species selected for the next harvest; and by reducing the basal area of undesirable species in order to reduce competition in the population, in general (Azevedo et al., 2012).

In this study, the three interventions that took place in the area (logging in 1985, the fall of trees caused by the windstorm in 1990 and the application of silvicultural treatments in 1994) enhanced the emergence of multiple timber species, multiple products and by-products of flora, favoring the natural regeneration of forest species, growth in height and basal area, contributing to the forest keeping its characteristics close to its original state.

The challenge of discussing a new timber harvesting proposal through spatial maps, verifying where the species with the largest basal area and the highest initial volume for the second cut can be found, may become an alternative to maintain the balance of the most commercial species exploited in the Amazon to avoid extinction.

Our biggest challenge is choosing new species to make up the second harvest cycle, since not all exploited species can recover the initial commercial volume of the first harvest. However, there are numerous publications focusing on the botany, phytosociology, ecology, technology of wood and little on subjects that select potential species for a second crop. Table 3 shows the commercially exploited forest species that succeeded and could not recover the initial volume, and those that were close to recovering the initial volume.

According to De Avila et al. (2015), the high intensity of exploitation coupled with the reduction of the basal area can cause damage to the forest harvest that can substantially influence the tree community (DBH ≥ 10 cm). One way to combat this environmental damage is to determine the minimum level of interventions and to avoid strong thinning. This can improve ecosystem recovery and maintain biodiversity at other trophic levels.

According to Silva et al. (2006), the diameter growth and the volumetric productivity of the dryland forests of the eastern Amazon are low. These factors should be taken into account when establishing cutting cycles in management plans. For a forest to be considered sustainable and have the same yield we must avoid heavy logging (80 $m^3$/ha) which would take a long time to recover. Very long cutting cycles are economically unfeasible. In the present study, after 27 years of logging, the volume of wood from 40-45 $m^3$. $ha^{-1}$ recovered.

Silva et al. (2006), studying growth models carried out in Flona do Tapajós with a cutting intensity of 75 $m^3.ha^{-1}$, in 30-year cutting cycles, considered a 200-year period with cuts of less than 0 to be simulated 0,7 $m^3.ha^{-1}.year^{-1}$. Sustainable production of 27 – 28 $m^3$ $ha^{-1}$ in 30-year cycles has been shown to be possible; and after harvesting in a second cutting cycle it is recommended to include 60 - 70% of potential species.

According to Braz et al. (2012), studying forest growth in the State of Amazonas, found that the time required for non-commercial species (DAP ≤ 35 cm) to enter commercial classes (DBH ≥ 45 cm) was 19 years. Subjects less than 35 cm DAP did not cooperate for volume in the first post-cut cycle. Only species from 35 cm of DBH can reach the minimum diameter (50 cm) for a second exploration.

A forest management plan aimed at the conservation of native species has to take into account the dynamics of forest growth Schaaf et al. (2005) and the maximum exploitation limit per species. These factors must be evaluated individually prior to exploration. For this, account should be taken of 100% forest inventory information. In addition, the evaluation of natural regeneration according to its structure is considered for each species. The classification of the species becomes the possibility to compose the cut rate.

And finally, the effect of the interventions that occur in this work can recover the total and commercial volume and basal area of commercial species and favor the emergence of new species with potential in the timber market, which can significantly increase the growth rates of commercial and potential trees (Wadsworth and Zweede, 2006).

## Conclusions

From the spatial distribution of the forest species, the productive potential of wood in its points of greatest volume was verified. Thus, the biomass growth and the interventions that took place during these periods were taken into account. Productivity data are generated to better plan the infrastructure for the future forest harvest, with greater productive efficiency and lower impacts to the forest.

