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Full Length Research Paper

Effect of scattered tree species on the diversity, abundance and biomass of pastures in a sedentary grazing system in South-western Uganda

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Tree species play a significant role in sustaining the productivity of grazing lands. However, information on appropriate species to use in restoring degraded grazing areas is limited. This study used 120 trees to assess the effect of 8 tree species on pasture diversity, abundance and biomass. This was done in a total of 960 quadrats of 1 m^2 established under tree canopies and 5 m away from the edge of tree canopies. In each quadrat, the different pasture species and their ground cover were recorded. The pastures were harvested, weighed and their biomass recorded. Results of analysis by Shannon–Wiener's index indicated that pasture diversity was almost the same under and outside tree canopies (H = 1.8 and H = 1.78 respectively), but pasture abundance was significantly higher under tree canopies ($p < 0.05$). *Ficus natalensis* and *Albizia coriaria* had the highest pasture abundance under their canopies. Pasture biomass never varied significantly under and outside tree canopies but between tree species, *F. natalensis* had a significantly higher positive influence on pasture biomass than other species. It was discovered that *F. natalensis* and *A. coriaria* have a higher potential for restoring degraded grazing areas in South-western Uganda.

Key words: *Brachiaria* spp., *Ficus natalensis,* livestock, Shannon-Wiener, tree canopy.

INTRODUCTION

In the livestock dominated farming communities of South-western Uganda, natural pastures constitute the major feed resource for livestock throughout the year (Tibezinda et al., 2016). However, lack of sufficient pastures remains one of the leading constraints of the low livestock productivity in the area (Creemers and Aranguiz, 2019). Other than climate related effects such as prolonged droughts which have reduced the abundance of pastures, the predominant and intensive sedentary practice of livestock grazing can hardly favor sustainable production of natural forage (Selemani et al., 2012). Nonetheless, increasing the productivity of

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existing grazing space by increasing the quantity of pasture species remains one of the key options available for livestock farmers engaged in sedentary grazing.

In an effort to address the challenge of pasture shortage, livestock farmers in South-western Uganda resorted to indiscriminate cutting of trees; a practice which is increasingly being adopted by sedentary pastoralists in the current decade (Roba and Oba, 2013). Livestock farmers have hoped that by clearing trees, more pastures will grow in the created spaces and therefore increase availability of adequate forage for their animals. Unfortunately, the act only exacerbates the declining productivity of grazing sites (Mganga et al., 2019) and in the sedentary grazing areas of South-western Uganda, the diversity and quantity of forage pastures is increasingly declining (de Vries, 2019).

Globally, several studies have documented integration of trees in grazing lands as one of the remedies recommended to enhance the quantity of pasture species (Murgueitio et al., 2011). This is based on the fact that scattered tree species in grazing lands are reported to improve the microclimate below their canopies (Siqueira et al., 2017) for the benefit of the underlying community. In a study by Abdulahi et al. (2016), it was revealed that trees scattered in grazing lands modify light intensity, temperature and can influence mineralization of nutrients, which factors support growth and biomass yield of pasture species. Therefore, in degraded grazing areas where pasture productivity and associated grazing operations are constrained, restoration of tree cover will contribute towards improving the productivity of such lands.

However, despite the facilitative effect of trees towards pasture productivity, in some cases tree species in grazing lands have suppressed pasture growth and biomass accumulation under their canopies. This has been through reduction of light intensity and competition for soil moisture (Whitecross et al., 2017; Lozano-Parra et al., 2018; Castillo et al., 2020). With such inhibitory evidence, identification of tree species with a complimentary effect on pasture diversity and quantity and therefore be used in restoration initiatives can only be established through localized scientific investigation. Therefore, this study sought to identify tree species with potential for restoring the productivity of degraded grazing lands in South-western Uganda, by assessing the effect of selected scattered tree species on the diversity, abundance and biomass of natural pastures.

MATERIALS AND METHODS

Study area

This study was carried out in Ruborogota and Masha sub-counties in Isingiro district (0.8435° S, 30.8039° E) and Kenshunga and Kikatsi sub-counties in Kiruhura district (0.1928° S, 30.8039° E) in South-western Uganda (Figure 1). This was between February and March, 2019; a period which marks the onset of the first rainy season. In particular, the study districts experience a semi-arid type of climate with average annual rainfall of 750 - 800 mm distributed in a bimodal pattern (Nagasha et al., 2019). Average temperatures range from 17° - 30°C, with highest peaks recorded in January and July. Soils are sandy loamy and are predominantly covered by savannah grassland type of vegetation with scattered Acacia tree species. Livestock farming dominated by cattle is the major livelihood activity; characterized by Ankole Longhorn, Holstein Friesian and crosses as the major breeds usually grazed in a sedentary practice (Tibezinda et al., 2016).

Data collection

Selection of the study tree species and data collection sites

This study used eight indigenous tree species which included; *Acacia abyssinica Benth., Acacia campylacantha A. Rich., Acacia gerrardii Benth., Acacia hockii De Wild., Albizia coriaria Oliv., Allophylus africanus Davies. & Verdcourt., Ficus natalensis Krauss ex Engl.,* and *Grewia mollis Juss*. Selection of the tree species was through focus group discussions with livestock farmers in two livestock dominated sub-counties in each study district. In every sub-county, 15 livestock farmers who had a representative number of the prioritized tree species and had greater uniformity in terms of sedentary grazing were selected for field assessments.

Study design and data collection

At every farmers field, mature (>20 cm DBH) and healthy trees of the respective species were considered. Canopy radius of each study tree species was taken with a measuring tape to determine the canopy size of tree species. Using wooden square frames, four quadrats of 1 m^2 were established in four directions under the tree canopy. A distance of 5 m away from the outer most edge of the tree canopy was measured and corresponding quadrats of 1 m^2 were established and demarcated (Figure 2). In each quadrat, data was collected on the different types of pasture species encountered and their proportional ground cover (%). To determine pasture biomass, all growing plants in a quadrat were manually harvested to ground level and sorted to remove non-pasture plants. The sorted pastures were collected in a polythene bag and weighed using a sensitive digital weighing scale. A total of 120 trees and 960 quadrats were covered in this study.

Data analysis

The data collected was entered in MS Excel computer package where descriptive statistics in form of tables and graphs were generated. Within Excel package, Shannon-Wiener's species diversity index (H) was used to determine pasture diversity under and outside tree canopies and between tree species, as described by Sagar and Sharma (2012);

Shannon index (H) = $-\Sigma si = 1\ pi\ ln\ pi$

Where; p = Proportion (n/N) of individual species found, ln = Natural log, Σ = Sum of calculations,
s = Number of species.

To identify the dominant pasture species under and outside tree canopies, MS Excel was used further to compute the Simpson dominance index (D) of pastures following the formula described by Sagar and Sharma (2012).

$D = \Sigma si = 1\ pi^2$, with components described as above.

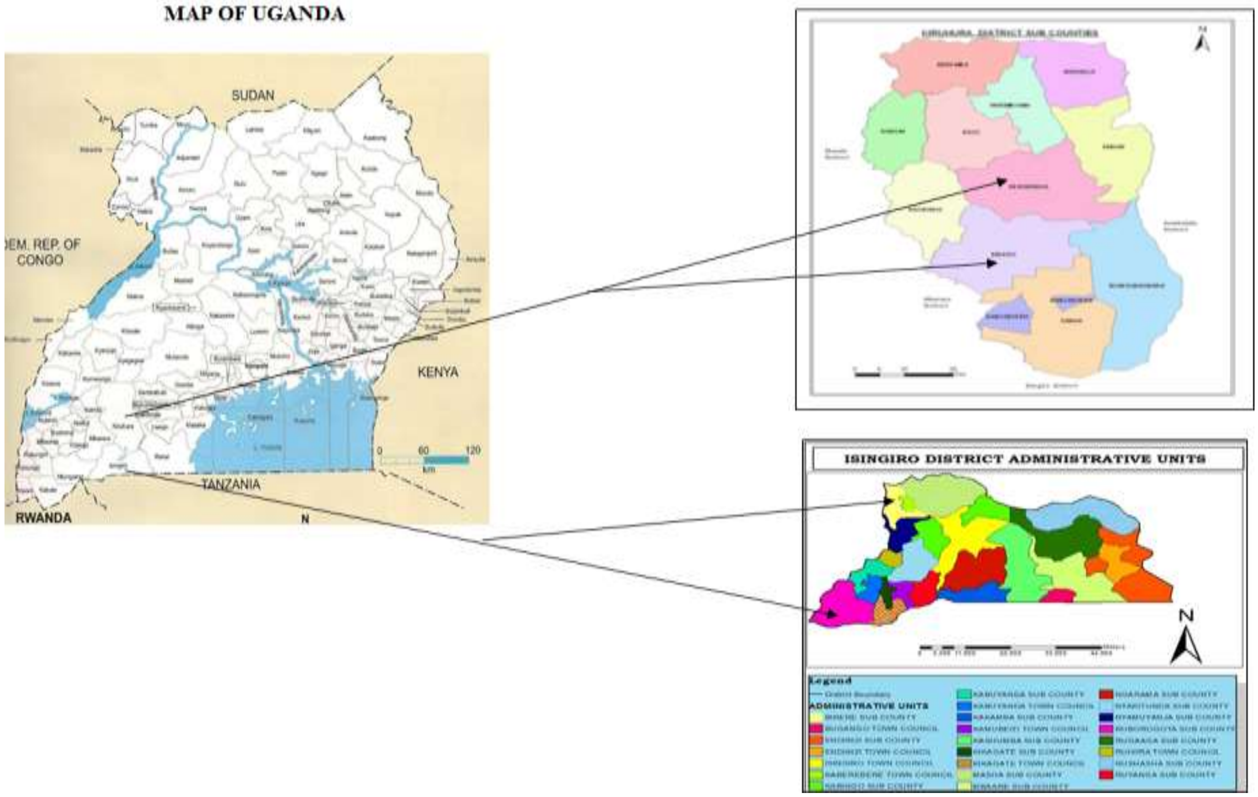

Figure 1. Map of the study area.

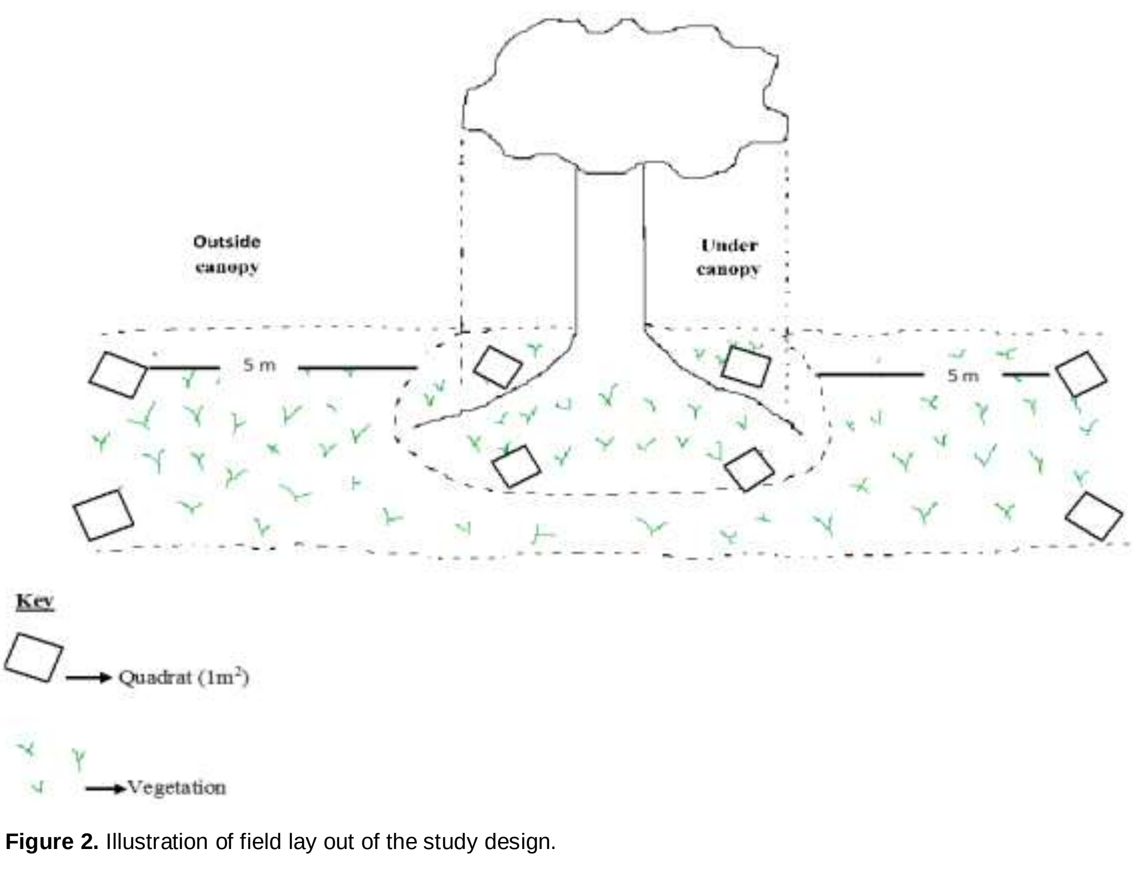

Figure 2. Illustration of field lay out of the study design.

To obtain statistical information on abundance and biomass of pastures, the data was imported into Minitab.19.0 software where it was subjected to Ryan-Joiner (similar to Shapiro-Wilk test) technique to test for normality. Any deviation from normal distribution was corrected using Johnson transformation technique. Analysis for differences in abundance and biomass of pastures under and outside tree canopies and between tree species was undertaken using Generalized Linear Models (GLM) at 95% CI. Fishers Least Square Difference (LSD) test at 95% CI was used to separate the means where significant differences existed. More so, the data was imported into R software and using the vegan package (Oksanen et al., 2019), rarefaction curves were generated to display pasture species richness under and outside tree canopies. Non-Metric Multidimensional Scaling (NMDS) analysis was as well undertaken to display the spatial relationships between tree canopy effects and pasture abundance.

RESULTS AND DISCUSSION

Canopy size characteristics of the study tree species

The tree species in this study were of significantly varying canopy size ($p < 0.05$). *A. campylacantha* had the widest canopy of 7.6 m while the shortest canopy was displayed by *A. hockii* (2.4 m) Table 1. Statistically, canopy radius of *A. abbysinica*, *A. campylacantha*, *A. coriaria* and *F. natalensis* was significantly wider than the canopy radius of *A. gerrardii*, *A. hockii*, *A. africanus* and *G. mollis* (Table 1).

Effect of scattered tree species on the diversity and abundance of pastures

A total of 14 different naturally growing pasture species were identified in the study, with two pasture species exclusive to under tree canopies. Analysis of diversity by Shannon-Wiener's method showed that pasture species diversity was almost the same under and outside tree canopies (H=1.8 and H=1.78 respectively). The dominant pasture species under and outside tree canopies were *Brachiaria ruziziensis, Cynadon dactylon, Brachiaria brizantha* and *Brachiaria decumbens* (Table 2). An exception existed in *B. decumbens* whose dominance index outside tree canopies was far lower than the other dominant pasture species.

The close uniformity in the diversity index of pastures under and outside tree canopies could be attributed to the dominant sedentary practice of grazing in the area. During grazing more so under sedentary practice, previous studies have documented that the intensive and non-selective grazing exerts excessive pressure on natural pastures thus limiting species recovery (Kavana et al., 2019). As an adaptation strategy therefore, pasture species that can withstand the resultant grazing pressure have ended up colonizing the pastoral communities (Souther et al., 2019). Such findings could explain the uniform diversity and dominance of certain pasture species under and outside tree canopies in this study.

From the rarefaction plot in Figure 3, visual displays of pasture species richness revealed existence of variation under and outside tree canopies and between tree species. At a standardized sampling depth of 20 pastures under and outside canopies of each tree species, *A. hockii,* and *F. natalensis* displayed the highest number of pastures ranging from 6 - 8 for both canopy sites. *A. campylacantha* had the lowest species richness ranging from 2 - 4 pastures under its canopies where beyond a sample of 10 pastures, the tree species had no more new pastures recorded under its canopies. For most tree species, it was revealed that no more new pastures would be encountered under and outside tree canopies beyond an approximate sampling depth of 50 pastures. More sampling would only favor *F. natalensis* and *A. coriaria* whose species richness increased to more than 10 pastures with a batch of 80 - 100 samples. Nonetheless, as revealed from the curves, beyond 100 pasture samples the two tree species would as well not register any more new pasture species under and outside their tree canopies.

The use of rarefaction curves is an important tool in assessing species richness which aspect gives one of the simplest and most popular measure of diversity (Bacaro et al., 2016). From the rarefaction curves displayed, the varying species richness under and outside tree canopies as well as between tree species reveals some level of association between certain tree species and pastures. Within grazing lands, a study by Li et al. (2017) revealed that plant species richness is positively correlated with availability of soil nutrient resources. This implies that under tree canopies of *A. hockii* and *F. natalensis* soil nutrient concentrations could have favored the growth of several pastures compared to other tree species. Such findings could as well explain the higher pasture species richness under *A. coriaria* at a higher sampling depth.

However, whereas A. *hockii*, *F. natalensis* and *A. coriaria* displayed higher pasture species richness under their canopies compared to other tree species, the relatively higher pasture richness outside tree canopies for most species implies that in the sedentary grazing areas of South-western Uganda, presence of tree species did not change the composition of pasture species. Interestingly, a study by Lopes et al. (2016) revealed that presence of trees in grazing areas changes the feeding behavior of cattle, leading to higher time of grazing and rumination under tree canopies compared to open pastures. With such findings, it could be true that tree canopies for several species favored grazing activities for livestock and partially influenced pasture species richness under their tree canopies (Figure 3).

Complementary to rarefaction curves, pasture diversity varied significantly under and outside tree canopies and between tree species ($p < 0.05$). As shown in Figure 4, significant variation in pasture diversity under tree canopies was between *F. natalensis* (H = 0.32) which had the highest diversity and *A. campylacantha* (H =0.17),

Table 1. Canopy size (m) of the study tree species.

Tree species	n	Mean	Std Dev
A. abbysinica	16	7.4[a]	2.19
A. campylacantha	14	7.6[a]	1.50
A. gerrardii	16	5.2	1.24
A. hockii	14	2.4	0.65
A. coriaria	14	6.6[a]	2.45
A. africanus	16	3.6	0.73
F. natalensis	15	6.1[a]	1.32
G. mollis	15	4.8	1.06

Canopy size of means which do not share a letter is significantly different at 95% CI.

Table 2. Composition of pastures and their dominance levels under and outside tree canopies.