**Table 3.** List of species exploited in 1985 and their respective commercial volumes and their variation in loss and gain in biomass ($m^3$ ha) after 27 years of exploitation in Jari native forest, AP.

| Species | 1984 | 1986 | 1988 | 1990 | 1994 | 1996 | 2004 | 2011 | 1984-2011 |
|---|---|---|---|---|---|---|---|---|---|
| *Bowdichia nítida* | 4.79 | 3.89 | 3.89 | 3.90 | 3.92 | 3.71 | 3.74 | 2.19 | -2.60 |
| *Carapa guianensis* | 3.85 | 3.13 | 3.17 | 3.38 | 3.21 | 3.60 | 4.00 | 4.48 | 0.62 |
| *Caryocar villosum* | 1.19 | 0.86 | 0.86 | 0.86 | 1.05 | 1.06 | 1.10 | 1.30 | 0.10 |
| *Combretaceae* | 2.64 | 1.50 | 1.51 | 1.52 | 1.54 | 1.55 | 1.35 | 1.36 | -1.28 |
| *Dinizia excelsa* | 0.78 | 0.39 | 0.39 | 0.56 | 0.57 | 0.58 | 0.80 | 0.82 | 0.04 |
| *Dipteryx odorata* | 14.31 | 9.90 | 9.93 | 9.95 | 10.02 | 9.63 | 8.76 | 7.35 | -6.96 |
| *Goupia glabra* | 54.86 | 37.16 | 36.59 | 36.37 | 35.14 | 34.80 | 35.47 | 34.43 | -20.43 |
| *Handroanthus serratifolius* | 5.40 | 4.89 | 4.90 | 4.70 | 4.90 | 4.91 | 4.60 | 4.19 | -1.21 |
| *Hymenaea courbaril* | 11.17 | 4.29 | 4.31 | 4.51 | 4.73 | 4.75 | 4.87 | 3.92 | -7.25 |
| *Hymenolobium excelsum* | 1.88 | 1.38 | 1.38 | 1.39 | 1.41 | 1.42 | 1.09 | 1.09 | -0.79 |
| *Hymenolobium petraeum* | 0.75 | 0.20 | 0.18 | 0.18 | 0.20 | 0.20 | 0.23 | 0.24 | -0.51 |
| *Hymenolobium sericeum* | 2.96 | 2.30 | 2.34 | 2.54 | 2.52 | 2.55 | 2.59 | 2.49 | -0.48 |
| *Licaria crassifolia* | 3.17 | 2.61 | 2.45 | 2.47 | 2.88 | 3.95 | 4.50 | 4.41 | 1.24 |
| *Manilkara bidentata* | 19.57 | 17.26 | 17.37 | 16.49 | 17.08 | 17.17 | 16.52 | 17.55 | -2.02 |
| *Manilkara huberi* | 57.20 | 40.11 | 39.44 | 39.08 | 38.65 | 39.07 | 37.60 | 36.19 | -21.01 |
| *Não identificada* | 14.99 | 13.07 | 12.01 | 9.78 | 8.11 | 6.35 | 2.44 | 4.21 | -10.78 |
| *Nectandra micranthera* | 12.64 | 11.06 | 10.69 | 10.41 | 11.22 | 11.39 | 13.23 | 11.94 | -0.70 |
| *Platymiscium* sp. | 2.09 | 1.60 | 1.62 | 1.81 | 2.02 | 2.03 | 2.25 | 2.08 | -0.01 |
| *Pouteria oppositifolia* | 23.09 | 22.08 | 22.21 | 22.16 | 21.85 | 21.62 | 21.76 | 19.78 | -3.31 |
| *Pouteria* ssp. | 77.81 | 75.04 | 72.37 | 69.70 | 69.77 | 69.00 | 69.49 | 69.02 | -8.78 |
| *Qualea paraensis* | 2.26 | 1.67 | 1.70 | 1.42 | 1.64 | 1.66 | 2.33 | 2.45 | 0.19 |
| *Ruizterania albiflora* | 7.16 | 5.67 | 5.68 | 5.13 | 5.60 | 5.72 | 7.30 | 7.66 | 0.50 |
| *Terminalia* ssp. | 3.02 | 2.17 | 2.18 | 2.20 | 2.40 | 2.42 | 2.48 | 2.86 | -0.16 |
| *Trattinnickia rhoifolia* | 7.73 | 6.79 | 6.86 | 6.88 | 7.21 | 0.44 | 7.37 | 6.59 | -1.14 |
| *Vatairea* sp. | 4.93 | 4.18 | 4.23 | 4.12 | 4.19 | 4.23 | 4.58 | 4.28 | -0.65 |
| *Vouacapoua americana* | 0.67 | 0.41 | 0.41 | 0.42 | 0.43 | 0.44 | 0.46 | 0.47 | -0.20 |
| Total | 340.92 | 273.59 | 268.67 | 261.94 | 262.26 | 254.24 | 260.92 | 253.34 | -87.58 |

The application of interpolation methods tends to result in spatial maps with precise information, especially when dealing with commercial volume of tree species, becoming a very important tool for forest management. Therefore, new tools for forest management that help in the recognition and monitoring of forest stands generate reliable and important information for forest management, contributing to the sustainability of the ecosystem, increasing the yield of available resources, besides reducing wood waste and operating costs in the exploration.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Fertilizer management and nutrient use efficiency on rice paddy in integrated system

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**Nitrogen budget under integrated production system in fish farming is one of the acceptable practices in maintaining ecological balance and circulation. This result reveals the patterns of pond water and nutrient flows which were strongly influenced by the main fish farming activities resulting to large amount of nitrogen inputs. The main nutrient input sources of nitrogen into the system were found to be pig manure, feed and maggot while outputs were in water, soil, harvested fish and rice; and also in unaccounted forms as a result to discharge. Nutrient composition of rice paddy in integrated production system records 17% N, 19% P and 64% K. The NPK value recorded in integration production system throughout the period of the experiment showed significant difference (p>0.05). There was a positive correlation between nitrogen in rice and rice growth r=0.8373 and 0.7709. The Increase in nutrient is correlated with the increase in the rating of rice growth. The increase in nutrient of unaccounted forms which accumulate in the sediments absorbed by the plant from the soil plays an important role in balance of an aquaculture system. Thus, in order to ensure sustainable productivity there is need to enhance the management of all the nutrient input channels while minimizing the nutrient output through crop intensification.**

**Key words:** Nutrient budget, nutrient input, nutrient output, pond effluent, soil, amendment.

## INTRODUCTION

The aquaculture industry faces growing pressure to operate under strict environmental safety standards. These standards lead to the development of integrated agriculture-aquaculture systems, designed to maintain a high biological carrying capacity (Twarowska et al., 1997; Thoman et al., 2001). Water exchange and cost in these systems are minimized through the use of biological, chemical and nutrient efficiency in the pond system. Fish farming has been an important development in recent decades in response to the growing global market demand (Costa-Pierce, 2002). Meeting the demand for fish farming in production systems has developed ranging from extensive to semi-intensive with increasing use of artificial food and high water quality (Crab et al.., 2007). Previous reports on nutrient budget reveal that 90% of nitrogen and phosphorous inputs is in the form of feed,

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whereby the major portion is lost and less being assimilated as biomass. The nitrogen waste such as ammonia and nitrite when produced during culture exceeding its recommended capacity in open waters leads to deterioration of water quality. Nevertheless, crop wastes accumulate in water, as in the case of uneaten food and metabolic wastes that increase as time goes on with the intensification of the cropping system (Lin, 1995). Previous studies show that nutrient balance in an open system for tilapia culture with only 21.4 and 18.8% nitrogen and phosphorus incorporated in the feed are retrieved at the time of harvest; the rest are losses that are downloaded as metabolic contaminants through effluent (Siddiqui and Al-Harbi, 1999). The residual nitrogen produced in the culture system (eg ammonia and nitrites) generally exceeds the assimilative capacity of the system, which impairs water quality creating a toxic environment for tilapia (Avnimelech, 1999; Hargreaves and Kucuk, 2001). The outcome of an integrated agriculture-aquaculture system is based on the balance between production and waste assimilation capacity in the pond environment which results into sustainable scale and gives account for the impact of its waste and growth of culture of aquatic organisms (Martins et al., 2010). The basic steps to establish a balance of nutrient in an integrated system is to evaluate the efficiency of fertilizer use, uneaten feed, crop waste accumulation in water, metabolic waste, water quality in biological and physio-chemical processes (Avnimelech and Kochba, 2009).