Pasture species	Dominance index under canopies	Dominance index outside canopies
Brachiaria ruziziensis	0.13	0.17
Cynadon dactylon	0.04	0.02
Brachiaria brizantha	0.02	0.02
Brachiaria decumbens	0.02	0.00
Panicum maximum	0.00	0.00
Neonotonia wightii	0.00	0.00
Hyparrhenia rufa	0.00	0.02
Pennisetum clandestinum	0.00	0.00
Chloris guyana	0.00	0.00
Setaria anceps	0.00	0.00
*Desmodium intortum**	0.00	0.00
Setaria sphacelata	0.00	0.00
*Microptilium atropurpureum**	0.00	0.00
Themeda triandra	0.00	0.00

Pasture species with an asterisk (*) were recorded under tree canopies only.

A. hockii (H = 0.16) and *A. africanus* (H = 0.23) which species had low pasture diversity.

In grazing lands, the contribution of tree species towards improving the productivity of below ground vegetation is attributed to the reduction in incoming solar radiation, decreasing sub-canopy evapotranspiration and enhanced mineralization of nutrients (Abdulahi et al., 2016). Such factors positively influence establishment and growth of different types of pastures. Banking on previous studies it could be true that the micro climate under the canopy of *F. natalensis,* led to a higher facilitative effect towards growth of different pasture species compared to other tree species especially A. *campylacantha* and *A. hockii.*

In terms of abundance, there was significant difference in pasture abundance under and outside tree canopies and between tree species especially for under the tree canopy site ($p < 0.05$). As displayed in Figure 5, the distinct patterns in the alignment of pasture abundance under and outside tree canopies and in relation to tree species reveal existence of significant differences. Most importantly, the relatively wider distance between pasture abundance positioning under the tree canopies of A. *campylacantha*, *A. coriaria*, *F. natalensis* and other tree species gives insights into the source of variation. More so, like in the case of pasture species diversity, higher pasture abundance was recorded under tree canopies of *F. natalensis*, *A. coriaria* while lower abundance was recorded under canopies of *A. hockii* and *A. campylacantha* (Figure 5).

Basing on the results of this study, it is evident that presence of tree species enhanced colonization of pasture species in the sedentary grazing areas of South-western Uganda. Besides, the significant differences in pasture abundance under the canopies of different tree species reveal that some tree species had more complementary support towards pasture growth and ground coverage. This is true especially for *F. natalensis*, *A. coriaria* under whose canopies highest abundance of pastures was recorded.

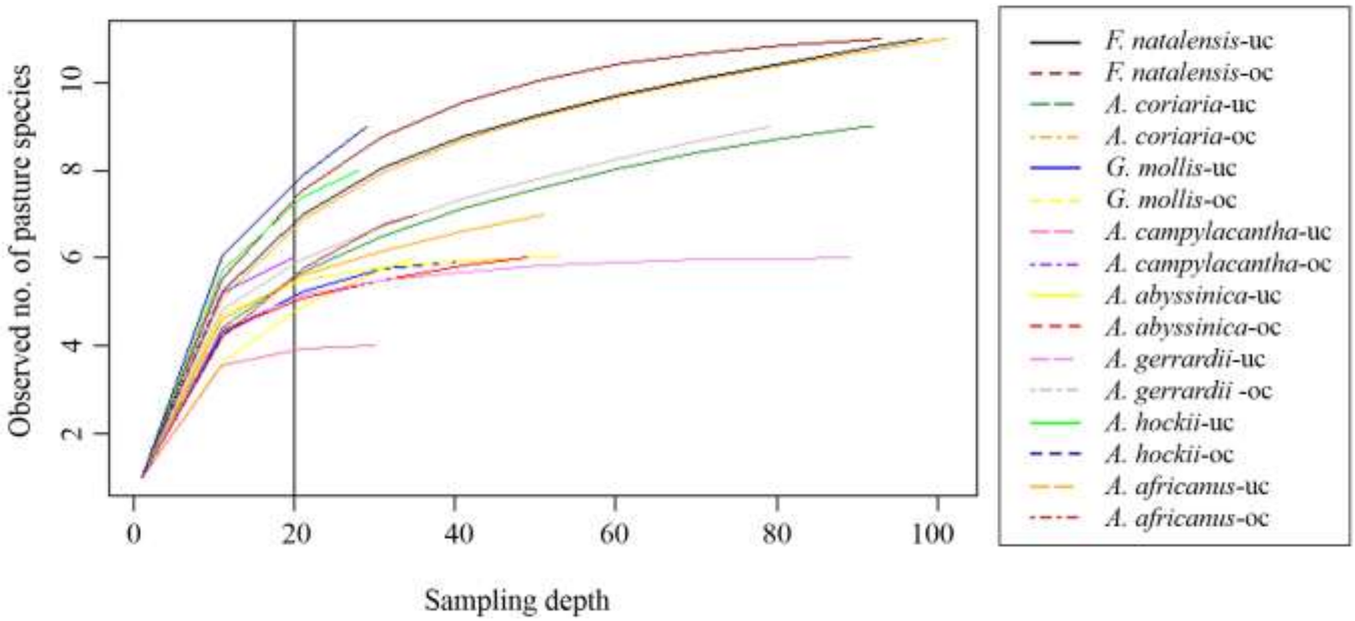

Figure 3. Rarefaction plot for pasture species richness under (uc) and outside (oc) tree canopies.

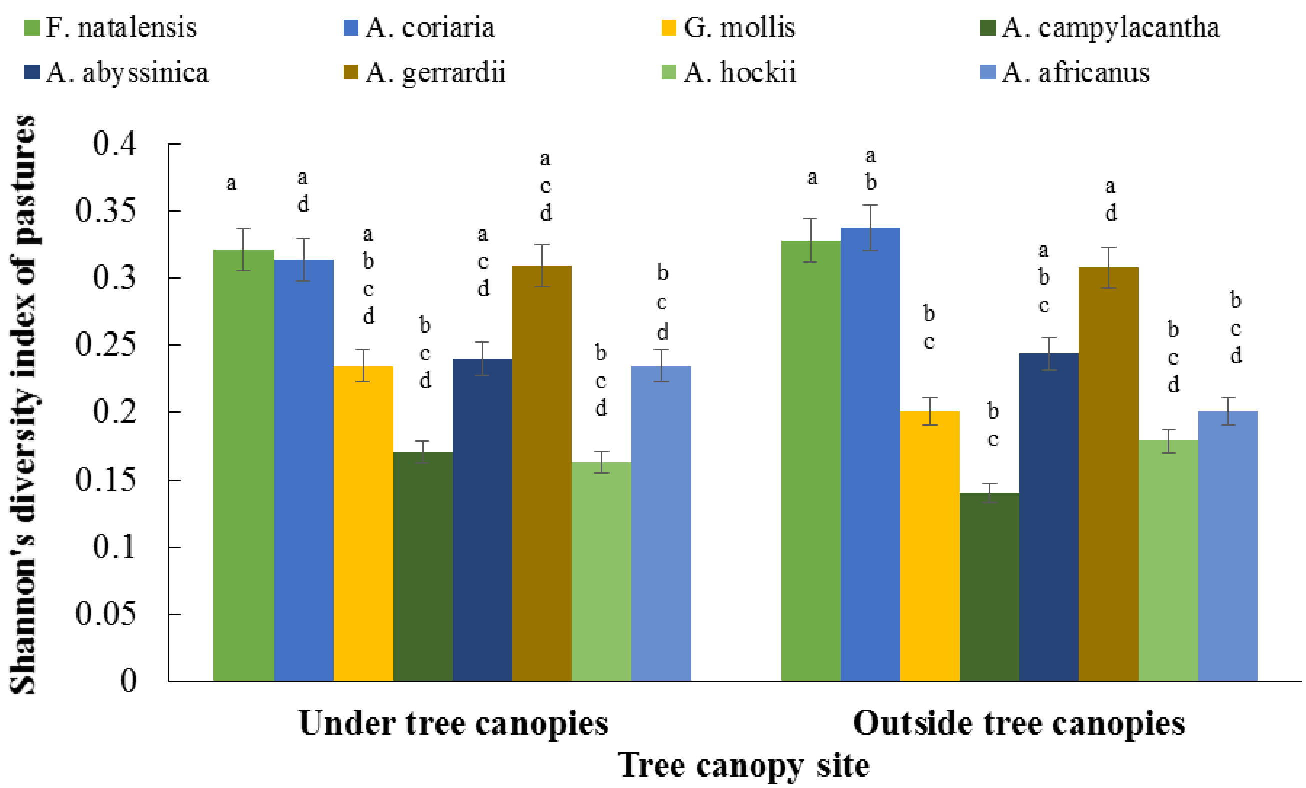

Figure 4. Pasture diversity under and outside canopies of the different tree species. Pasture diversity of bars which do not share a letter is significantly different at 95% CI.

The findings revealed by the results above could be explained by differences in plant traits between tree species. An earlier study by Castillo et al. (2020) revealed that differences in tree traits such as canopy structure, nutrient uptake and litter quality can affect soil processes thus impacting on the growth and colonization of pasture

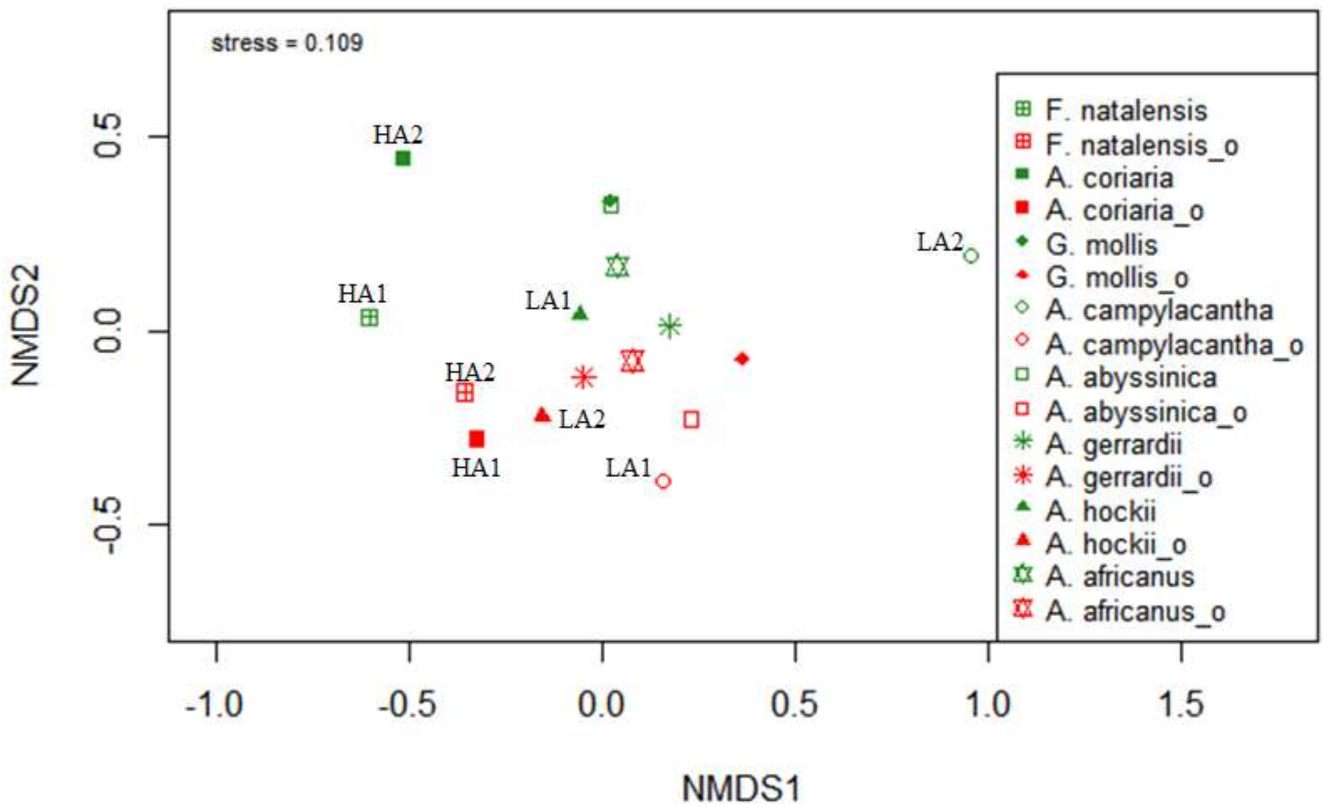

Figure 5. NMDS ordination plot displaying the canopy effects of different tree species on pasture abundance. HA= High Abundance; LA=Low abundance; o = outside tree canopy.

species. In a study by Ssebulime et al. (2018), it was discovered that the leaf litter of *A. coriaria* and *F. natalensis* comprise a high concentration of nutrients which factor could have improved soil fertility under these tree species and supported growth and greater ground coverage of the pastures. More so, a study by Fujita (2014) reports that below tree canopy ground conditions of *F. natalensis* tend to favour germination and seedling survival of plants compared to the open environment. Therefore, since *A. coriaria* and *F. natalensis* never had the widest canopies as reflected in Table 1, enhancement of soil fertility linked to these tree species could justify the findings of this study.

Worth noting, *F. natalensis* and *A. coriaria* which had higher pasture diversity and abundance under tree canopies compared to other tree species still recorded higher pasture diversity and abundance outside tree canopies. Whereas in some studies tree species have been reported to have negative effects on below ground grass community (Abate et al., 2012), in this study such results could imply that the extent of positive influence on pasture growth and ground coverage by the two species extended beyond their tree canopies.

Much as the physiological attributes of tree species was an aspect beyond the scope of this study, the deciduous nature of *F. natalensis* and *A. coriaria* gives the closest factor to explain the observed results. Probable reasons could be that during leaf shedding, some of these high nutrient rich leaves, could have been deposited in a close distance outside the tree canopies and improved the soil fertility conditions for the benefit of the pastures (Ssebulime et al., 2018). More so, in a study by Buyinza et al. (2019), it was revealed that as *A. coriaria* sheds its leaves between late January and February, the species increases its water use about one month prior to the start of the wet season, between mid-February and early March. Such results could be banked on to ascertain that in the phase of this study, there was higher water use by the two tree species under their canopies which lowered pasture growth and subsequent ground coverage compared to outside their canopies.

Effect of scattered tree species on pasture biomass

Above ground biomass of pastures never varied significantly under and outside tree canopies ($p > 0.05$). Nonetheless, six out of the eight tree species studied had slightly higher pasture biomass under tree canopies. This was under *A. coriaria*, *A. africanus*, *A. gerrardii*, *A. campylacantha*, *A. abyssinica* and *G. mollis* (Figure 6). Similar to pasture diversity, the uniformity of pasture

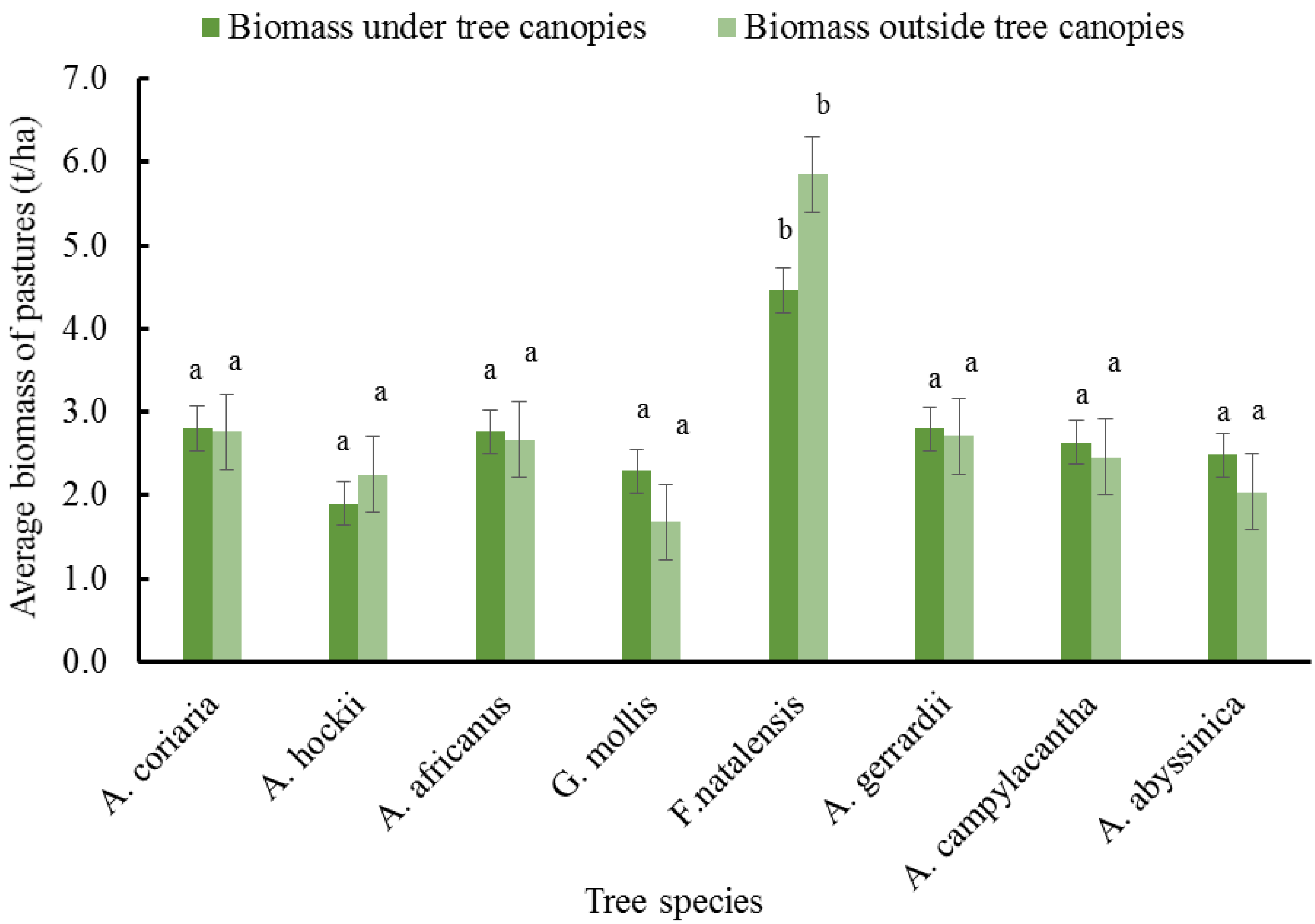

Figure 6. Average biomass of pastures under and outside canopies of the study tree species. Biomass in bars that do not share a letter is significantly different at 95% CI.

biomass under and outside tree canopies could be attributed to the non-exclusive foraging pattern of livestock in the area. In such unprotected sites, overgrazing which accrues from continuous pasture utilization has been documented to hinder biomass productivity (Hassan et al., 2017).