Problem associated with integrated system is the nutrient discharges effluent from fish farms which causes rapid eutrophication in ponds and receiving water bodies, along with organic matter accumulated during the growing season, and its impact has been a major environmental concern (Phillips et al.., 1993; Piedrahita, 2003). The approach used to mitigate the impact to the environment through the discharge and disease spread by contaminated water is using water recycling in integrated agriculture-aquaculture systems (Timmons et al., 2002; Piedrahita, 2003) thereby manipulating the environment to improve nutrient efficiency of the farm as a whole (Prein, 2002; Lightfoot et al., 1993). This is perceived as a type of integrated resources management which encourages the increase in production through understanding of chemical and biological processes of the ponds (Boyd, 1986). The pond sub-system should be integrated as much as possible with activities to maximize production while minimizing nutrient discharges.

## MATERIALS AND METHODS

The experiment was carried out at the University of Ibadan in the Department of Aquaculture and Fisheries Management, fish farm. The research facilities involved an earthen pond with rice paddy, maggot house and pig sty.

Pond sedimentation was sampled at a depth of 20 cm in two (2) sampling locations A and B in rice paddy. Soil samples were collected initially, weekly monitored and at harvest. The composite sediments samples were air-dried at room temperature; sample was dried at 105°C to a constant weight for the determination of bulk density (Boyd, 1998), then sampled for further chemical analysis: Organic matter (Walkley-Black), total nitrogen (Kjeldahl), available phosphorus (Bray-2) and exchangeable potassium ($BaCl_2$ 0.1 N solution). The data were aggregated to estimate soil nutrients stocks of the Integrated farming systems.

Other parameters of samples were analyzed and recorded to determine nutrient contents in fish feed, maggot, water (ground water, rain water) and pig manure. Representative samples of each material used as a nutrient input to the pond were analyzed.

### Pond nutrient budget

The nutrient inputs were separated into on-farm and off-farm sources. The on-farm sources are: pig manure and maggots. The off-farm sources included: fish stocked, commercial fish feed, and nutrients introduced through water recharge before stocking and rainfall into the pond. The nutrient outputs were separated into harvests and losses. The harvests included: fishes, rice yield, rice plant and aquatic plant. The losses included: outflow water (leakage, evaporation), pond effluent, and accumulation in pond sediments.

### Nitrogen budget

Input = Output ± Unaccounted

Where Input is: fish feed, maggot, and pig manure and water exchange; Outputs are: soil, pond effluent, and fish; unaccounted: are assumed as taken by rice. The general balance equation was calculated according to the methods of Nhan et al. (2007) and Teichert-Coddington et al. (2000).

WE in + Fert in + Fe in = PE out + S out + F out ± UN

Calculated as described above; WE- –water exchange; Fert - fertilizer (Pig manure); Fe- fish feed; PE- pond effluent; S- soil sediment; F- fish; UN- unaccounted nutrient (rice yield, rice plant).

### Statistical analysis

Descriptive statistics was used to present growth performances. Correlation and regression was used to analyse the nutrient budget data. Correlation and regression were used to investigate relationships of fish, rice growth data and water quality data.

## RESULTS

### Soil characteristics

The nature of soil component was measured before and during the paddy growing period. The composited soil samples in the rice fish field after 16 weeks of culture period showed reduction in the amount of silt and increase in clay content in line with Kajiru et al. (2015) and Frei et al. (2007). In this study, the textual classes of the soil ranged from sandy clay loam to loamy sand.

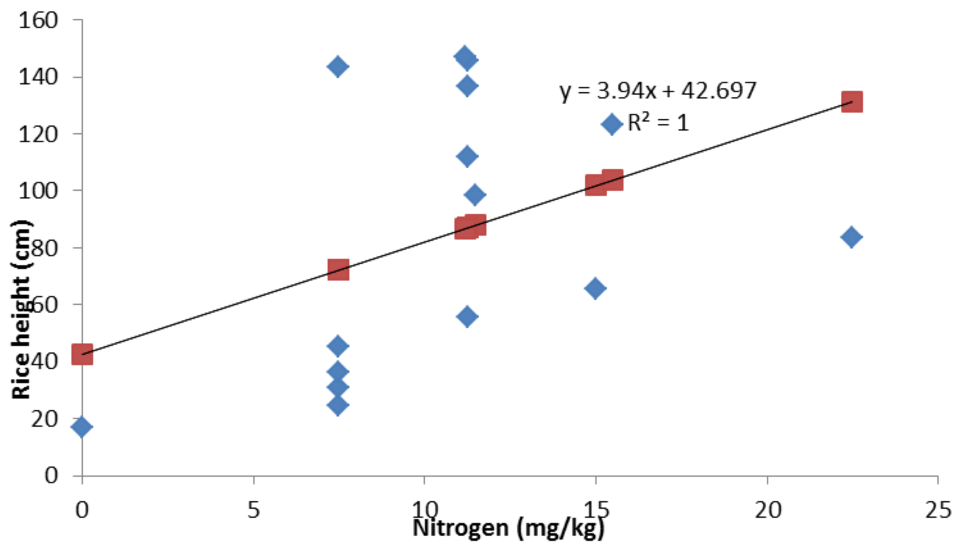


**Figure 1.** The relationship between rice plant height and nitrogen for sixteen weeks.

**Nutrient inputs**

Laboratory analysis for nutrients in pig manure indicated that the manure samples contained 0.124% nitrogen, 1.848% phosphorus, 0.685% potassium, 8.040% calcium and 1.610% magnesium. Fish feed nutrient indicated 45% protein, 14% fat, 7 - 8% ash, 2.6 - 2.7% fibre, 1% phosphorus while maggot (supplementary feed) indicated 4.75% protein, 2.5% fat, 0.755% ash and 0.477% fibre.

**Nutrient amendment**

Mean nitrogen concentration was recorded as 9.89±5.80 mg/kg. The nitrogen concentration in rice paddy before transplant was 12.1 mg/kg followed by a decline in the study period and remained low at 7.5 mg/kg (Figure 1) till six weeks of the rearing as shown in Table 2. Nitrogen concentration increase (22.5 mg/kg) trend in the later part of the study which showed highest concentration level in week nine. Available phosphorus concentration in paddy fluctuated as shown in Table 2 without any definite trend and concentration in rice paddy before transplant was 12.1 mg/kg which showed an increase during the rearing period. The mean phosphorus concentration was recorded as 11.46±5.88 mg/kg. The highest concentration (18.5 mg/kg) was recorded in week six, eight and nine and dropped remarkably at week sixteen (2.3 mg/kg). Potassium concentration in rice paddy before transplant was 31 mg/kg which showed an increase during the rearing period. Mean nitrogen concentration was recorded as 38±21.9 mg/kg. Potassium concentration showed alternate decline and surge throughout the study period. An exponential increase in potassium concentration (80 mg/kg) was observed in paddy from week eight followed by a sharp decline of 17 mg/kg at the end of rearing cycle. Organic carbon concentration was recorded as 134.35±41.57 mg/kg. Over the study period, organic carbon concentration in rice paddy before transplant was 103.05 mg/kg followed by an increase during the rearing cycle. A sharp increase (181.5 mg/kg) was recorded from week nine of the experiment followed by a decline toward the end of the rearing cycle. Organic carbon contents translate to organic matter contents in the soils. The organic matter present in rice paddy was 10.31 mg/kg before transplant and at harvest was 12.69 mg/kg; which showed a steady increase and decline towards the rearing cycle. The rice paddy pH values ranged from 6.4 (slightly moderate acidic) to 7.75 (neutral soil reaction) with a mean pH of 6.77±0.47. The optimum soil pH for rice production ranges from 5.5 to 8.92, from neutral soil to alkaline (Landon, 1991) which can be produced in non-irrigated condition and under flooded condition system. pH values remained within the recommended range all through the rearing cycle. The pH value before planting was 6.45 and at the last planting season, pH was recorded as 6.74. The pH value obtained in this study showed significant difference ($p>0.05$) and it was seen that there was a positive relationship.