However, irrespective of the canopy size, the slightly higher pasture biomass under the canopies of most species reveals that probably there was a more favorable micro-climate under tree canopies of the respective species which enhanced nutrient uptake and subsequent biomass accumulation. Although the physiological attributes of tree species were not covered in this research, the aspect presents a probable insight into the findings of this study. In a study by Mazía et al. (2016), it was revealed that most deciduous and leguminous trees enhance the biomass of grasses growing beneath them. In this study 3 trees species out of the 6 species where higher pasture biomass under tree canopies was recorded are leguminous while *A. coriaria* is deciduous. Between tree species, pasture biomass varied significantly under and outside tree canopies ($p < 0.05$). Under the canopies, the highest and statistically significant pasture biomass was 4.5 t/ha which was recorded under *F. natalensis* while the lowest pasture biomass was 1.9 t/ha which was recorded under *A. hockii* (Figure 6). In comparison with previous studies, biomass of natural pastures in grazed areas has been documented in values of 0.596 - 1.59 t/ha as the lower range and 0.7 - 2.83 t/h as the higher range (Oñatibia and Aguiar, 2016; Ishaq et al., 2019). Basing on the previous findings elsewhere, this study has revealed that presence of *F. natalensis* in the sedentary grazing areas of South-western Uganda has a higher complementary support towards pasture biomass for the benefit of livestock. Since above ground plant biomass is linked to soil nutrient levels (Li et al., 2017), it is highly likely that the leafy litter of *F. natalensis* contains a high concentration of nutrients which improved soil fertility and led to higher pasture biomass.

Surprisingly, the highest and statistically significant pasture biomass outside tree canopies (5.9 t/ha) was still recorded in pastures away from tree canopies of *F. natalensis* (Figure 6). It is probable that the positive effect of *F. natalensis* trees on pasture biomass stretched to more than 5 m beyond their canopies. However, the fact that the biomass outside tree canopies was higher than under the canopy calls for detailed analysis. Since no previous literature has been documented in relation to the findings, such observation warrants scientific investigation.

Conclusion

This study has shown that in the sedentary grazing lands of South-western Uganda, scattered tree species especially *F. natalensis* and *A. coriaria* improve the abundance of forage pastures. Whereas most tree species did not lead to significance increase in pasture species diversity and biomass, *F. natalensis* had a significantly higher positive influence on pasture diversity and biomass. Therefore, this study recommends that *F. natalensis* and *A. coriaria* can be used to restore degraded grazing lands in South-western Uganda. Further research on the effect of deciduous attributes of the two tree species on pasture productivity is needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Influence of distance between plants and pruning of axillary buds on morphological and productive characteristics of tomato plants

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This research was carried out for 120 days in the horticulture area of the Universidad Nacional de Concepción, Facultad de Ciencias Agrarias to evaluate how morphological and productive characteristics of tomato plants were influenced by the distance between plants and the pruning of axillary buds. The design used in the experiment was the randomized block method with factorial arrangement (3x3); factor A was the pruning of axillary buds (no pruning, pruning between 1 and 5 cm, pruning between 6 to 10 cm of sucker length) and factor B was the distance between plants (40, 59 and 60 cm). Three repetitions were carried out. Plant height, fruits per bunch, polar and equatorial fruit diameters, and fruit yield per plant were determined. The results indicated significant differences of pruning of axillary buds for all determinations carried out. Only fruit yield per plant showed a significant difference by planting density. Pruning of axillary buds (6 to 10 cm sucker length) gave the best morphological and productive results. It is shown here that pruning of axillary buds has a significant influence on tomato production.

Key words: *Lycopersicum esculentum,* sucker length, fruit yield.

INTRODUCTION

Sowing density is a key factor in the crops' level of exposure to sunlight. This affects the level of transformation of solar energy into biomass, which is essential for increasing crop productivity (Castilla, 2001).

The importance of pruning lies in the fact that a rapidly growing plant organ can compete with leaves for easily translocated nutrients, causing leaf senescence and a reduction in photosynthetic capacity. The growth resulting from branch pruning is quite fast, temporarily altering the ratio between the root and above-ground part of the plant. Likewise, the removal of foliage and branches reduces the amount of accumulated carbohydrates and, even more importantly, reduces the leaf area available for carbohydrate production (Salisbury and Ross, 1994).

Pruning is an important practice in tomato cultivation that can improve fruit quality and yield. Given this, pruning

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is a necessary practice for the planting of tomato varieties of indeterminate growth. Pruning is carried out when the first side stems appear, which are removed as well as on the oldest leaves, thus improving the ventilation of the root collar and making it easier to mound soil around the base of plants (Vera et al., 2015).

Machado et al. (2007) evaluated different planting densities and types of pruning of tomato plants. Total and commercial production was best with spacing of 20 cm between plants and average fruit weight was best with 50 cm spacing. Increasing the population density of plants increased fruit production but, in contrast, reduced the average weight of fruits.

Ponce et al. (2011) determined the effect of four levels of branch pruning on tomato cultivation, concluding that none of these levels produced a positive effect on fruit yield or quality. However, there was a positive effect observed between different varieties. The highest yield (963.5 g/plant) was obtained with the variety CHF1. Equatorial and polar fruit diameters of 54.44 and 34.1 mm were obtained respectively, with fruit weight of 26.4 g.

The objective of this research was to evaluate the influence of distance between plants and different levels of pruning of axillary buds on morphological and productive characteristics of tomato plants.

MATERIALS AND METHODS

The experiment was carried out in the experimental terrain of the Facutad de Ciencias Agrarias, Universidad Nacional de Concepción, Concepción, Paraguay, at coordinates 230° 40'13" latitude, 570°41'85" longitude, and 160 meters above sea level (mamsl).

The area's climate is characterized by annual average temperatures of 14 and 26°C, with maximum temperatures reaching 45°C in the summer months and minimum temperatures of 4°C in winter with light levels of frost (DMH, 2018).

According to the soil analysis, the soil in the experimental area has the following physical and chemical characteristics at a depth of 0 - 0.20 cm: sandy loam texture, pH (H_2O) 5.67; organic matter (Walkley Black, modified): 1.67%; $Ca^{+2}_{(KCl\ extraction)}$, $Mg^{+2}_{(KCl\ extraction)}$ and $K^{+}_{(Mehlich)}$: 5.06, 1.27 and 0.19 cmol/LS(liter of soil), respectively; P(Mehlich) and $S_{(Acetic\ acid)}$: 28.94 and 11.73 mg/LS respectively; Al^{+3}: 0.05; cation exchange capacity (CEC): 9.71 cmol/LS, and base saturation (V): 67.21%.

The design used in the experiment was the randomized blocks method with factorial arrangement (3x3). Factor A was pruning of axillary buds (no pruning, pruning between 1 to 5 cm of sucker length, pruning between 6 to 10 cm of sucker length) and Factor B was the distance between plants (40; 50 and 60 cm). Three repetitions were carried out, giving a total of 27 experimental units (EU). The total plot size was 240 m^2.

The soil was prepared with ridges of 20 cm in height. The ridges were 1 m apart from each other and contained 1 row of tomatoes sowed using the distances between plants already mentioned. During the preparation of the ridges, cattle manure was added uniformly in doses of 4 kg m^{-2} in all the experimental units.

Subsequently, photoselective netting with 50% light retention and a drip irrigation system were installed. Afterwards, the soil's surface was covered using white plastic sheeting.

The seeds were sown in expanded-polystyrene germination trays with 105 cells. One hybrid Hs 1188 seed was planted in each cell. Plants were transplanted 30 days after sowing. Additionally, galvanized wire was installed so that the plants could be tied for support. 25 days after transplanting, pruning of axillary buds began. This was done according to a planned schedule: twice a week during the vegetative phase and once a week once flowering had begun.

To control diseases and pests, a contact fungicide (copper oxychloride, 3 g L^{-1} of water) was used preventively at 15-day intervals and a systemic fungicide was used (Tricur, 15 mL in 5 L of water). A systemic insecticide was also used (Imidacloprid, 8 mL in 5 L of water). In addition, a bactericide was used to control an outbreak of bacterial diseases.

Harvesting began 120 days after transplanting and was carried out daily until the fruits reached commercial maturity. Determinations were made by selecting 5 plants from each experimental unit and evaluating the following variables: 1) Plant height (the length of the main stem after the formation of the 9th floral bunch was measured using a tape measure); 2) number of fruits per bunch (fruits $bunch^{-1}$): the number of fruits in each bunch from the selected plants was counted and averaged; 3) Polar diameter (PD) and equatorial diameter (ED) of fruit (mm $fruit^{-1}$): These measurements were taken using a vernier caliper; 10 fruits were selected from each experimental unit for measurement; 4) mean fruit weight (g): 10 fruits were selected from each experimental unit; these were weighed, and a mean was obtained and 5) Fruit yield per plant (kg pl^{-1}): This was measured by weighing all the commercial fruits from the selected plants using precision scales; an average was calculated.

The data were subjected to variance analysis (ANOVA) using the Fisher test for each of the variables, and the averages from each treatment were compared using the Tukey test at 5% probability level.

RESULTS AND DISCUSSION

Plant height and number of fruits per bunch

Table 1 shows the average results for plant height and number of fruits per bunch obtained when varying the factors mentioned above. Significant differences were observed between the recorded means for the factor of pruning axillary buds. However, no statistical differences were observed for the factor of distance between plants. No significant effects were found for any of the variables studied in this experiment with regards to interaction between the two factors (Tables 1 to 3).

Analysis of the effect of pruning axillary buds on plant height shows that pruning between 6 and 10 cm SL produced plants with the greatest height, with an average of 177 cm. This does not statistically differ from pruning axillary buds between 1 and 5 cm in length, which produced an average height of 169 cm. However, it differed from no pruning, which produced the lowest value of 155 cm.

An analysis of the results in Table 1 for the number of fruits per bunch shows that the most favorable values were observed in plants pruned at 6 to 10 cm SL, with 4.53 fruits per bunch. These results were lower than those obtained by Max et al. (2016).

Monge (2016) investigated the effects of different types of pruning and planting density on the agronomic

Table 1. Plant height (PH) (cm) and number of fruits per bunch (NFB) (mm) as a function of pruning of axillary buds and plant density.

Factor	PH (cm)	NFB (mm)
Test F		
Pruning of axillary buds (A)	5.35*	35.97**
Distance between plants (B)	3.47[ns]	1.68 [ns]
Interaction A x B	0.69[ns]	1.18 [ns]
Pruning of axillary buds		
Pruning between 6 to 10 cm SL	177.07[a]	4.53[a]
Pruning between 1 to 5 cm SL	169.00[ab]	3.16[b]
No pruning	154.64[b]	2.22[c]
Distance between plants (cm)		
40	177.03	3.02
50	164.44	3.47
60	159.23	3.42
MSD	17.92	0.71
OA	166.90	3.31
CV	8.83	17.61

ns, *, **, F tests not significant and significant at 5 and 1%, respectively; for each column and each variable, means with different letters are significantly different at 5% probability by Fisher's test; CV, Coefficient of variation; MSD, Minimum significant difference; OA, Overall Average; SL, Sucker length.

Table 2. Polar (PD) (mm) and equatorial (ED) (mm) diameters in tomato fruits as a function of pruning of axillary buds and plant density.

Factor	PD (mm)	ED (mm)
Test F		
Pruning of axillary buds (A)	64.01**	99.39**
Distance between plants (B)	0.32 ns	1.93 ns
Interaction A x B	0.72 ns	1.61 ns
Pruning of axillary buds		
Pruning between 6 to 10 cm SL	86.36[a]	66.42[a]
Pruning between 1 to 5 cm SL	76.59[b]	58.56[b]
No pruning	69.43[c]	51.04[c]
Distance between plants (cm)		
60	77.84	59.89
50	77.77	58.23
40	76.77	57.89
MSD	3.88	2.82
OA	77.46	58.67
CV	4.11	3.94

ns, *, **, F tests not significant and significant at 5 and 1%, respectively; for each column and each variable, means with different letters are significantly different at 5% probability by Fisher's test; CV, Coefficient of variation; MSD, Minimum significant difference; OA, Overall Average; SL, Sucker length.

characteristics of pepper plants. In contrast to the present study, characteristics were not affected by these factors. Jovicich et al. (2004) examined the same factors and found that plant height was higher in plants with pruning

Table 3. Mean fruit weight (MFW) (g) and fruit yield per tomato plant (kg plant^{-1}) as a function of pruning of axillary buds and plant density.

Factor	Mean fruit weight (g)	Yield (kg plant^{-1})
Test F		
Pruning of axillary buds (A)	480.85**	96.11**
Distance between plants (B)	3.26 ns	16.17**
Interaction A x B	2.33 ns	2.44 ns
Pruning of axillary buds		
Pruning between 6 to 10 cm SL	220.00^{a}	8.85^{a}
Pruning between 1 to 5 cm SL	126.44^{b}	6.89^{b}
No pruning	90.11^{c}	5.36^{c}
Distance between plants (cm**)**		
60	151.89	7.77^{a}
50	142.11	6.99^{b}
40	142.56	6.34^{b}
MSD	11.15	0.65
OA	145.52	7.03
CV	6.30	7.59

ns, *, **, F tests not significant and significant at 5 and 1%, respectively; for each column and each variable, means with different letters are significantly different at 5% probability by Fisher's test; CV, Coefficient of variation; MSD, Minimum significant difference; OA, Overall Average; SL, Sucker length.

of 2 stems/plant compared to plants with pruning of more than 2 stems/plant. Grijalva et al. (2008) did not observe significant differences for this variable.

Seifi et al. (2012) mentioned that plant height is occasionally greater when planting density increases. However, authors such as Aminifard et al. (2012) obtained opposing results or, just as has been seen in the present study, authors observed no statistical differences between different planting densities (Reséndiz et al., 2010).

In the research conducted by Arebalo et al. (2018) to evaluate the influence of different levels of early pruning on tomato plants, no statistical differences were detected for the variable of the number of fruits per bunch. These results differ from those reported by Sánchez and Ponce (1998) that investigated the density and pruning in tomato, and found significant differences for this determination. These results are similar to those achieved in this investigation, regarding pruning, but not for density.

Polar and equatorial diameters of fruits

Table 2 shows the mean values of the variables analyzed. The data indicate that there were significant differences regarding the pruning of axillary buds; however, no statistical differences were detected for distances between plants.

The highest polar diameter (PD) was recorded in plants with pruning of between 6 to 10 cm SL followed by pruning between 1 to 5 cm SL. The lowest value was found for no pruning. Between the best and worst-performing pruning techniques, there was a mean difference of 16.93 mm; this is a marked difference in fruit size.

The distance between plants did not significantly affect the polar and equatorial diameters of tomato fruits (Table 2). These data are similar to those obtained by Ponce et al. (2012). In contrast, Salguero and Curay (2016) reported that polar and equatorial diameters of tomato fruits were significantly affected by planting density: equatorial diameter of fruits varied between 79.8 and 104.5 mm with an OA of 94.7 cm. Those values were higher than values achieved in the present study probably due to the genetic material used.

Average fruits weight and yield per plant

As can be seen in Table 3, pruning of axillary buds and distance between plants produces significant differences in yield per plant. No significant differences are detected in fruit weight.

The best results for average fruit weight (Table 3) in relation to the pruning of axillary buds were observed for pruning between 6 and 10 cm SL, with 220 g fruit^{-1}. This was statistically superior to pruning axillary buds between 1 to 5 cm (126.44 g fruit^{-1}) and no pruning (90.11 g fruit^{-1}).

A yield of 8.85 kg plant^{-1} was obtained when pruning axillary buds of 6 to 10 cm SL on average. This was statistically superior to pruning between 1 to 5 cm SL, and to no pruning, with values of 6.89 and 5.36 kg plant^{-1}, respectively. The distance of 60 cm between plants produced the highest value of 7.77 kg plant^{-1}. The data obtained in this work is slightly higher than that reported by Mendoza et al. (2018), who achieved a mean of 6.55 kg plant^{-1}.

For studies of population density and pruning of tomato plants, Machado et al. (2007), Sanchez et al. (2017) and Arébalo et al. (2018), in early pruning works in tomato, reported an increase in the average weight of the fruit with the lowest density. Likewise, Villegas et al. (2004), achieved an increase in fruit yield through the combination of higher population density and pruning pruning; the results of all these works coincide with those of the present investigation.

On the other hand, Sánchez and Ponce (1998) and Sánchez del et al. (2017) working with different levels of pruning and planting densities in tomato and Carrillo et al. (2003), investigating with different densities, found no significant effects for performance, in contrast to those found in this investigation.

Conclusions

The factor of pruning axillary buds proved to have a significant influence on tomato production: there was a notable difference observed in plants that were pruned for all determinations carried out. Pruning between 6 to 10 cm SL led to better performance, allowing for the assumption that pruning suckers within this length range is a good management alternative for tomato crops.

With regards to the factor of the distance between plants, a lower population density (60 cm apart) apparently contributed to more productive plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Participatory upland rice (*Oryza sativa* L.*)* seed rate determination for row method of sowing under irrigated eco-system

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A field experiment was conducted in 2013 cropping season at Bedloale and Werer sites in Amibara district, Afar Regional State, to determine optimum seeding rate for row method of sowing for productivity of rice (*Oryza sativa* L.) under irrigated ecosystem. The experiment was conducted with five levels of seed rate treatments (50, 60, 70, and 80 kg ha^{-1} and local practice) laid out in a randomized complete block design (RCBD) with three replications. The results of agronomic analysis showed that there was no statistical difference for all parameters at Bedolale site. However, at Werer site total number of tillers per plant, number of productive tillers per plant, total biomass and grain yield were significantly affected by seed rate. Compared to the control, 50 and 60 kgha^{-1} seed rates have shown a grain yield and superior performance in total biomass yield advantage. Moreover, the partial budgeting analysis results showed that sowing of 60 kg ha^{-1} NERICA-4 seed yielded positive gross margin at Werer site. While at Bedolale site, the two least cost treatments, that is, 50 and 60 kg ha^{-1} could be economically viable. In general, from the results of this study, it can be concluded that 60 kg ha^{-1} drilling is optimum seed rate for row method of sowing.

Key words: Seed rate, rice productivity, NERICA-4, sowing method, row planting.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important staple food and grown across the world. It is the second most widely consumed cereal in the world next to wheat (Kumari et al., 2014). Rice is becoming increasingly popular in Africa with about 16 million metric tons of annual consumption and 14 million tons of production, creating a deficit of 2 million metric tons, which is filled by imports (Somado et al., 2008).

Introduction of the crop dates back to recent decades; evidences have indicated that cultivation of the crop in

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Ethiopia was first started at Fogera and Gambella plains in the early 1970s (MoARD, 2005). In Ethiopia, research in rice was started informally by Tana Beles project and by the Koreans who were in Ethiopia for a different mission. Nowadays, production of rice is expanding as the hot to warm moist climates are potentially suitable for rice culture as they fulfill all the requirements of the crop and produced mainly by small scale farmers in many parts of the country and with large scale farmers in few places (MoARD, 2010).

Low land areas of Ethiopia are dominantly settled by pastoral or agro-pastoral peoples who are seasonally moving in search of pasture and water for their animals. Recently, the government of Afar implemented permanent settlement program for agro-pastoralists. This program is benefiting agro-pastoralists who are just starting crop production. Rice is the staple food for Afar peoples and one of the potential crops suitably grown in the region. Although the crop is staple food for the people of the region, almost all the consumption demand is fulfilled by buying rice like other consumption goods, rather than satisfying their demand via cultivating the crop. However, now a day's agro-pastoralist has started production of the crop due to the opportunities that have been created by the state government settlement program.