**DISCUSSION**

The soil characteristics presented in Table 1 reveal an increase in clay content. It has being recorded that clay content is suitable for rice production because of their capacities to retain plant nutrient and soil water (moisture). These high clay content restrict the percolation of water through soil, hence encouraging ponding of bundled fields (Kajiru et al., 2015) as well as extends and improves the water use efficiency of the harvested rainwater by the rice plant. This can be due to nutrient

**Table 1.** Rice paddy soil characteristics samples in integrated culture system.

| Parameter (unit) | Before cultivation (initial) | Final |
|---|---|---|
| **Texture** | | |
| Sand (%) | 84.2 | 83.6 |
| Silt (%) | 12.4 | 7.0 |
| Clay (%) | 3.4 | 9.4 |
| Texture | Loam | Loam |
| | | |
| Organic carbon (mg/kg) | 1.03±0.1 | 0.80±0.7 |
| Organic matter (mg/kg) | 1.77±0.2 | 1.37±0.2 |
| Total nitrogen (mg/kg) | 12.2±3.7 | 11.2±14.1 |
| Available phosphate (mg/kg) | 9.52±14 | 2.39±0.3 |
| Potassium (mg/kg) | 31±42 | 17±28.3 |
| pH (mg/l) | 6.5±0.1 | 6.7±0.2 |

**Table 2.** Mean value of rice paddy soil characteristics and nutrients in integrated culture system.

| Week | Nitrogen | Phosphorus | Potassium | pH |
|---|---|---|---|---|
| Initial | 12.2±3.7 | 9.52±1.4 | 31±42 | 6.5±0.1 |
| 3 | 7.5±0 | 12.5±0 | 30±0 | 7.5±0 |
| 4 | 7.5±0 | 12.5±0 | 30±0 | 6.75±0 |
| 5 | 7.5±0 | 12.5±0 | 110±70.7 | 6.75±0 |
| 6 | 7.5±0 | 37.5±17.7 | 30±0 | 6.75±0 |
| 7 | 22.5±10.6 | 12.5±0 | 80±0 | 6.75±0 |
| 8 | 15±0 | 37.5±17.7 | 80±0 | 7±0 |
| 9 | 45±21.2 | 37.5±17.7 | 80±0 | *6.25*±0 |
| 10 | 11.5±7.0 | 10.85±0.5 | 37.5±4.5 | 6.3±0.1 |
| 11 | 22.5±10.6 | 12.5±0 | 80±0 | **7**±0 |
| 12 | 15.5±7.0 | 13.8±0.4 | 48.5±0.7 | 6.8±0.1 |
| 13 | 22.5±10.6 | 12.5±0 | 80±0 | 6.75±0 |
| 14 | 7.5±0 | 12.5±0 | 30±0 | 7±0 |
| 15 | 22.5±10.6 | 12.5±0 | 30±0 | 6.75±0 |
| 16 | 11.2±14.1 | 2.39±0.3 | 17±0.7 | 6.7±0.2 |

accumulation from ammonia present in water. The nitrogen value recorded in integration production system throughout the period of the experiment showed significant difference ($p>0.05$) and it was seen that there was a strong positive relationship.