Soil nutrient application rates, schedule of nitrogen fertilizer application, irrigation amount and scheduling, seeding rate and planting methods are among the major agronomic practices, which limit rice productivity and production. Seeding rate is one of most important agronomic aspect which need due attention. When the plant density exceeds an optimum level, competition among plants for light above ground and nutrients below ground becomes severe (Baloch et al., 2002). Consequently, plant growth slows down and the grain yield decreases. However, very low plant density may not enable to attain the yield plateau.

Given to the fact that rice is a recently cultivated crop in Ethiopia, to the best of our knowledge, very few studies so far have been conducted with regard to optimum cultural practices' determination for the rice producing areas of Afar region particularly in Amibara Woreda. This urges that a lot has to be done on rice agronomic experiments so as to have appropriate rice crop management recommendations.

This research was therefore, initiated to elucidate the effect of seeding rate on rice yield and yield components for row method of sowing under irrigated condition with reasonable economic return to be recommended and disseminated to farmers.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted on farmer's field and on-station (Werer) during the main cropping season at Werer and Bedolale Kebeles in Amibara district of Afar Region, Ethiopia. Geographically, the experimental site was located at 09° 60' N latitude and 40° 09' E longitude and at an elevation of 740 m.a.s.l. The total rain fall during 2013 cropping season was 516.3 mm. The major portion of the total annual rainfall was received between July and October. The total rainfall received between these months in the 2013 cropping season was 306.8 mm which was 59.42% of the total rainfall in the year. The average yearly minimum and maximum temperatures were 31.2 and 39.4°C, respectively. The annual evapotranspiration (2796.9 mm) of the area was much more exceeded than annual rain fall (516.3 mm) which resulted 2280.6 mm deficit (Table 1) owing to this the area is fully irrigated. The soil of the experimental site was Vertisol. The map of the Woreda where the experimental sites was located is as shown in Figure 1.

Soil sampling, preparation and analysis

Composite soil sample per replication, each made from three sub-samples was collected in a diagonal pattern from 0 to 30 cm soil depth for Werer site. Likewise, in the same manner three sub-samples from each replication for each treatment was collected and composited into one sample per treatment for Bedolale site before planting to assess physico-chemical properties of the soil under study. Uniform slices and volumes of soils were obtained in each sub-sample by vertical insertion of an auger. The samples were air-dried, ground to pass through a 2 mm sieve, except for analysis of organic carbon and nitrogen, where the samples were passed through 0.5 mm sieve. Working samples were obtained from each submitted samples and analyzed for selected physico-chemical properties such as texture, soil pH, EC, organic carbon, total N, available phosphorus, and available potassium using standard laboratory procedures.

Total N in the soil was determined by the Kjeldahl method (Dewis and Freitas, 1975). Organic carbon content of the soil was determined by reduction of potassium dichromate by organic carbon compound and determined by reduction of potassium dichromate by oxidation reduction titration with ferrous ammonium sulfate (Walkley and Black, 1934). The organic matter was calculated by multiplying the organic carbon using the factor 1.724. Particle size distribution (texture) was determined by hydrometer method (differential settling within a water column) using particles less than 2 mm diameter (FAO, 2008). This procedure measures percentage of sand (0.05 - 2.0 mm), silt (0.002 - 0.05 mm) and clay (<0.002 mm) fractions in soils. Available P was determined following the method of Olsen and Dean (1965). The pH of the soil was measured in 1:2.5 (weight/volume) soil samples to $CaCl_2$ solution ratio using a glass electrode attached to digital pH meter (Page et al., 1982). The soil analysis was carried out by soil and water laboratory of Werer Agricultural Research Center.

Treatments and experimental details

A participatory trial was conducted in one selected agro pastoralist field at Bedloale Kebele and Werer experimental site in Amibara district. As to the team formation three (Farmer's Research Groups, FRGs) were formed and training was organized on FRG concept and rice production techniques for FRG member agro-pastoralists.

Four seeding rates (50, 60, 70, and 80 $kgha^{-1}$) and one control (60 $kgha^{-1}$ broadcasting/local practice) was laid out in a randomized complete block design (RCBD) with three replications using a total plot size of 20 m^2 (4 m width and 5 m length) with a spacing of 2 m and 3.6 between plots and blocks, respectively and no spacing between plants (drilling). NERICA-4 variety of rice was used as a

Table 1. Average rainfall, average temperature, evapotranspiration and relative humidity for the year 2013.

Month	R.F. (mm)	Temperature (°C)		R.H. (%)	Evap. (mm)
		Min.	Max.		
January	14.5	12.7	32.5	52	199.9
February	3.4	16.4	34.2	37	223.5
March	85.7	21.4	36.4	49	251.2
April	79.2	22.7	36.8	50	228.2
May	8.1	23.8	38.0	43	277.5
June	9.5	26.4	39.4	37	313.3
July	161.9	22.0	34.8	58	212.4
August	75.1	22.3	33.7	63	192.1
September	22.6	22.3	35.4	55	212.9
October	47.2	19.6	34.4	50	282.9
November	9.1	17.6	32.6	56	222.6
December	0.0	13.3	31.2	49	180.5
Total	516.3				2796.9
Mean	43.0	20.0	35.0	49.9	233.1

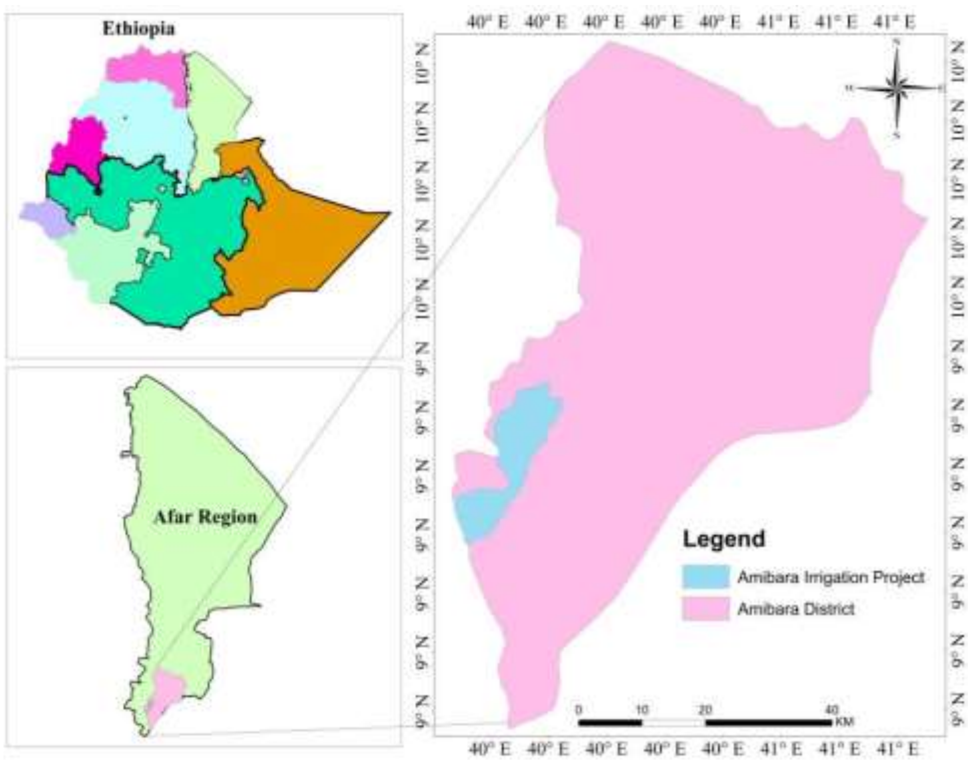

Figure 1. Geographical map of the study sites.

test crop. Each plot received uniform doses of 100 kgha^{-1} Urea and 50 kgha^{-1} di-ammonium phosphate (DAP) fertilizers. Urea was applied in two equal splits, at tillering and at panicle initiation stage; whereas full dose of DAP was applied at sowing. As to the seeding rate, it was used as per the treatment. Besides, necessary compensations were made to the seed rate amount based on the germination rate obtained by the germination test conducted before sowing. All necessary management was carried out as per the

Table 2. Selected chemical properties of the experimental soil.

Seed rates (kgha^{-1})	Bedloale site					
	pH	EC_e (ds/m)	%OC	%OM	%TN	AP (ppm)
50 (drilling)	8.3	0.402	0.397	0.684	0.034	8.280
60 (drilling)	8.3	0.505	0.494	0.852	0.043	9.655
70 (drilling)	8.4	0.394	0.462	0.796	0.040	8.155
80 (drilling)	8.4	0.408	0.631	1.087	0.054	10.405
60 (broadcasting)	8.4	0.457	0.585	1.009	0.050	8.280
	Werer site					
50 (drilling)	8.3	0.463	0.254	0.437	0.022	17.279
60 (drilling)	8.4	0.409	0.306	0.527	0.026	14.655
70 (drilling)	8.4	0.439	0.273	0.471	0.024	14.030
80 (drilling)	8.5	0.512	0.137	0.235	0.012	21.841
60 (broadcasting)	8.4	0.502	0.338	0.583	0.029	18.311

EC_e=Electrical conductivity, OC=Organic carbon, OM=Organic matter, NT=Total Nitrogen, AP=Available Phosphorus.

research recommendations. Eventually, field visit at Bedloale and Werer sites was organized to show the experience of the team (FRGs).

Data collection

At maturity, number of tillers (productive and total), panicle length and plant height was taken per plant basis from randomly selected six plants. While biomass yield and grain yield were taken per plot base and then converted in to per hectare basis. Grain yield was adjusted to 12% moisture content and biomass yield/ha was determined after sun drying to bring moisture content to near minimum level. Exceptionally, for broadcasting/control 1 m × 1 m quadrat was used for biomass and grain yield determination. However, for row method of sowing a dimension of 2.4 m length and 3 m width (7.2 m^2) was considered as net harvested plot size. Thousand-grain weight was recorded by counting 1000 grains of representative seeds samples collected from each plot. It was also adjusted to 12% moisture content and weighed using an electronic balance.

Economic analysis

The economic analysis of an on-farm experiment begins with partial budget analysis[1]. This partial budget analysis was conducted to find out which seeding rate is economically feasible (Table 5). Dominance analysis was carried out to eliminate treatment which was not considered by agro-pastoralists' (Table 6). Moreover, marginal analysis was performed to consider the increase costs (Table 7). Although the calculation of net benefits accounts for the costs that vary, it was necessary to compare the extra (or marginal) costs with extra (or marginal) net benefits. Higher net benefits may not be attractive if it requires very much high costs.

Statistical analysis

Analysis of variance (ANOVA) was performed using SAS software version 9.1 (SAS Institute, 2004). Fisher's least significant difference (LSD) test at 5% probability was used for mean separation (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Physical and chemical properties of the experimental soil

The result of the physical and chemical properties of the soil of the study sites is presented in Table 2. The soil used for the experiment was silt clay loam and clay in texture for Bedolale and Werer sites, respectively. Soil pH of the Bedolale site was 8.3 to 8.4, which is moderately alkaline according to Jones (2002). However, the Werer site had pH which ranges from moderately alkaline to strongly alkaline (8.3-8.5) (Ibid.). The electrical conductivity values of saturation extract have ECe value ranging from 0.394 to 0.512 dS m^{-1}, these values can be rated as normal since it is less than 4 dS m^{-1} for both locations. The organic carbon content of the soil ranges from 0.397 to 0.631%, which is low in accordance with Landon (1991) at Bedolale site. Likewise for the Werer site which it ranges from 0.254 to 0.338%. Also, organic matter contents of the experimental sites ranges from very-low to low for both sites in accordance with Tadesse (1991). Total nitrogen of the present soil was low (<0.05%)

[1] Partial budgeting is a method of organizing experimental data and information about the costs and benefits of various alternative treatments. In partial budgeting only those costs that are affected by the alternative treatments are being considered and not all production costs are included. In this instance costs which vary among treatments are seed and weeding costs. Therefore, seed and weeding cost for the control and seed cost for the rest treatments were considered as a variable cost for the analysis. Tables 5 and 6 are the steps involved in partial budgeting analysis. For interpretation, marginal rate of return (Table 7) is considered.

Table 3. Effect of seeding rate on yield and yield components of upland rice variety NERICA-4 at Bedolale, 2013.

Seed rates (kgha^{-1})	TNTPP	NPTPP	PL (cm)	PH (cm)	BY (tha^{-1})	GY (kgha^{-1})	TSW (g)
50 (drilling)	7.3	6.7	20.3	91.1	17.4	4415.0	25.3
60 (drilling)	7.4	6.9	20.4	88.2	17.8	4434.1	25.5
70 (drilling)	6.0	5.9	20.7	87.8	18.0	4293.5	25.4
80 (drilling)	6.8	6.5	18.5	84.3	16.4	3774.3	24.8
60(broadcasting)	5.7	5.5	20.3	88.8	15.7	3793.9	24.3
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns
CV (%)	22.8	23.0	7.5	5.6	13.3	14.3	2.1

ns = Not significant, TNTPP = total number of tillers per plant, NPTPP = number of productive tillers per plant, PL = panicle length, PH = plant height, BY = biomass yield, GY = grain yield, TSW = thousand seed weight.

Table 4. Effect of seeding rate on yield and yield components of upland rice variety NERICA-4 at Werer, 2013.

Seed rates (kgha^{-1})	TNTPP number of tillers/plant	NPTPP	PL (cm)	PH (cm)	BY (tha^{-1}) Yield tha^{-1}	GY (kgha^{-1}) (yield kgha^{-1}	TSW (g)
50 (drilling)	6.0^{ab}	5.3^{ab}	18.5	68.2	12.7^{a}	2739.3^{a}	24.1
60 (drilling)	6.6^{a}	6.0^{a}	19.3	70.3	13.1^{a}	2960.6^{a}	23.7
70 (drilling)	5.4^{bc}	4.9^{abc}	18.1	69.8	11.8^{ab}	2650.4^{ab}	23.5
80 (drilling)	4.7^{c}	4.1^{c}	17.5	65.2	9.7^{c}	2156.1^{b}	23.5
60 (broadcasting) control	4.9^{bc}	4.3^{bc}	18.6	63.0	10.2^{bc}	2152.1^{b}	23.2
LSD (0.05)	1.1	1.2	ns	ns	1.9	527.6	ns
F-probability	*	*	-	-	*	*	
CV (%)	11.3	12.9	5.5	5.3	9.3	11.5	1.4

*, **, *** significant at $P \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ probability levels, respectively; ns= not significant. Means in a column followed by the same letters are not significantly different at p > 5% level of significance.

for Werer site and it ranges from low to medium (0.034-0.054%) for Bedolale site according to Tekalign (1991). Regarding on available P, it was medium (10 - 25 ppm) for Werer site and ranges from low to medium (8.155 - 10.405 ppm) for Bedolale site based on the classification (Horneck et al., 2011). This indicated that P is limiting nutrient for optimum crop growth and yield in the experimental sites. In general, the properties of the experimental soil in both sites were conducive, albeit there was an evidence suggesting replenishment of nutrients such as nitrogen and phosphorus for proper growth of the crop.

Bedloale site

The analysis of variance exhibited that seed rate had brought no significant effect on all parameters considered in this study (Table 3). None of the parameters were significantly (P > 0.05) affected by seeding rate implying the treatments considered in this particular site was not high enough to encounter yield loss due to competition of above and below ground resources. In addition, compared to other row sown treatments, broadcasted control has given statistically similar result which is difficult to justify. Therefore, based on the result, we recommended 50 kg ha^{-1} seeding rate since it permit to save unnecessary usage of extra seeds.

Werer site

The yield components and other parameters, viz. panicle length, plant height and thousand grain weight were not significantly (P > 0.05) influenced by seed rate (Table 4). Since the result obtained from aforementioned parameters was non-significant, thus it is not logical to compare such figures because the analysis of variance revealed statistically non-significant difference among the treatments considered in this study.

Total and productive tillers per plant

In this study, total number of tillers and productive tillers (Table 4). Higher total number of tiller per plant was

Table 5. Calculation of partial budget.

Site		Seeding rates ($kgha^{-1}$)				
		50 $kgha^{-1}$ Drilling	60 $kgha^{-1}$ Drilling	70 $kgha^{-1}$ Drilling	80 $kgha^{-1}$ Drilling	60 $kgha^{-1}$ (Broadcasting)
Bedolale site	AY ($kgha^{-1}$)	4415.00	4434.10	4293.50	3774.30	3793.90
	AY* ($kgha^{-1}$)	3752.75	3768.99	3649.48	3208.16	3224.80
	GB (ETB ha^{-1})	37,527.50	37,689.85	36,494.75	32,081.55	32,248.15
	CS (ETB ha^{-1})	600.00	720.00	840.00	960.00	720.00
	WC (ETB ha^{-1})	-	-	-	-	200.00
	TVC (ETB ha^{-1})	600.00	720.00	840.00	960.00	920.00
	NB (ETB ha^{-1})	36,927.50	36,969.85	35,654.75	31,121.55	31,328.15
Werer site	AY ($kgha^{-1}$)	2739.3	2960.6	2650.4	2156.1	2152.1
	AY* ($kgha^{-1}$)	2328.92	2516.51	2252.84	1832.69	1829.29
	GB (ETB ha^{-1})	23289.15	25165.10	22528.40	18326.85	18292.85
	CS (ETB ha^{-1})	600.00	720.00	840.00	960.00	720.00
	WC (ETB ha^{-1})	-	-	-	-	180.00
	TVC (ETB ha^{-1})	600.00	720.00	840.00	960.00	900.00
	NB (ETB ha^{-1})	22689.15	24445.15	21688.40	17366.85	17392.85

Costs other than seed cost were kept constant because of an assumption that all treatments received the same management practices except 60 kg ha^{-1} broadcasted which had incurred additional 200 and 180 Birr ha^{-1} for weeding (labor cost) for Bedolale and Werer site, respectively. CS: Seed cost is 1200 Birr/100kg; Grain price is 1000 Birr/100kg; GB=gross benefit without deduction of costs; VC=variable cost/costs that vary; TVC=Total variable cost; NB=net benefit = GB-TC; AY=average yield; AY*=adjusted yield, 15% is adjusted for analysis (literature recommends 5-30%). All the monitory value were stated in Ethiopian birr (ETB)

obtained from 60 kg ha^{-1} seeding rate which was drilled while the lowest was observed from 60 kg ha^{-1} broadcasted (control). Compared to the control seeding rate other than, 60 kg ha^{-1} which was drilled was statistically at par with the control. Likewise, number of productive tillers per plant was consistent with the total number of tillers. Thus, the higher number of productive tillers was recorded from 60 kg ha^{-1} seed rate which was drilled whereas the lowest was observed from 60 kg ha^{-1} seed rate which was broadcasted (control). Total number of tillers per plant and number of productive tillers per plant from 60 kg ha^{-1} seed rate found to be increased by 34.69 and 39.53%, respectively compared with the control.

Among yield components, productive tillers are very important because the final yield is mainly a function of the number of panicles bearing tillers per unit area and number of grains per panicle (Chatterjee and Maiti, 1985). In this trial, maximum number of tillers per plant and productive tillers per plant were achieved from 60 kg ha^{-1} seed rate which was row sown. Concomitant with the present finding, Angassa (2017) had reported higher total and productive number tillers per plant from using lower (40 kg ha^{-1}) seed rate compared to higher seed rate under irrigated condition. On the other hand, Tekle and Wedajo (2014) had reported higher total number of tillers and productive tillers from using 100 kg ha^{-1} seed rate under rainfed condition.

Biomass yield (t ha^{-1})

Statistical analysis of data showed significant difference ($P \leq 0.05$) due to the effect of seed rate on biomass yield of rice (Table 4). Significantly, the highest biomass was observed from 60 kg ha^{-1} seed rate followed by 50 kg ha^{-1} seed rate while the lowest biomass yield was observed from 80 kg ha^{-1} seed rate. Lower seeding rate (50 and 60 kg ha^{-1}) gave higher biomass yield. Comparatively, lower seeding rate have shown superior performance over higher seed rate and the broadcasted control. Lower seed rates (50 and 60 kg ha^{-1}) have 24.51 and 28.43% biomass yield advantage, respectively, over the control. Concurrent with the result of this study, Angassa (2017) had reported higher biomass yield with lower seeding rates (40 and 60 kg ha^{-1}). In addition, it has been reported that biomass yield was found to decrease as seed rate increase beyond 60 kg ha^{-1}.

Grain yield (kg ha^{-1})

The result indicated that seeding rate had significant ($P \leq 0.05$) effect on grain yield of rice (Table 4). Maximum yield was obtained from lower seeding rates (50 and 60 kg ha^{-1}) which were not statistically different. The lowest grain yield was observed from 60 kg ha^{-1} broadcasted

Table 6. Dominance analysis.

Seeding rates (kgha^{-1})	Bedolale site		Werer site	
	TVC (ETB)	NB (ETB)	TVC (ETB)	NB (ETB)
50 kgha^{-1} drilling	600.00	36,927.50	600.00	22689.15
60 kgha^{-1} drilling	720.00	36,969.85	720.00	24445.15
70 kgha^{-1} drilling	840.00	35,654.75	840.00	21688.40
80 kgha^{-1} drilling	960.00	31,121.55*	960.00	17366.85*
60 kgha^{-1} (broadcasted)	920.00	31,328.15	900.00	17392.85

*The dominated treatment that was eliminated. VC is variable costs that are considered (the seed costs that vary only keeping other production costs constant)

Table 7. Marginal analysis.

Seed rates kgha^{-1}	TVC	MC	NB	MNB	MRT
Bedolale site					
50 kgha^{-1} drilling	600.00	-	36927.50	-	-
60 kgha^{-1} drilling	720.00	120	36969.85	42.35	0.3529
70 kgha^{-1} drilling	840.00	120	35654.75	-1315.1	-10.9591
60 kgha^{-1} (broadcasting)	920.00	80	31,328.15	-4326.6	-54.0825
Werer site					
50 kgha^{-1} drilling	600.00	-	22689.15	-	-
60 kgha^{-1} drilling	720.00	120	24445.10	1755.95	14.63
70 kgha^{-1} drilling	840.00	120	21688.40	-2756.70	-22.97
60 kgha^{-1} (broadcasting)	900.00	60	17392.85	-4295.55	-71.59

MC=Marginal cost (ETB); NB=net benefit (ETB); MNB=marginal net benefit (ETB); MRT=marginal rate of return (ETB).

(control). The lower seeding rate 50 and 60 kg ha-1 which was drilled have shown superior performance over the control with 27.28 and 37.57% yield advantage, respectively. The result is in agreement with findings of Angassa (2017) who reported higher grain yield with lower seeding rates (40 and 60 kg ha^{-1}).

Result of economic analysis

Bedolale site

The agronomic data evaluation and the statistical analysis result of this particular on-farm experiment reveal that there is no yield difference among treatments. The yield differences between different seed rate treatments [50, 60, 70, 80 and 60 kg ha^{-1} (broadcasted)] were statistically non-significant. It is needless to show the economic analysis of such statistically non-significant treatments, rather it is advisable to consider the least cost treatment. However, for this experiment though the treatments are non-significant there is still a room to have a positive return of using either of the two least cost treatments. Sowing of 60 kg ha^{-1} NERICA-4 seed yielded 35% profit (if Agro-pastoralists incur 1 birr they will cover their 1 birr and got additional 35 cents profit). Therefore, it is up to the agro-pastoralists to choose among the two least cost treatments, that is, 50 and 60 kg ha^{-1}.

Werer site

As revealed by statistical result, grain yield was significantly affected by different seed rate. Sowing of 60 kg ha^{-1} NERICA-4 seed resulted in positive return. Besides, using of seeding rate which exceed 60 kg ha^{-1} was encountered loss. In fact, still it is up to the agro-pastoralist to choose between two least cost treatments. Nonetheless, from economic point of view it would be better if they made a choice of 60 kg ha^{-1} which was drilled, since using of 60 kg ha^{-1} seed rate yielded 1463% profit (if agro-pastoralists incur 1 birr they will cover their 1 birr and got additional 14.63 birr profit), which is far above the acceptable level (Table 7). Therefore, it is advisable to use 60 kg ha^{-1} seed rate at Werer site.

Conclusion

This experiment follows a participatory approach by which agro-pastoralists were involved from problem identification to final decision of the research output. Thus, the recommendations to be made are farmer oriented. Farmers' and/or agro-pastoralists' assessments have been carried out to gain their opinions about the treatments they have seen in their fields. Besides, agronomic evaluation and statistical data analysis have also been done ahead of economic analysis.

Statistical analysis declared yield difference among the seed rate for Werer site whereas at Bedolale site no yield difference was observed. Economic analysis dictated that using of 60 kg ha^{-1} yielded profit compared to other seed rate considered at Werer site. However, for Bedolale site, since the test was non-significant, it was needless to show economic analysis for such result. Hence, it is up to agro-pastoralists to choose between the two least cost treatments (50 and 60 kg ha^{-1}). Therefore, based on the choice of the agro-pastoralist's, statistical and economic analysis, it can be concluded that the seed rate of 60 kg ha^{-1} is advisable and could be appropriate for rice production in the test area even though further testing is required to put the recommendation on a strong basis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Performance of faba bean (*Vicia faba* L.) varieties grown under broomrape (*Orobanche* spp.) infestation in South Tigray, Ethiopia

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Broomrapes (*Orobanche* spp.) are the serious roots parasitic weed to legumes crop production in many countries. In Ethiopia, *Orobanche crenata* is a dominant parasite and a major constraint to faba bean (*Vicia faba* L.) cultivation, especially in the Northern parts of the country. Presently, it reduces crop production and has forced farmers to stop growing faba bean crop. Thus, it is necessary to find new sources of resistance, understand means of resistance mechanisms to facilitate faba bean resistance breeding, and identify the best performed with high yielding variety to sustain their production and productivity. Thus, to evaluate the performance and their degree of resistance, twenty faba bean varieties were tested under the area affected by Orobanche infestation, at Korem experimental site of Alamata Agricultural Research Center, Tigray, Ethiopia during 2017 cropping season by using Randomized Complete Block Design (RCBD). All difference among faba bean varieties was analyzed using Tukey's Standardized Range (P ≤ 5%) Test. Higher level of broomrape infection was observed during host-pod setting stage. During evaluation, 13 out of 20 tested faba bean varieties were the superior yielding varieties (312.5 - 3129.17 kg ha^{-1}), whereas the remaining seven varieties completely lost yield due to 100% Orobanche infestation. Cluster analysis was then carried out and the varieties were grouped into different clusters with different sizes based on their level of resistance or susceptibility. Three varieties 'Ashenge, Dedia and Obse' were selected for their best performance. Accordingly, Ashenge variety was selected as partially resistant with highest yield provided due to lowest occurrence of Orobanche infestation within variety. Future breeding program therefore, should mainly focus on these three selected varieties to improve the problem of faba bean production by using conventional and molecular breeding methods.

Key words: *Orobanche* spp., *Vicia faba*, resistance/tolerance.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the earliest domesticated food legumes in the world (Singh et al., 2013) that is grown under rain fed and irrigated conditions in many countries. It has high nutritional value

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and is used almost daily in human diet of many Ethiopians (Teklay et al., 2015). Due to its high nutritional value and its ability to grow over a wide range of climatic and soil conditions (Yahia et al., 2012), faba bean is the fourth most important legume worldwide after pea, chickpea and lentil. It is grown under different cropping systems as dry grains, green pods, animal feed and green-manure in the world. The main faba bean producers are China (2.1 Mt), Ethiopia (0.8 Mt), France (0.65 Mt), Egypt (0.4 Mt) and Australia (0.25 Mt) (FAOSTAT, 2016).

Faba beans require a cool season for best growth with moderate amounts of rainfall 650 to 1000 mm per annum (Gasim and Link, 2007), considered to tolerate frost, but are susceptible to drought and water logging (Subash and Priya, 2012). Faba beans are moderately tolerant to acid soil conditions than most legumes (Singh et al., 2010) and can tolerate a wide range of soil types with pH 6.5 to 8.0 (Rajan et al., 2012), but grow best in loamy soils. The crop takes about four or five months for the pods to mature enough for the seeds to be harvested based on environmental condition (Mussa et al., 2008). In Ethiopia, the major faba bean producing regions are Oromia (Wollega, Shoa, Bale), Amhara (Gondar, Gojam, Wollo), Southern parts of the country (Gamo Gofa), Benishangul-Gumuz and highland parts of Tigray (CSA, 2016/2017).

However, there are different biotic (diseases, insect pests, and parasite weeds) and abiotic (drought, salinity, fertility etc.) constraints that limit the production and productivity of the faba bean (Mussa et al., 2008). Recently, in addition to the previous common diseases such as chocolate spot, faba bean rust, aschochyta blight, virus, nematode, etc., the crop is threatened by new gall forming disease which become a very dangerous problem that is seriously affecting faba bean production (Dereje et al., 2012). This new disease mainly affects the leaves and stems with typical symptoms of green and sunken on the upper side of the leaf, bulges to the back side of the leaf and then develops light brownish color lesion and chlorotic galls (Hailu et al., 2014). It is the most destructive disease that causes yield loss up to 30-100%. Broomrapes (Orobanche spp.) remain one of the constraints of faba bean production that is known by the aggressive root parasitic weeds and are completely dependent on the host due to the lack of chlorophyll and functional roots (true roots) (Eizenberg et al., 2010).

Legumes are parasitized mainly by two different species of broomrapes, namely crenate broomrape (*Orobanche crenata* Forsk.) and foetida broomrape (*Orobanche foetida* Poir) (Rubiales, 2014). Although other Orobanche species can infect leguminous plants, they are generally of little economic importance. *O. crenata*, however, has a wide host range among legumes crop in temperate climates. It was known in the past as an agricultural weed in Europe, but currently it is not common as such any more. It is found in native and disturbed habitats throughout the central and southern parts of Europe, and extends to the eastern coast of Africa and southwards. In addition, it was imported to various other parts of the world and is currently found as a garden weed. *Crenate broomrape* (*O. crenata* F.) is the most damaging, widespread, and most dominant parasite compared to foetida broomrape (Fernández et al., 2012). The damage caused by this parasite on faba bean crop is significant and estimated yield losses is about 7 to 80% depending on the level of infestation (Maalouf et al., 2011).

Broomrapes have a single stem and can release more than 100,000 seeds that are known to remain viable for decades in the soil (Eizenberg et al., 2010). This provides the parasite with a great genetic adaptability to environmental changes, including host resistance, agronomical practices and herbicide treatments. Orobanche spp. parasitizes a number of crops globally, with legumes being some of the most severely damaged, and only germinates in response to specific chemicals released by the host plant (germination stimulant compound present in the root exudate). However, before being able to respond to this external stimulus, the seeds need to be exposed to moist conditions at a suitable temperature (optimum 15-20°C) for a certain period of time. Following germination, the seedlings attach to the host roots and grow at the expense of the host plant's resources (Joel et al., 2007). As they are root parasitic weeds, most of their infection and pathogenesis process take place underground, which complicates diagnosis of infection and control (Rubiales et al., 2009). This continuing invasion of Orobanche is exacerbated (worsened) by the lack of knowledge of the farmers and the official agricultural experts, about the biology and exact means of spread of this parasite and un-sufficient availability of breeding for Orobanche resistance faba bean varieties.

The particular characteristics (underground development, attachment to the host roots) of this pathogenic weed hamper the development of effective control strategies. A number of methods for controlling broomrape in faba bean are practiced, including agronomical practices (like intercropping, fertilizer application) and chemical treatments (application of glyphosate at low rates) (Rubiales et al., 2016; Eizenberg et al., 2010). Because of the extreme difficulty of controlling broomrape, prevention is most importance. Traditionally, the sources of infection can be reduced by controlling the use of contaminated seed lots, farmyard manure from broomrape fed cattle, and hand weeding. However, the most desirable control strategy is the use of tolerant or resistant cultivars. Moreover, the levels of resistance available in faba bean cultivars are low and of narrow genetic basis in spite of the many efforts made by national and international programs. Resistance against broomrape is a particularly difficult character to assess as it is highly influenced by environmental factors (Rubiales et al., 2016).

In Ethiopia, the Orobanche infestations become quickly distributed over large area of cultivating lands. Northern parts of the country, especially Gonder, southern and western part of Wollo and southern Tigray were the most dominated area by this parasite. The estimated yield losses in faba bean due to *O. crenata* is as high as 75 to 100% yield loss depending on host susceptibility, level of infestation and environmental conditions (Teklay et al., 2013).

Heavy Orobanche infestation does not only lead to a complete crop failure, but make soils Orobanche-sick over a long period of time. Because of unidentifiable nature and difficulty of parasite to the farmers, the only option they took is, planting other cereal crops instead of faba bean, which are not affected by this parasite weed. However, this practice leads to a decrease in the progress of faba bean production and productivity thoroughly. Presently, the continuous spread of Orobanche limits the choice of rotational crops and often force farmers to stop growing this most valuable crop (Teklay et al., 2013). Thus, in Ethiopia, particularly the northern part, productivity of the crop is decreasing due to the infestation of this parasite weed. Hence, there is a need to evaluate faba bean performance with high yielding varieties to find new sources of resistance, and to understand the underlying resistance mechanisms in order to facilitate faba bean resistance breeding. Therefore, the main objective of this study is to evaluate the performance of faba bean varieties for resistance against broomrape species.

MATERIALS AND METHODS

Experimental materials

Twenty faba bean varieties were obtained from different Agricultural Research Centers in Ethiopia as listed in Table 1. One standard check (as resistance control) was obtained from Alamata Agricultural Research Center. The selection of these varieties was based on their productivity and high productive faba bean varieties across different agro-ecologies were selected. All of the varieties were evaluated for their response to the parasites in highly infested field with *Orobanche* spp.

Description of the study area

The experiment was conducted at Korem districts of South Tigray Zone, located at 12°31'N latitude and 39°33'E longitude, at research station of Alamata Agricultural Research Center (AARC) during the main cropping season of 2017. The area represents highlands of South Tigray Zone with moderate rainfall (average annual rainfall of 600 mm), which extends mostly from half of June to late September, with a dominant soil type (clay) and slightly acidic with a pH of 6.4 (Teklay et al., 2013).

Experimental design and management

This experiment was evaluated using Randomized Complete Block Design (RCBD) with three replications. The space between replication was 1.5 m. Each plot consists of 4 rows of 3 m length, with 0.1 and 0.4 m intra and inter row spacing respectively (3 m × 1.6 m = 4.8 m² plot size). At planting, 100 kg ha^{-1} DAP was applied. All the culture practices were applied during the growing to ensure good crop stands. Hand weeding, other than broomrape was done. However, herbicide was not applied to avoid interference with broomrape development.

Data collection

Data collection from plot and plant bases

Days to 50% emergence, stand count after emergence, days to 50% flowering, plant height (cm), days to 90% maturity: stand count at maturity, 100-seed weight after being adjusted to 11.6% moisture content, yield per hectar (kg), biomass (kg), harvest index (HI) = $\left(\frac{Grain\ yield}{Total\ biomass\ yield}\right) \times 100$, number of nodes per plan, number of pods per node, number of pods per plant, pod length (cm), and number of seeds per pod were the data collected during the experiment. All data were recorded from five plants randomly selected from the two middle rows and computed for their mean value.

Data on broomrapes infestation

Number of *Orobanche* shoots per plot: The total number of broomrapes grown per plot was recorded at three host growing stages (flowering, pod setting and maturity) and calculated for their average values.

Number of *Orobanche* per plant: Number of *Orobanche* spp. grown per single plant was recorded from five randomly selected plants at three host growing stages (flowering, pod setting and maturity) and computed for their mean values.

***Orobanche* incidence (%):** Percentage of faba bean plants hosting *Orobanche* shoots per plot was measured. This implies that, first the percentage of host plant that persist with having yield under *Orobanche* infestation was measured, then subtracted from hundred to gain the percentage of *Orobanche* incidence.

Data analyses

All collected data were subjected to ANOVA (one way ANOVA) using SAS version 9.2. Simple descriptive statistics (such as means and coefficient of variation) was used in order to compare variation between varieties or traits. The significance of the mean difference between varieties was evaluated by the Tukey's Standardized Range (HSD) Test at P ≤ 0.05.

Principal component analysis (PCA)

Principal component analysis was used for multivariate analysis after the means of all traits were computed and properly arranged, and for grouping faba bean varieties into different groups based on their response to the level of *Orobanche* infection.

Cluster analysis

Cluster analysis was carried out for tested traits and grouping of twenty faba bean varieties into different clusters based on their degree of resistance. All analyses were conducted using SAS

Table 1. Description of faba bean Varieties used for the study.

S/N	Name of varieties	Maintainer	Year of release
1	Holetta-2 (BP 1802-1-2	HARC	2001
2	Selele (Selele kasim 91-13)	HARC	2002
3	Wayu (Wayu 89-5)	HARC	2002
4	Moti (EH 95078-6)	HARC	2006
5	Gabelecho (EH96009-1)	HARC	2006
6	Obse (EH 95073-1)	HARC	2007
7	Welki (ETH 96049-2)	KARC	2008
8	Dosha (Coll 155/00-3)	HARC	2009
9	Mesay	HARC	1995
10	Tumsa (EH-99051-3)	HARC	2010
11	Hachalu (EH009102-4-1)	HARC	2010
12	Bulga-70	HARC	1994
13	Degaga	HARC	2002
14	Didea	HARC	2014
15	Tesfa	HARC	1995
16	Gachena (ETH 91001-13-2)	HU	2008
17	Ashenge (Resistant)- check	Alemata ARC	2014
18	Mosisea (EH-99047-1)	Sinana ARC	2013
19	Aloshe	Sinana ARC	2017
20	Shallo	Sinana ARC	2000

statistical software version 9.2.