On the whole, the mean soil nutrients recorded as shown in Table 2 during the study were within the range reported by Nwilene et al. (2008) for optimum growth and rice production. Phosphorus obtained in this study showed no significant difference ($p<0.05$) and it was seen that there was a positive relationship. Since rice plant is a low demanding crop, the observed plant available phosphorus value would satisfy the phosphate demand by the rice. In addition, the availability of phosphorus in rice paddy is a function of soil pH (Inusa et al., 2013; Kajiru et al., 2015). Hence, the availability of the soil phosphorus may be negatively affected by the high pH values of the soils. The potassium recorded in integrated production system through the period of experimental study showed significant difference ($p>0.05$). On the whole, the mean soil nutrients recorded in this study were within the range reported by Nwilene et al. (2008) for optimum growth and rice production. Under tropical and subtropical conditions, requirement for N, $P_2O_2$ and $K_2O$ are reported to be 80, 30 and 30 kg/ha respectively. This variation in organic matter in soil influence physical, chemical and biological properties of soil such as soil texture, water retention, nutrient content and retention and microbial activities in soil (Frei et al., 2007). Application of livestock manure increases soil organic matter content, leading to improved water infiltration and water holding capacity, as well as an increased cation exchange capacity. Rasowo et al. (2008) reported that manure and urine raise the pH level and accelerate the decomposition of organic matter and activity. It is reported that cultivation of rice is even possible with the

**Table 3.** Total Nitrogen budget used in the nutrient input and output calculations.

| Week | Input | | | | | Output | | | | Unaccounted |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water | Pig Manure | Feed | Maggot | Total | Water | Soil | Fish | Total | |
| 0 (g/kg) | 2.75 | 1.24 | 91.20 | 47.40 | 142.59 | 2.99 | 1.22 | 45.60 | 49.81 | 88.57 |
| % | 1.96 | 0.87 | 63.78 | 33.57 | 100.00 | 2.10 | 0.86 | 32.00 | 34.96 | 65.04 |
| 4 (g/kg) | 2.99 | 0 | 72.00 | 47.40 | 122.39 | 2.99 | 0.75 | 36.00 | 39.74 | 78.95 |
| % | 2.50 | 0 | 58.80 | 38.70 | 100.00 | 2.44 | 0.61 | 32.00 | 35.05 | 61.90 |
| 7 (g/kg) | 0.01 | 0 | 72.00 | 47.40 | 119.41 | 3.97 | 1.15 | 43.20 | 48.32 | 65.97 |
| % | 0.01 | 0 | 60.30 | 39.70 | 100.00 | 3.32 | 0.96 | 36.00 | 40.28 | 55.44 |
| 10 (g/kg) | 0.02 | 0 | 72.00 | 47.40 | 100.00 | 0.02 | 1.15 | 28.00 | 29.16 | 89.10 |
| % | 0.01 | 0 | 60.30 | 39.70 | 100.00 | 0.01 | 0.96 | 31.00 | 30.97 | 68.06 |
| 13 (g/kg) | 0.02 | 0 | 72.00 | 47.40 | 119.42 | 0.02 | 1.15 | 29.00 | 30.17 | 80.08 |
| % | 0.01 | 0 | 60.30 | 39.70 | 100.00 | 0.01 | 0.96 | 24.00 | 25.16 | 73.68 |
| 16 (g/kg) | 0.02 | 0 | 72.00 | 47.40 | 119.42 | 0.01 | 1.12 | 29.00 | 30.13 | 88.15 |
| % | 0.01 | 0 | 60.30 | 39.70 | 100.00 | 0.01 | 0.94 | 24.00 | 24.95 | 74.10 |

**Table 4.** Nutrient budget for sixteen weeks in the fish cum rice and pig Integrated production system.

| Weeks | Unaccounted (g/kg) | Mean Rice Height (cm) |
|---|---|---|
| Initial | 88.57 | 10.12±2.12 |
| 4 | 78.95 | 30.67±3.12 |
| 7 | 65.97 | 55.51±5.53 |
| 10 | 89.10 | 98.33±9.68 |
| 13 | 80.08 | 136.51±10.40 |
| 16 | 88.15 | 146.97±11.65 |

pH up to 9.0 but high pH values of the soil could negatively influence the availability of the micronutrient as well as phosphorus (Kajiru et al., 2015).