RESULTS AND DISCUSSION

Days to 50% flowering

Analysis of variance showed that there was highly significant difference (P≤0.01) among faba bean varieties in days to 50% flowering (Table 2). The varieties Moti, Ashenge and Mosise flowered early within 46.67, 46.67 and 47.33 days, respectively, whereas Hachalu variety (56 days) was the latest, followed by Dosha and Tumsa varieties (54 days). At flowering stage, there were a few number of *Orobanche* emergence per plant. Thus, the result of this study showed that days to 50% flowering were not much influenced by *Orobanche* infestation even if the variation observed was due to genetic character of the varieties (Table 3).

Days to 90% maturity

The mean for days to maturity for tested varieties ranged from 114 days to 122 days, which was found to be highly significantly different (P≤0.01) (Table 2). The varieties that were highly affected by *Orobanche* infestation or susceptible and highly susceptible varieties were pushed to mature early which take days to 90% maturity ranging from 114 to 119. Concerning the mean value of the crops' life cycle, significantly higher days to 90% maturity was observed in partially resistant and tolerant varieties such as Ashenge (122.33), Obse (122), and Dedia and Mosise (120.67) (Table 3), which showed that these varieties kept their optimum days to maturity, and might be due to their high defense mechanisms and ability to tolerate the parasite. This result indicated that maturity date was affected by *Orobanche* infection. Rubiales (2014) also reported similar result.

Plant height

There were highly significant variations among tested faba bean varieties (P≤0.01) in plant height which was influenced by *Orobanche* infestation and varieties' character. The maximum plant height was recorded from Ashenge, Dedia, Gachena and Degaga varieties with height of 78-90 cm, whereas, the shortest plant heights were recorded from Tesfa, Wayu and Gebelcho varieties with height of 51.9 - 55.47 cm (Table 3). The result of this investigation revealed that *Orobanche* infestation had significant effect on plant growth by reducing the height of the plants.

Number of nods per plant

The influence of broomrapes in addition to varieties' genetic variability did exert highly significant variation (P ≤ 0.01) on number of nods per plant (Table 4).

Table 2. Level of significance for phenological traits from analysis of variance for faba bean varieties grown under broomrapes.

Character/trait	Mean square		Mean	R^2
	Varieties (*df* =*19*)	Error (*df* = *38*)		
Stand count after emergence	10.3^{NS}	11.45	48.22	0.47
Number of plant harvested with yield	202.42**	0.74	8.48	0.99
Days to 50% flowering	21.9**	0.36	50.33	0.97
Days to maturity	21.9**	1.20	117.6	0.90
Plant height	283.36**	28.85	66.87	0.83
Number of *Orobanche* per plot	3417.65**	108.6	77.7	0.98
Number of *Orobanche* per plant	3.59**	0.25	3.05	0.97

** =highly significant ($P < 0.01$), NS = none significant, df = degree of freedom and R^2 = coefficient of determination.

Table 3. Combined mean of *Orobanche* emerged plot^{-1} and plant^{-1} (at three host-growing stages) and its effects on faba bean varieties for phenological traits.

Variety	Mean of *O.* emerged at three host growth stage		OI (%)	DF 50%	DM 90%	NNP	PH
	NOPP	NOPT					
Holleta-2	73.3^{fgh}	3^{cd}	76^{cd}	49^{d}	115^{fg}	6^{ab}	67.5^{bcd}
Salale	70.4^{fgh}	2.76^{cd}	66.69^{g}	49.33^{d}	116.33^{def}	5^{bc}	67.6^{bcd}
Wayu	61^{h}i	2.95^{cd}	72.76^{def}	53^{bc}	118^{bcd}	6.33^{ab}	55.33^{ef}
Moti	84.56^{cde}	3.9^{ab}	100^{a}	46.67^{f}	114^{g}	4.33^{c}	69^{bcd}
Gebalcho	75.2^{ef}	3.2^{bcd}	100^{a}	49.33^{d}	115^{fg}	5.33^{bc}	51.97^{f}
Obse	67.67^{fgh}	3.2^{bcd}	57.15^{h}	48^{def}	122^{a}	5.33^{bc}	70.4^{bcd}
Welki	78.2^{def}	2.78^{cd}	81.9^{b}	52^{c}	116.33^{def}	5.33^{bc}	66.33^{cde}
Dosha	92.67^{bcd}	2.69^{cd}	100^{a}	54^{b}	120.67^{ab}	4^{c}	65^{cde}
Mesay	43ij	2.4^{d}	73.72^{de}	53^{bc}	118.67^{bc}	6.33^{ab}	62.17^{def}
Tumsa	95.67^{bc}	4.3^{a}	100^{a}	54^{b}	114^{g}	5.33^{bc}	60.33^{ef}
Hachalu	77^{ef}	3.18^{bcd}	100^{a}	56^{a}	118.67^{bc}	5.33^{bc}	58.6^{ef}
Bulga-70	79.56^{def}	2.64^{cd}	74.6^{de}	49.33^{d}	118.67^{bc}	5.33^{bc}	64.8^{cde}
Degaga	70.56^{fgh}	3^{b}	68.36^{fg}	49^{d}	115^{fg}	6.33^{ab}	78^{abc}
Dedia	79.78^{def}	2.89^{cd}	54.24^{h}	48.67^{de}	120.67^{ab}	6.33^{ab}	80.3^{ab}
Tesfa	118.1^{a}	4.2^{a}	100^{a}	48^{def}	115.33^{ef}	5.33^{bc}	55.47^{ef}
Gachana	104.2^{ab}	3.27^{bcd}	70.77^{efg}	49.33^{d}	119^{abc}	7^{a}	79.5^{ab}
Ashange	33j	1.5^{e}	38.5i	46.67^{f}	122.33^{a}	7.33^{a}	90^{a}
Mosise	85.44^{cde}	2.6^{d}	79.16^{bc}	47.33^{ef}	120.67^{ab}	6^{ab}	74.33^{bcd}
Aloshe	98^{bc}	3.49^{abc}	100^{a}	52^{c}	116.67^{def}	4^{c}	58.4^{ef}
Shalo	65.89^{gh}	2.69^{cd}	82.78^{b}	52^{c}	114.33^{g}	5.33^{bc}	62.33^{def}
CV (%)	13.41	16.43	1.9	1.20	0.93	11.36	8.03

NOPP = Number of *Orobanche* per plot, NOPT = number of *Orobanche* per plant, OI=*Orobanche* incidence, DF = Days to 50% flowering, DM = Days to maturity, NNP = Number of nods per plant, PH = Plant height and CV = Coefficient of variation. Means with the same letter per column are not significantly different.

Significantly higher number of nods plant^{-1} (7) was observed on variety Ashenge (resistance control) and Gachena, followed by Dedia, Degaga, Wayu, Messay and Mosissie (6); whereas, significantly lower number of nods (4) was observed on Dosha and Aloshe varieties. The number of pods borne on each nods was varied and variety dependent ranging from 0 to 1.7 pods per nod (Table 5).

Number of pods per plant

Number of pods per plants was highly ($P \leq 0.01$) affected by broomrapes and faba bean varieties (Table 4). The

Table 4. Level of significance for yield and yield related traits from analysis of variance (ANOVA) for tested faba bean varieties grown under broomrapes at Alemata Agricultural Research Center (Korem) experimental site.

Character/trait	Source of variation		Mean	R^2
	Varieties (*df =19*)	Error(*df = 38*)		
Number nods per plant	2.38**	0.47	5.6	0.73
Number of pods per plant	62.29**	0.26	5.78	0.99
Number of seeds per pod	7.29**	0.01	1.94	0.99
Pod length	29.17**	0.2	3.8	0.99
Hundred seed weight	7229**	1.57	37.33	0.99
Grain yield in kg ha^{-1}	1814121.5**	675.18	595.7	0.99
Biomass yield in kg ha^{-1}	5105394**	68716.97	3064.2	0.97
Harvest index	640.78**	0.5	14.9	0.99
Number of *Orobanche* per plot	3417.65**	108.6	77.7	0.98
Number of *Orobanche* per plant	3.59**	0.25	3.05	0.97

** =highly significant (P < 0.01), NS = none significant, df = degree of freedom and R^2 = coefficient of determination.

number of pods available on a plant depends on number of nods per plant and pod produced on each nod. Highest number of pods per plant was recorded from varieties Ashenge (control) (11.67) and Dedia (11.3), followed by Degaga and Wayu with 10 and 9.3, respectively. The result of this finding showed that only 13 varieties performed with pod setting out of 20 tested varieties with different values in pods number ranging from 7 (Mosissie) to 11.67 (Ashenge), whereas the other seven varieties did not set any pod due to their high susceptibility to *Orobanche* infestation and scored zero (Table 5). However, according to Ashenafi and Mekuria (2015) and Teama et al. (2017), about 10-15 pods per plant were produced from these susceptible varieties under non *Orobanche* infestation area.

Number of seeds per pod

The number of seeds obtained from pods was dependent on the performance of faba bean varieties established under *Orobanche* infestation. The highest seed per pod was obtained from partially resistant and tolerant varieties. Accordingly, the maximum value of seeds from single pod was recorded from variety Ashenge/resistance control as well as Gachena (4.00) and Obse (3.8), while the minimum number of seeds was recorded from Shalo, Welki and Bulga-70 with the value of 2.00 seeds per pod, excluding highly susceptible varieties that had scored zero values (Table 5). However, the varieties with lowest seed per pod in this study were highly productive under non-*Orobanche* infestation area, as indicated in the finding of Ashenafi and Mekuria (2015) at Sinana and Agarfa districts of Bale Zone; whereas Welki, Shallo, Hachalu, Tumsa, Gebelcho, Dosha, Moti and Mosise varieties produced about 3.2 to 4.3 seeds pod^{-1}. This implies that *Orobanche* infection causes yield reduction by 95-100% in susceptible varieties.

Pod length

Orobanche infestation and varieties' genetic variability exerted significant effect on pod length. Also, there were highly significant variations (P ≤ 0.01) in pod length among tested faba bean varieties (Table 4). The highest pod length was recorded from varieties Gachena (8.67 cm), Obse (8.33 cm) and Ashenge (7.33 cm) followed by Dedia (6.67 cm) variety. Without considering susceptible varieties, which scored zero values, the minimum value of pod length was recorded from Welki and Bulga-70 varieties with 4 cm length (Table 5).

Hundred-seed weight

In addition to genetic character of faba bean varieties, the level of *Orobanche* infestation did exert significant effect on 100 seed weight (P ≤ 0.01) (Table 4). In some varieties, hundred seed weight depended on seed size, in which largest seeds gave higher values of weight. Unfortunately, of those varieties performed with yield, a susceptible 'Gachena' variety produced heaviest seed weight (83.67 g) followed by Obse variety (77.00 g) than any other partially resistant and tolerant varieties. However, for most varieties tested in this study, on the other hand, the reduction in hundred seed weight was positively correlated with the level of *Orobanche* infestation, in which hundred seed weight was highly influenced under high levels of parasite infestation. For instance, the susceptible or slightly tolerant varieties except Gachena were scored lower 100-seed weight than that of partially resistant and tolerant varieties such as Ashenge, Dedia and Obse with seed weight of 65, 68.3 and 77 g, respectively; whereas, the highly susceptible

Table 5. Combined mean of *Orobanche* emerged per plots and per plants at three host growing stages and its effects on faba bean varieties for yield and yield related traits.

Variety	Mean No. of *O.* at 3 host growth stage		OI (%)	NPE	SCM	PL	NPP	NSP	HSW g	GY kg ha^{-1}	BM kg ha^{-1}	HI (%)
	NOPP	NOPT										
Holleta-2	73.3^{fgh}	3^{cd}	76^{cd}	47.33	11.33^{fg}	5.67^{cd}	10^{ab}	3^{b}	38^{g}	386.1^{i}	3648.6^{d}	12.9^{gh}
Salale	70.4^{fgh}	2.8^{cd}	66.7^{g}	45	15^{d}	6^{bc}	8.33^{cd}	3^{b}	48.7^{ef}	869.45^{e}	2958.3^{h}	26.5^{d}
Wayu	61^{hi}	2.95^{cd}	72.8^{def}	46.67	12.67^{ef}	5.67^{cd}	9.33^{bc}	3^{b}	40^{g}	497.2^{h}	3309.7^{e}	15^{fg}
Moti	84.6^{cde}	3.9^{ab}	100^{a}	49.67	0^{h}	0.0^{f}	0.0^{e}	0.0^{d}	0^{h}	0.00k	1822.2k	0^{j}
Gebalcho	75.2^{ef}	3.2^{bcd}	100^{a}	48	0^{h}	0.0^{f}	0.0^{e}	0.0^{d}	0^{h}	0.00k	2348.6^{j}k	0^{j}
Obse	67.7^{fgh}	3.2^{bcd}	57.2^{h}	43.67	18.67^{c}	8.33^{a}	7.67^{d}	3.8^{a}	77^{b}	1418.06^{c}	3804^{c}	37^{b}
Welki	78.2^{def}	2.8^{cd}	81.9^{b}	49.67	9^{h}	4^{e}	7.33^{d}	2^{c}	49.7^{ef}	312.5^{j}	3026.4^{gh}	10.3^{hi}
Dosha	92.7^{bcd}	2.7^{cd}	100^{a}	49.67	0^{i}	0.0^{f}	0.0^{f}	0.0^{d}	0^{h}	0.00k	451.33^{fgh}	0^{j}
Mesay	43^{ij}	2.4^{d}	73.7^{de}	47	12.33^{ef}	4.67^{de}	8.33^{cd}	3^{b}	46^{f}	559.7^{g}	3068^{fg}	18.3^{e}
Tumsa	95.7^{bc}	4.3^{a}	100^{a}	50	0^{i}	0.0^{f}	0.0^{e}	0.0^{d}	0^{h}	0.00k	2945.2^{h}	0^{j}
Hachalu	77^{ef}	3.2^{bcd}	100^{a}	46	0^{i}	0.0^{f}	0.0^{e}	0.0^{d}	0^{h}	0.00k	1481.9kl	0^{j}
Bulga-70	79.6^{def}	2.64^{cd}	74.6^{de}	46	11.67^{fg}	4^{e}	8^{cd}	2^{c}	50^{e}	387.5^{i}	2651.4^{hi}	14.6^{fg}
Degaga	70.6^{fgh}	3^{b}	68.4^{fg}	46.33	14.67^{d}	6^{bc}	10^{ab}	3^{b}	59.67^{d}	1154.17^{d}	3558.3^{d}	32.4^{c}
Dedia	79.8^{def}	2.9^{cd}	54.2^{h}	46	21^{b}	6.33^{bc}	11.33^{a}	3^{b}	68.3^{c}	1709.72^{b}	4261^{b}	40^{b}
Tesfa	118.1^{a}	4.2^{a}	100^{a}	47.67	0^{i}	0.0^{f}	0.0^{e}	0.0^{d}	0^{h}	0.00k	1120.8 l	0^{j}
Gachana	104.2^{ab}	3.3^{bcd}	70.8^{efg}	48	14^{de}	8.67^{a}	7.33^{d}	4^{a}	83.67^{a}	698.6^{f}	4212.5^{bc}	16.6^{ef}
Ashange	33^{j}	1.5^{e}	38.5^{i}	45	27.67^{a}	7.33^{ab}	11.67^{a}	4^{a}	65^{c}	3129.17^{a}	7136^{a}	43.9^{a}
Mosise	85.4^{cde}	2.6^{d}	79.2^{bc}	48	10^{gh}	5.33^{cd}	7^{d}	3^{b}	60^{d}	479.17^{h}	1880.6k	19^{e}
Aloshe	98^{bc}	3.5^{abc}	100^{a}	47	0^{i}	0.0^{f}	0.0^{e}	0.0^{d}	0^{h}	0.00k	2570.8^{hij}	0^{j}
Shalo	65.9^{gh}	2.7^{cd}	82.8^{b}	50.33	8.67^{h}	4.67^{de}	7.67^{d}	2^{c}	60.67^{d}	312.5^{j}	2959.7^{h}	9.7^{i}
CV	13.41	16.43	1.9	3.93	6.52	11.79	8.88	3.99	3.35	4.36	9.68	6.84

NOPP = Number of Orobanche per plot, NOPT = Number of Orobanche per plant, OI = Orobanche incidence, NPE = number of plant established, SCM = Stand count at maturity, PL = pod length, NPP = number pod per plant, NSP = number of seeds per pod, HSW = 100-seeds weight, GY kg ha^{-1} = grain yield per hectar, BM kg ha^{-1}= biomass per hectar, HI = harvest index. Means with the same letter per column are not significantly different.

varieties did not produce any seed and scored zero values (Table 5).

Grain yield

There were highly significant ($P \leq 0.01$) variations (Table 4) in grain yield among faba bean varieties. As the result indicated, out of twenty faba bean varieties conducted under *Orobanche* infestation, about thirteen varieties (Table 5) performed with obtaining yield in different degree of response to parasite. The maximum grain yield was obtained from Ashenge (resistance control), Dedia and Obse varieties with the values of 3129.17, 1709.7 and 1418.06 kg ha^{-1}, respectively, followed by Degaga (1154.17 kg ha^{-1}) and Selale (869.45 kg ha^{-1}) varieties. The other seven varieties did not produce yield and any other yield related traits (complete yield loss) due to their high susceptibility to the parasite, which also delayed at pod setting stage.

Biomass yield

The result obtained from analysis of variance for above ground dry matter of tested faba bean varieties indicated that there were highly significant ($P \leq 0.01$) differences between faba bean varieties (Table 4), in which the broomrapes infestation had serous effect on biomass yield. The effect of parasite on faba bean varieties was not only for grain yield, but also had great influence on crop production by reducing growth and development. Accordingly, the higher number of *Orobanche* within variety causes lower number of branches per faba bean varieties and vice-versa. However, in this study, some varieties were performed as well with good biomass performance, which might be due to their best mechanical defense.

The highest mean dry biomass weight (7136 kg ha^{-1}) was recorded from Ashenge variety (resistance control), followed by Dedia (4261 kg ha^{-1}), Gachena (4212.5 kg ha^{-1}), Obse (3804 g ha^{-1}) and Degaga (3558.3 kg ha^{-1}) varieties. On the other hand, the lowest mean dry biomass was recorded from Dosha, Tesfa, Hachalu, Moti and Gebalcho varieties with dry biomass of 451.3, 1120.8, 1481.9, 1822.1 and 2348.6 kg ha^{-1}, respectively (Table 5).

Harvest index

The performance of grain yield over biomass of twenty faba bean varieties grown under broomrapes infestation was evaluated by analyzing the percentage of theirharvest index. Analysis of variance shows that there were highly significant ($P \leq 0.01$) variations (Table 4) among tested varieties in harvest index. The maximum harvest index value was recorded from Ashenge (43.85%), Dedia (40%), Obse (37%), and Degaga (32.44%), followed by Selale variety (26.52 %). Whereas, in other varieties, the harvest index value was less than 20%, including highly susceptible varieties which were scored with 0% value of HI (Table 5). The result of this study indicated that higher grain yield producing varieties were higher in harvest index, while varieties which have a lower yield have a lower harvest index.