Nitrogen budget in integrated production system over the study period revealed that fish feed as the major input of nitrogen is shown in Table 3. Nitrogen in form of feed ranged from 58.80 to 63.78% and maggot input through feed ranged from 33.57 to 39.70% of the total inputs; the result is in agreement with Nhan et al. (2007) and Prein (2002). It has being reported previously that the predominant inputs of nitrogen in water exchange unit are feed, which accounted for 82-95% nitrogen of total inputs. Feeds nitrogen gain by ponds occurred primarily from feed 36% and fertilizer at 1.1% in the present study; also, contribution of feed to the total nutrient inputs is in the same range as reported. In addition, nitrogen through feed was significantly higher throughout the study period. Nutrient budget showed that some portions of the nutrient were deposited in soil followed by nutrient contained in water at harvest, and relatively large fractions were unaccounted as observed in Table 3. Percentage nitrogen accumulates into the soil and water (effluent) ranged from 0.61 to 0.96% and 0.01 to 3.97%. In addition, nutrient budget revealed that during the rearing cycle, large percent of the nitrogen went unaccounted for. Unaccounted nitrogen ranged from 95.75 to 99.05% of the total inputs. Moreover, further accounting of the nutrient budget revealed that rearing 1 kg fish resulted into 72 g N loss throughout the study period. Nutrient budget showed in Table 3 that the total of inputs were incorporated into harvested fish and rice yield; the remainder in the system as uneaten feed, excreted material went to support high level of phytoplankton, heterotrophic activity and rice growth.

These unaccounted forms were deposited in fish and rice growth in line with Rukera et al. (2011) and Inusah et al. (2013) as shown in Table 4. It was reported that the major output of nutrient in water exchange ponds were in discharge water. This experiment showed in Table 3 that in an integrated production system losses of nutrient are through sediment and is higher than water borne loss. Integrated production system unit excess of nutrient input, which especially originated from eaten feed, keep accumulating in the system and in turn may support growth of natural food organism, ultimately fish growth and increase in the yield of rice. Furthermore, in this experiment it was observed that the increased unaccounted nutrient was absorbed by the plant from the soil. Rukera et al. (2011) mentioned that the sediment which accumulates the nutrient plays an important role in balance of an aquaculture system; it can act as a buffer in water nutrient concentration and helps in minimizing loss from the system. However, from the nutrient budget data, it is apparent that larger percentage of the nutrient inputs went unaccounted in the integrated production unit. The nitrogen content of the nutrient budget at the end of the experiment provides insight into the physical characteristics of potential effluent waste and magnitude of denitrification.

Boyd and Tucker (1998) mentioned that the most probable loss of nitrogen is by ammonia gas volatilization which is further enhanced by vigorous aeration and high pH and nitrogen fixation by blue-green algae. In this study, some of the potential sources of nitrogen were not accounted for such as inorganic nitrogen inputs through precipitation which was considered insignificant and nitrogen fixation by blue-green algae. In accounting for the nutrient budget, it revealed that rearing 1 kg fish resulted into 70 g (58%) of nitrogen used up in integrated production system.

The correlation coefficient was computed to assess the relationship between rice growth, fish growth and ammonia. There was a positive correlation between nitrogen in rice and rice growth r=0.8373 and 0.7709 as shown in Figure 1, leading to a strong positive correlation between the two variables. Increase in nutrient correlated with increase in the rating of rice growth. Meanwhile, a negative correlation between nitrogen in fish and fish growth r=-0.8367 leads to a strong negative correlation between the two variables.

## Conclusion

Thus, it is worth pointing out that the use of nutrient rich water such as nitrogen, phosphorus and total dissolved solids to irrigate alleviates a potential problem of pollution. Therefore, maintaining soil structure and fertility through its use, as well as the risk of soil degradation had been reduced. The study provides management practice in integrated production system for nutrient budget; this system unit nutrient input originated from eaten feed which accumulates and supports growth of natural food organism. The increase in unaccounted nutrient which accumulates in the sediments was absorbed by the plant from the soil, plays an important role in balance of an aquaculture system, acts as a buffer in water nutrient concentration and helps in minimizing loss from the system. Thus, in order to ensure sustainable productivity there is need to enhance the management of all the nutrient input channels while minimizing the nutrient output through crop intensification. Farmers need to better regulate water and nutrient flows between the pond and the other IAA-farm components to maximize the productivity and profitability while minimizing nutrient discharges of the whole farm.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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