Level of *Orobanche* infestation at different host-growing stages

Infestation of *Orobanche* spp. on faba bean varieties at field study was determined by using different parameters on five randomly selected faba bean plants, from each plots, at three host-growing stages (at flowering stage, pod setting stage and maturity stage); the infestation of broomrapes was recorded as per plot and per plant at each growth stage (Table 6).

At host-flowering stage

(a) Number of *Orobanche* shoot emerged per plot: A significant ($P < 0.05$) variation was observed on total number of Broomrapes emerged $plot^{-1}$ in faba bean varieties at host flowering stage. The maximum number of *Orobanche* that emerged per plot was observed in highly susceptible varieties than tolerance and resistance varieties. As a result, the higher number of *Orobanche* that emerged per plot was recorded from Moti (30.33), Gebelcho and Tesfa (29) varieties, followed by Hachalu and Tumsa with values of 26.67 and 26, respectively, whereas statistically minimum number of broomrapes emerged per plot was recorded at variety Obse (6) and Dedia (8); also, there was no emerged *Orobanche* in resistance control (Ashenge) variety at host-flowering stage. However, in the remaining varieties, the number of broomrapes emerged per plot ranged from 11 to 24 (Table 6).

(b) Number of Orobanche emerged per plant: The effect of broomrapes infestation on faba bean varieties at host-flowering stage was comparatively lower due to minimum number of emerged *Orobanche* with faba bean varieties at this growing stage. However, there was significant variation ($P \leq 0.05$) among faba bean varieties in number of *Orobanche* emerged per plant. Higher number of *Orobanche* was recorded from Tesfa (2.1), Tumsa (2), Moti (1.9), and Gebalcho (1.9) varieties, whereas in other varieties there were either very few or no *Orobanche* emerged per plant at this growing stage (Table 6).

At host-pod setting stage

(a) Number of Orobanche per plot: The maximum infestation of broomrapes to faba bean varieties was observed at host-pod setting stages (Figures 1 and 2). There were highly significant ($P \leq 0.01$) difference (Table 4) in number of broomrapes emerged per plot among faba bean varieties at this stage. The higher number of Orobanche that emerged per plot was recorded from Tesfa, Aloshe, Tumsa, Gachena and Dosha varieties with 320.33, 262.67, 241, 236 and 223 respectively, followed by variety Mosise (205.67) and Moti (204). Whereas significantly minimum number (15) of emerged parasite $plot^{-1}$ was recorded from .resistance control (Ashenge) variety (Figure 3). Further, in the remaining varieties the number of broomrapes emerged per plot ranged from 100 to 186 (Table 6).

(b) Number of *Orobanche* per plant: Number of broomrapes emerged per plant highly increased, except in partially resistant variety, at host-pod setting stage comparatively than both at flowering and pod maturity stages. There was highly significant difference ($P \leq 0.01$) (Table 6) between tested faba bean varieties in number of *Orobanche* emerged per single plant. The maximum number of *Orobanche* $plant^{-1}$ was recorded from Tesfa (10.67), Moti (9), Aloshe (8), Hachalu (7) and Gachena (7) varieties, followed by Gebalcho (6.73), Holleta-2

Table 6. Mean of broomrape infestation on faba bean varieties at different host-growing stages.

Variety	At host-flowering stage		At host-pod setting		At Host-maturing stage	
	NOPP	NOPPT	NOPP	NOPPT	NOPP	NOPPT
Holeta-2	19.33^{cde}	1.07^{c}	168.33^{gh}	6.67^{cd}	32.67^{de}	1.33^{def}
Salale	15.33^{e}	0.93^{c}	139.67^{i}	5.73^{cd}	56.33^{c}	1.53^{cde}
Wayu	19.33^{cde}	1.07^{c}	136^{i}	6.3^{cd}	27.67^{def}	1.47^{cde}
Moti	30.33^{a}	1.93^{a}	204^{def}	9^{ab}	19.33^{efg}	0.87^{fg}
Gebalcho	29^{a}	1.93^{a}	177.33^{fgh}	6.73^{cd}	19.33^{efg}	0.93^{efg}
Obse	6^{g}	0.73^{c}	100.33^{j}	4.67^{d}	96.67^{a}	4.27^{a}
Welki	20^{cde}	1.07^{c}	184^{efg}	6.27^{cd}	30.67^{def}	1^{def}
Dosha	22.67^{bcd}	0.87^{c}	223^{cde}	6.4^{cd}	32.33^{de}	0.8^{fg}
Mesay	18.33^{cde}	1.2^{bc}	102.33^{j}	4.67^{d}	10.33^{gh}	1.47^{cde}
Tumsa	26^{ab}	2^{a}	241^{bc}	10^{a}	20^{efg}	0.93^{efg}
Hachalu	26.67^{ab}	1.8^{ab}	186^{efg}	7^{bc}	18.67^{fg}	0.73^{fg}
Bulga-70	21^{cd}	1.27^{bc}	183.6^{efg}	4.67^{d}	34^{d}	2^{c}
Degaga	11.33^{f}	1.07^{c}	173^{fgh}	6.4^{cd}	27.33^{def}	1.2^{def}
Dedia	7.67^{g}	0.67^{c}	150^{hi}	5.67^{cd}	81.67^{b}	3.2^{b}
Tesfa	29^{a}	2.07^{a}	320.33^{a}	10.67^{a}	5^{h}	0.47^{g}
Gachana	13.67^{ef}	1.00^{c}	236^{bcd}	7^{bc}	63^{c}	1.8^{cd}
Ashange	0.0^{h}	0.00^{d}	15k	1.53^{e}	84^{ab}	3^{b}
Mosise	17de	0.93^{c}	205.67^{cde}	5.33^{d}	33.67^{d}	1.53^{cde}
Aloshe	24.33^{bc}	1.93^{a}	262.67^{b}	8^{abc}	7^{gh}	0.53^{g}
Shalo	19.67^{cde}	1.07^{c}	150^{hi}	6^{cd}	28^{def}	1^{def}
CV (%)	9.17	15.8	9.85	12.75	11.98	13.48

NOPP = Number of *Orobanche* per plot, NOPPT = Number of *Orobanche* per plant, CV = Coefficient of variation. Means with the same letter per column are not significantly different.

Figure 1. Level of Orobanche infestation in highly susceptible varieties.

Figure 2. Level of Orobanche infestation in partially tolerant varieties.

(6.67, Dosha and Degaga (6.4), Wayu and Welki (6.3) and Shallo (6) varieties, while the lower number of

Figure 3. Level of Orobanche infestation in partially resistance variety (Ashenge).

Orobanche per plant (1.53) was recorded from Ashenge (resistance control) variety (Table 6).

At host-maturing stage

(a) Number of Orobanche per plot: There were significant ($P \leq 0.01$) differences among faba bean varieties in number of Broomrapes emerged per total plot area at host maturing stage; that is, the maximum number of *Orobanche* emerged $plot^{-1}$ was recorded from Obse (96.67), Ashenge/resistance control (84) and Dedia (81.67) varieties, whereas the lower number of *Orobanche* that emerged per plot was recorded from highly susceptible varieties, Tesfa (4), Aloshe (5), Mesay (10), Gebelcho 19 and Hachalu (20) at this stage.

(b) Number of *Orobanche* per plant: The number of emerged broomrapes within single plant of faba bean varieties was inversely increased in partially resistant and tolerant varieties at host-maturity stage (Figure 6). There were significant differences ($P \leq 0.01$) observed among faba bean varieties for the number of *Orobanche* shoots per plant at maturity stage (Table 4 and Figures 4 to 6). The higher number of *Orobanche* shoots per plant was recorded from varieties Obse, Dedia, Ashenge, with value of 4.27, 3.2, and 3 respectively, followed by Bulga-70 (2), Gachena (1.8), Salale, Wayu, Mesay and Mosissie (1.5) varieties. Whereas the number of broomrapes emerged per plant decreased ranging from 0.47-1.3 in highly susceptible and/or susceptible varieties (Table 6), this was due to the host plants delay at the growing stage.

Figure 4. Level of Orobanche infestation in highly susceptible varieties during host physiological maturity.

Figure 5. Level of Orobanche infestation in partially tolerant varieties during host physiological maturity.

Figure 6. Level of Orobanche infestation in partially resistant variety (Ashenge) during host physiological maturity.

Effect of broomrapes on faba bean varieties and host-plant responses

Based on average results of *Orobanche* infestation during the three host growing stage, there were significant variation among faba bean varieties in their response to *Orobanche* spp. for most studied parameters. Combined mean of broomrape infestation at three host growth stage was analyzed for the total number of emerged broomrapes per plant and per plot to evaluate the performance of faba bean varieties grown with broomrapes infestation. Accordingly, the effect of *Orobanche* infestation on tested traits was statistically different between faba bean varieties at each growing stage. Highly significant differences ($P \leq 0.01$) were observed among varieties for the number of *Orobanche* shoots per faba bean plant at pod setting stage, and most of the tested traits of faba bean varieties were highly influenced by parasites, due to the fact that number of *Orobanche* that emerged per plot and per plant was highest at host pod setting stage when compared with at host-flowering and pod maturity stages.

At host-flowering stage, the number of emerged *Orobanche* spp. within varieties was much lower and had less effect on host plant. But, at pod maturity stage there were two phenomenon about number of parasite emerged within faba bean varieties: the first being that the number of broomrapes per plot and per plant become reduced in highly susceptible and slightly tolerant varieties, due to the number of plant per plot becoming delayed and the parasite also delayed with host plants; and the second, contrastingly one in which the number of emerged *Orobanche* per plant and per plot was increased in partial resistance and tolerance varieties at this host growing stage, with the host-plant not affected by parasite infection because of the pods already physiologically matured.

Accordingly, the maximum number of broomrape that emerged per plot at pod setting stage was in susceptible varieties, like Tesfa (320.33), Aloshe (262), Tumsa (241), Gachena (236), Dosha (223), Moti and Mosissie (205.67) varieties, whereas statistically lower number (15.0) of broomrapes was recorded from resistance control (Ashenge) variety. In general, nevertheless and without considering the resistance control variety, the number of broomrapes that emerged and its infection to varieties was positively associated with the reduction in performance for most traits of studied faba bean varieties. When the number of *Orobanche* that emerged per plot and per plant increases, the performance of faba bean varieties was highly influenced. However ‘Dedia and Obse’ varieties showed tolerance even though the number of emerged broomrapes was high within the varieties as compared with other varieties.

Although the infestation levels of the parasite vary from variety to variety, most of the varieties showed parasitism at field infested by broomrapes. The response of faba bean varieties to *Orobanche* infection was also varied. Accordingly, *Orobanche* incidence was significantly lower in varieties selected for their partial resistance than that of the susceptible varieties. Meanwhile, the incidence was estimated to be 100% in susceptible varieties, which indicates total yield loss. The overall broomrapes incidence ranged from 35.08-100% for tested faba bean varieties at Korem experimental site where the experiment was conducted. The evaluation of grain productivity per host-plants under *Orobanche* infested soil gives more details about the effect of parasitism on hosts. For instance, Ashenge, Dedia, Obse and Degaga varieties performed with yield of 3129, 1709.17, 1418.06 and 1154.17 kg ha^{-1}, respectively, whereas Shallo, Welki, Holleta-2, Bulga-70, Mosise, Wayu, Mesay, Gachena and Selale varieties performed with yield that ranged from 312-869 kg ha^{-1}; meanwhile that of the highly susceptible varieties were completely lost yield (zero). This shows that there were significant variations in impact levels of broomrapes infestation on faba bean grain production as well as variation in degree of host resistance/ susceptibility.

Principal component analysis (PCA)

Principal component analysis is appropriate on a number of observed variables and used as predictor or criterion

Table 7. Principal component analysis for the measured traits in faba bean varieties, and their groups based on their resistance/susceptibility levels.

Variable/trait	Group of principal component analysis		
	PC 1	PC 2	PC 3
Number of plant established (NPE)	-0.19	0.13	0.48
Number of plant harvested with yield($NPHY_W$)	0.30	0.00	0.00
Days to 50% flowering (DF)	0.14	-0.58	0.28
Days to 90% maturity (DM)	0.18	-0.27	-0.50
Plant height (PH)	0.24	0.25	-0.15
Number of nods per plant (NNP)	0.23	0.09	0.13
Pod length (PL)	0.28	0.12	0.18
Number pods per plant (NPP)	0.28	-0.01	0.28
Number of seeds per pod (NSP)	0.29	0.07	0.19
Hundred seeds weight (HSW)	0.27	0.14	0.20
Grain yield per hectar (GY kg ha^{-1})	0.28	-0.01	-0.23
Biomass yield per hectar (BM kg ha^{-1})	0.26	0.04	0.11
Harvest index (HI)	0.29	0.04	-0.15
Number *Orobanche* per plot (NOPP)	-0.19	0.51	-0.15
Number of *Orobanche* per plant (NOPT)	-0.22	0.46	-0.02
Orobanche incidence (OI)	-0.30	0.00	0.03
Eigenvalue	10.75	1.32	1.10
Difference	9.42	0.23	0.32
Explained variance (%)	67	8	7
Cumulative variance (%)	67	75	82

variables in subsequent analysis. It is typically used to analyze groups of correlated variables representing one or more common domains and to find optimal ways of combining variables into small number of subsets. PCA is mainly used as a tool in exploratory data analysis and for making predictive models. It is the simplest of the true eigenvector-based multivariate analyses. Accordingly, the mean values of sixteen quantitative traits tested for twenty faba bean varieties were computed for principal component analysis and then grouped into three principal components. The Eigen values of the three principal components for the performance of varieties which pulled out from the mean values of tested traits were 10.75, 1.32 and 1.1 with the variances of 67, 8 and 7% (Table 7), accounting for about 82% of the total variances or cumulative variation observed in faba bean performance from tested quantitative traits.

In first principal component, greater percentage of variation (about 67%) accounted due to plant height, number of nods per plant, number of pod per plant, pod length, number of seed per pod, grain yield/ha, biomass yield/ha and harvest index. The major contributors for the observed variation in the second principal component were days to flowering, plant height and number of *Orobanche* attached to plant, whereas the variation observed in the third principal component was mainly attributed to number of plant established, days to maturity, pods per plant and *Orobanche* incidence. All the values under each principal component were in the absolute values due to the fact that they represent the Eigenvector of PCA. In general, about 82% of variation was observed in the three principal components, in which greater percentage of variation accounted in the first principal component, that was largely explained by yield and yield related traits when compared to phenological traits (Table 7).

Cluster analysis

Cluster analysis was carried out for faba bean tested under field infested by *Orobanche*; thereafter, the varieties were grouped into four clusters of different sizes (Table 8 and Figure 7), with different members within a cluster assumed to be more closely related in terms of the traits under consideration with each other. Cluster 1 and 2 were the largest with 7 varieties whereas clusters 3 and 4 had 5 and 1 varieties, respectively. However, the varieties closed under the same cluster had been divided into sub partitions, with some varieties found to have good performance than the other within the same group. For example, the varieties Bulga-70 and Mosise had good degree of tolerance than the other varieties in the same group of cluster 2, and varieties Dedia and Obse were best performed than the other varieties within a cluster C-3 (Figure 1). The result obtained from cluster

Table 8. Grouping of 20 faba bean varieties in to different clusters based on their degree of resistance or susceptibility to *Orobanche* infestation under infested field study.

Cluster	Degree	No. of varieties in cluster	Designations
C1	Highly susceptible	7	Moti, Gebelcho, Dosha, Tumsa, Hachalu, Tesfa and Aloshe
C2	Susceptible	7	Welki, Shalo, Holetta-2, Bulga-70, Mosissie, Wayu and Mesay
C3	Intermediate tolerance	5	Selale, Degaga, Gachena, Obse and Dedia
C4	Partial resistance	1	Ashenge (check)

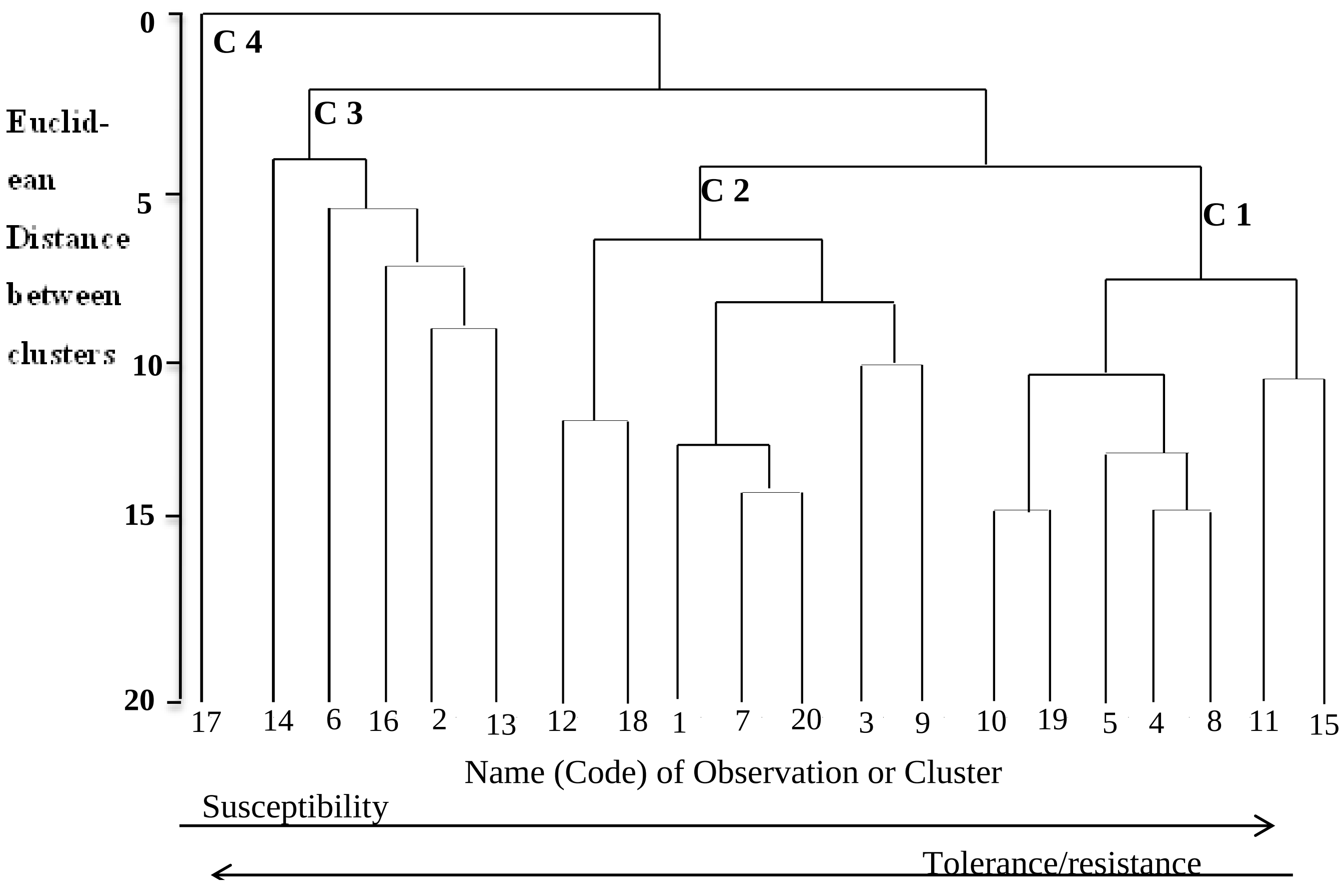

Figure 7. Dendrogram of 20 faba bean varieties evaluated for Orobanche spp. at field experiment based on average linkage cluster analysis between groups.

analysis and described as Dendrogram (Figure 1) indicated that the susceptibility levels increased from left to right, whereas the tolerance or resistance levels increased from right to left as described by the direction of the arrows.

Extent and pattern of faba bean performance under field infested by *Orobanche* infestation

The effect of *Orobanche* infestation on faba bean varieties was evaluated starting from host flowering up to pod maturity stage at field infested *Orobanche* spp. At flowering stage, the result showed that there was no significant influence of parasite on the host plants, because no more *Orobanche* weed emerged within plants. However, days to host-maturity were greatly affected by the parasite, that is, varieties with high level of *Orobanche* infection were faster to complete their life cycle than varieties within lower *Orobanche* infestation. Accordingly, the mean for days to maturity for tested varieties ranged from 114 days (susceptible) to 122 days (tolerance). Faba bean growth and developments are highly influenced by the parasite infestation. In line with this study, Rubiales et al. (2005) also reported that faba bean growth, height, and expansion of roots and branch were seriously affected under *Orobanche* infestation study.

However, in partially resistant and tolerant varieties, all phenological traits, yield and yield related traits were less affected by the parasite infection which might be due to their best host-resistance mechanisms and ability to tolerate the damage of parasites. Accordingly, the maximum plant height, nod per plant, pod per nod, pod length, seed per pod and grain yield was recorded from partially resistant or tolerant varieties Ashenge, Dedia, Obse, Degaga and Gachena in different units.

In susceptible varieties such as Welki, Shallo, Holleta-2, Bulga-70, Mosise, Wayu and Mesay, there was reduction in plant height, less ability to produce pod on their nods, reduction in number of pod per plant with few seeds per pod and minimum amount of yield recorded due to high level of *Orobanche* infection to them. However, according to Ashenafi and Mekuria (2015), those varieties were highly productive under area of non-*Orobanche* infestation.

In highly susceptible varieties such as Moti, Gebelcho, Dosha, Tumsa, Hachalu, Tesfa and Aloshe, there was extreme number of *Orobanche* that emerged per plot and per plant, which causes complete yield losses. The early parasite attachments also appear in highly susceptible varieties which might be due to the high production of stimulant by host-plants that initiate the *Orobanche* seed germination and attachments. However, at non-infested field (like Arsi and Bale), the yield produced by these susceptible varieties was great different from that of the infested field. For example, Hachalu (3436 - 4012 kg ha^{-1}), Tumsa (3497 - 3878 kg ha^{-1}), Moti (3621- 3703 kg/ha), Gebelcho (3703 - 4362 kg ha^{-1}), and Welki (4074 - 4104 kg ha^{-1}), were considered highly productive varieties (Ashenafi and Mekuria, 2015), and the faba bean varieties Dosha (3891 kg ha^{-1}), Tumsa (3437 kg ha^{-1}) and Hachalu (3271 kg ha^{-1}) at south Tigray were evaluated under *Orobanche* free site (Teama et al., 2017); whereas, in infested soil/field, the percentage reduction in grain yield of these varieties were estimated at 95- 100%.

In this study, based on their degree of resistance/ susceptibility, twenty tested faba bean varieties at infested field were grouped into four clusters: the first included the highly susceptible varieties (Moti, Gebalcho, Dosha, Tumsa, Hachalu, Tesfa and Aloshe) which were the most affected by *Orobanche* spp. with the *Orobanche* incidence close to 100%; the second group included the susceptible (Holetta-2, Wayu, Welki, Mesay, Bulga-70, Mosise and Shallo) varieties, with *Orobanche* incidence (71-90%); the third group were the varieties with intermediate tolerance to parasites ('Dedia', Obse, Selale, and Gachena) with incidence (60-70%); and the last group included tolerance/resistance Ashenge (resistance control) with lower parasitize or *Orobanche* incidence (<50%). Even though varieties tested under *Orobanche* infestation showed different performance in most cases, a few varieties fell under the same cluster. For instance, the third cluster "Dedia and Obse" varieties had best performed than any other varieties, next to Ashenge variety, but were however grouped with Selale, Degaga and Gachena varieties in the same cluster. This was probably due to those varieties that have related genetic background as against *Orobanche* infestation effect along with related morphological characteristics.

CONCLUSIONS AND RECOMMENDATION

Faba bean is the main source of human food in developing countries including Ethiopia and as animal feed in industrialized countries. In addition to food, many farmers in Ethiopia used the faba bean as cash crop for income purpose. However, there are different newly emerged biotic (diseases, insect pests and parasite weeds) and abiotic (drought, salinity, fertility etc.) constraints that limit the production and productivity of the faba bean.

Orobanche species are one of the serious parasitic weeds causing considerable losses in many major legumes crops especially faba bean. It is difficult to control, due to its high fecundity and long-term viability of its seed in the soil. Although other *Orobanche* species can infect leguminous plants, they are generally of little economic importance. *O. crenata*, however, has a wide host range among legumes crop, especially in Ethiopia. This parasite is known by its stout, unbranched stem, with smaller flowers. Therefore, this study was conducted under area considered highly infested by *O. crenata* located in South Tigray of Ethiopia, Korem district, to evaluate the performance of faba bean varieties under infestation.

The effect level of broomrape on faba bean varieties was determined using different parameters on five randomly selected faba bean plants, from each plot at three host-growing stages (flowering stage, pod setting stage and maturity stage). The number of emerged *Orobanche* within varieties and the infection level increased during the host-pod setting stage, but become decreased at host maturity stage especially in susceptible varieties; however, contrary to susceptible varieties, in partially tolerant or resistant varieties the number of emerged *Orobanche* increased after the host plants become physiologically matured. The extent and pattern of faba bean performance was also determined by cluster analysis for tested varieties. As a result, faba bean varieties were grouped into four clusters with different number of varieties in each cluster. Varieties with complete (100%) yield loss were grouped in cluster-1 (as highly susceptible); varieties with little amount of yield were grouped in cluster 2 (as susceptible); varieties from which medium yield was obtained were grouped in cluster 3 (as intermediate/partial tolerance); and varieties with higher yield were grouped in cluster-4 (as partial resistance).

Although the levels of effect of broomrape to faba bean plants and the degree of host-resistance was tested, there was no clear determination about 'why the poor number of broomrapes emerged with resistance and tolerance varieties' in this study. Therefore, more studies are needed to determine what happens when faba bean is challenged with *Orobanche* infestation, especially about: (i) deposition of obstructing compounds in the root xylem vessels of the host and mechanisms of resistance, and (ii) ways of water and nutrient flow towards the attached parasites, structure of host cell walls and lignification of the endodermal cells. In a previous study, resistance breeding attempts to achieve complete resistance to broomrape in legumes (that is, immune plants), but they did not succeed due to the complexity nature of its traits and possibly multi-genic nature of this trait. It is important to investigate repeatedly for resistance faba bean cultivars by assessing incomplete and quantitative resistance. In addition, the selection process in the faba bean program for resistance to *Orobanche* infestation on field evaluations is preferred, where natural populations of parasite occur, even though it is difficult to achieve the homogeneity of parasite seed distributions in the soil, and that of the environmental conditions can also affect the host–parasite interaction, hampering the reliable evaluations of host resistance.

Consequently, breeding for resistance is the most economic, feasible and environmental friendly method of control. In this investigation, the number of *Orobanche* seed that emerged per plot and its attachments to host plant, especially at host pod setting stage were the appropriate screening methods and effective selection indices. However, this study was conducted only on single location and under *Orobanche*-infested field. Therefore, future studies and research works are needed to investigate further the parasite-host reaction across a location or conducting the experiment both under infested and non-infested areas. Besides, the next breeding program should be mainly targeted on the improvement of faba bean plants by re-evaluation of partially resistant and tolerant varieties gained by this finding. Also, crossing of Ashenge variety with Dedia variety or Ashenge with Obse variety can lead to a positive response, including molecular analysis using QTLs and MAS, biochemical studies for solving these serious problems, as well as provide high yielding faba bean material that are tolerant or resistance to *Orobanche* spp.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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An update of sweet potato viral disease incidence and spread in Ethiopia

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Sweet potato (*Ipomoea batatas* (L.) Lam.) is an important root crop for poor farmers in developing countries. Since the late 1980s, viral diseases have increasingly become a threat to sweet potato production in Ethiopia. This review paper presents the role of sweet potato production for ensuring food security, the level of sweet potato virus research, including the types of viral species identified and their current level of incidences in Ethiopia. *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), Sweet potato virus 2 (SPV2), Sweet potato virus G (SPVG), and *Cucumber mosaic virus* (CMV) were reported in Ethiopia, where the first two are the most common and exist at high incidences. In addition, this paper discusses the virus vectors, virus transmission methods to new farms, factors exacerbating the rate of viral incidence and the methods used to reduce the incidences. Moreover, it highlights methods of sweet potato viruses' detection and cleaning of infected materials in use and the challenges encountered towards the efficient utilization of the methods. Finally, we suggest major intervention techniques that will integrate all key players in managing the impact of the virus on sweet potato production to improve productivity and ensuring food security in Ethiopia. The findings obtained from this review could be an input for the current research on sweet potato improvement (both planting materials and routines) in Ethiopia.

Key words: Sweet potato, research, virus, detection, planting, infection, production.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an important root crop in developing countries. It is grown in 115 countries, where China is the leading producer. Amongst the main crops produced in the world, sweet potato is ranked seventh on the base of production volume (FAO, 2017). In most developing countries, it ranks fifth in the order of food importance (Som, 2007) and is the third main crop after cassava and maize in East Africa (FAO, 2014).

There are many reasons why sweet potato is important

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and preferred by growers. It is a food, feed and an important raw material for the industry (Bovell-Benjamin, 2007). Growers choose to grow sweet potato crops because of its ability to tolerate a wide range of growth conditions, it has lower demand for agricultural inputs, high yielding potential per unit area per unit time, ease of cultivation and an effective vegetative propagation method (Woolfe, 1992). Moreover, it has a high nutritive value (primarily of carbohydrates and vitamins) and is suitable to grow on marginal lands. Due to all these merits, sweet potato remains a competitive crop for food security in developing countries (Gibson, 2009).

Sweet potato improvement researches have been started in the early 1980's in Ethiopia, and so far, 26 improved varieties were released with their appropriate production packages (Shonga et al., 2013). On the other hand, little is known and less attention was given to sweet potato viruses and the associated diseases until very recently. Furthermore, there is no adequately documented information on viral diseases (virus species, incidence, impacts on yield and efforts made so far to reduce its incidence) in Ethiopia. Thus, this research gap has been a problem for researchers in identifying, prioritizing and tackling constraints to sweet potato production, as well as in designing appropriate disease management strategies for Ethiopia. Therefore, the objectives of this paper are 1) to review the literature on sweet potato viral species identified and the level of incidence and impact of the diseases on sweet potato production in Ethiopia 2) to identify research questions and bring to the attention of researchers and stakeholders 3) suggest possible alleviation strategies.

Production status of sweet potato and its role in food security in Ethiopia

Sweet potato production and its role to combat food insecurity is currently increasing in Ethiopia. Based on the production volume, Ethiopia is ranked the seventh sweet potato producer in the world (FAO, 2017). Sweet potato stands second, after potato *(Solanum tuberosum)* in area coverage among the root crops grown in the country, but is ranked first in terms of production per hectare (Central Statistical Agency, 2015). Sweet potato is cultivated on 130,000 ha of land in Ethiopia, with an annual total production of 2, 0089, 290 tons (FAO, 2017). Over 95% of the sweet potato cultivations are in the densely populated areas in the southern, southwestern and eastern parts of the country (Central Statistical Agency, 2010). Oromia Regional State and Southern Nations Nationalities and Peoples Region (SNNPRS) are the two major producers contributing 52.15 and 47.15% respectively to the annual sweet potato production (Central Statistical Agency, 2010).

The contribution of sweet potato to poor farmers of Ethiopia has so far been underestimated. Farmers of Ethiopia cultivated sweet potato for several years either as a main or as a supplementary source of food. Farmers produce sweet potato mainly for own consumption and also to some extent as sources of income. Sweet potato is a food security crop for at least 20 million Ethiopians (Tofu et al., 2007). It is highly valued when there is shortage of other crops (Emanna, 1990). This is because it withstands drought and performs well on less fertile soil without significant compromises of yield. Sweet potato crop has potential to improve food and nutritional security (especially the orange fleshed with pro vitamin A precursor) for poor farmers (Tsou and Hong, 1992). It is amongst the underutilized crops in most Sub-Saharan Africa countries, including Ethiopia, compared to other sweet potato producing countries in Asia. Recently, there are efforts by various NGOs and Government institutions to introduce sweet potato to other regions in the northern, eastern and western parts of the country, to diversify their crop production (Shiferaw et al., 2014; Aldow, 2017). However, sweet potato yields can vary drastically due to viral diseases (Alemu, 2004; Tesfaye et al., 2013).

Sweet potato-infecting viruses identified in Ethiopia

There is no clear evidence of when or how sweet potato viruses were introduced into Ethiopia. However, *Sweet potato feathery mottle virus* (SPFMV) was first identified around three decades ago, at a place called Nazret [(Scientific Phytopathological Laboratory, 1986)]. There has been no sweet potato virus study in Ethiopia before the study by Alemu (2004). Since then, a number of surveys have been conducted to document the incidences, severities and identities of sweet potato viruses; mostly performed in southern Ethiopia (Alemu, 2004; Adane, 2010; Tesfaye et al., 2011, 2013). The infecting viruses were tested in samples obtained from sweet potato germplasm collections maintained at the research sites and in farmers' fields, mostly located in southern Ethiopia. The presence of five sweet potato infecting viruses in Ethiopia was confirmed by these survey studies (Table 1), out of the thirty virus species known to infect sweet potato worldwide (Clark et al., 2012). Moreover, the surveys also revealed that SPFMV is the most frequently detected virus in southern Ethiopia, followed by SPCSV. None of the other viruses tested [*Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), *Sweet potato chlorotic fleck virus* (SPCFV), Sweet potato caulimo-like virus (SPCaLV), *Sweet potato mild speckling virus (SPMSV) and C-6 virus*] were detected in these surveys. However, a recent report indicated that all these six viruses have later been detected in germplasm, imported into Ethiopia for the purpose of screening for diseases incidence and other traits (Shiferaw et al., 2017). A recent work has reported

Table 1. List of areas surveyed, number of sweet potato samples tested and sweet potato specific viruses detected in Ethiopia.

Location of sampling	Total no. of samples tested	Numbers of samples reacted positive to virus and mixed infection*								References
		SPFMV	SPCSV	SPFMV+SPCSV (SPVD)	SPFMV +SPVG	SPFMV+SPCSV +SPVG	CMV	SPVG	SPV2	
Southern Ethiopia	318	196	0	0	0	0	-	3	-	(Alemu 2004)
Hawassa ARC	57	22	21	7	0	0	-	0	1	(Adane 2010)
Wondo Genet ARC	127	79	13		0	0	0	0	2	
Hawassa Research Center	6	6	6	0	0	0	0	0	0	Dugassa and Feyissa (2011)
SNNPRP and eastern Oromia	970	146	125	90	0	0	0	44	0	
Symptomatic samples	235	134	115	88	25	0	0	28	0	Tesfaye et al. (2011)
Asymptomatic samples	735	13	10	0	7	0	0	15	0	
Hawassa ARC	32	+	+	+	0	+	+	+	0	Wondimu et al. (2012)
Southern Ethiopia Farmer field	166	ni	ni	83	ni	0	ni	ni	ni	Tesfaye et al. (2013).
Research stations		ni	ni	+ (46- 100%)	ni	0	ni	ni	ni	

Presence of the viruses were confirmed by test methods listed in table 2. SPVD: Sweet potato virus dieases, SPFMV: Sweet potato feathery mottle virus,* SPCSV*: Sweet potato chlorotic stunt virus,* SPVG*: Sweet potato virus G,* CMV*: Cucumber Mosaic Virus,* SPV2: Sweet potato virus 2, +: detected but number not indicated**,** **-**: not tested for, ni: no information if tested or not, and ARC: Agricultural Research Centre.

the detection of sweetpotato badinaviruses, *sweet potato mastreviruses*, *sweet potato virus C* and some viroids in high yielding sweet potato varieties from Ethiopia (Dereje, unpublished).

SPFMV and SPCSV, their mixed infections: A threat to sweet potato production in Ethiopia

Sweet potato viral diseases are the second most important limiting factor to sweet potato production next to the weevil in Ethiopia (Fite et al., 2014). SPFMV and SPCSV are the most frequently detected viruses in Ethiopia. For example, high infection of these viruses reported in sweet potato germplasm collections in the research fields at the Hawassa and Wendo Genet Agricultural Research Centers (Adane, 2010). The extent of SPFMV and SPCSV incidence and its economic importance has been described previously in many more sweet potato producing locations in the SNNPRS (Alemu, 2004; Tesfaye et al., 2011, 2013). SPFMV and SPCSV can occur as single infections or as mixed infections. Single infection of these viruses results in mild symptoms (and many times as symptomless infections). However, when both viruses occur as mixed infection, the symptoms are more severe and results in what is known as Sweet potato viral disease (SPVD). Single and multiple infections of sweet potato plants with SPFMV, SPCSV and SPVG and sweet potato virus II (SPV2) are also not uncommon in Ethiopia (Adane, 2010; Dugassa and Feyissa, 2011; Tesfaye et al., 2011). Recent studies also confirmed that SPFMV and SPCSV infections and their co-infection have become serious problems in the farmers' and sweet potato multipliers' fields (Dereje, unpublished) (Mebrate, 2018).

Summary of virus survey literature review reveals few studies that covered only limited locations were conducted on sweet potato virus diseases in Ethiopia (Figure 1). Moreover, most of the studies were limited to locations mostly in SNNPRS (Adane, 2010; Tesfaye et al., 2011; 2013). Unfortunately, no study has been carried out in other sweet potato growing areas of Ethiopia, except a single study data that was generated from samples collected from Hararge zone, eastern Ethiopia (Tesfaye et al., 2011). Therefore, extensive surveys that cover all the

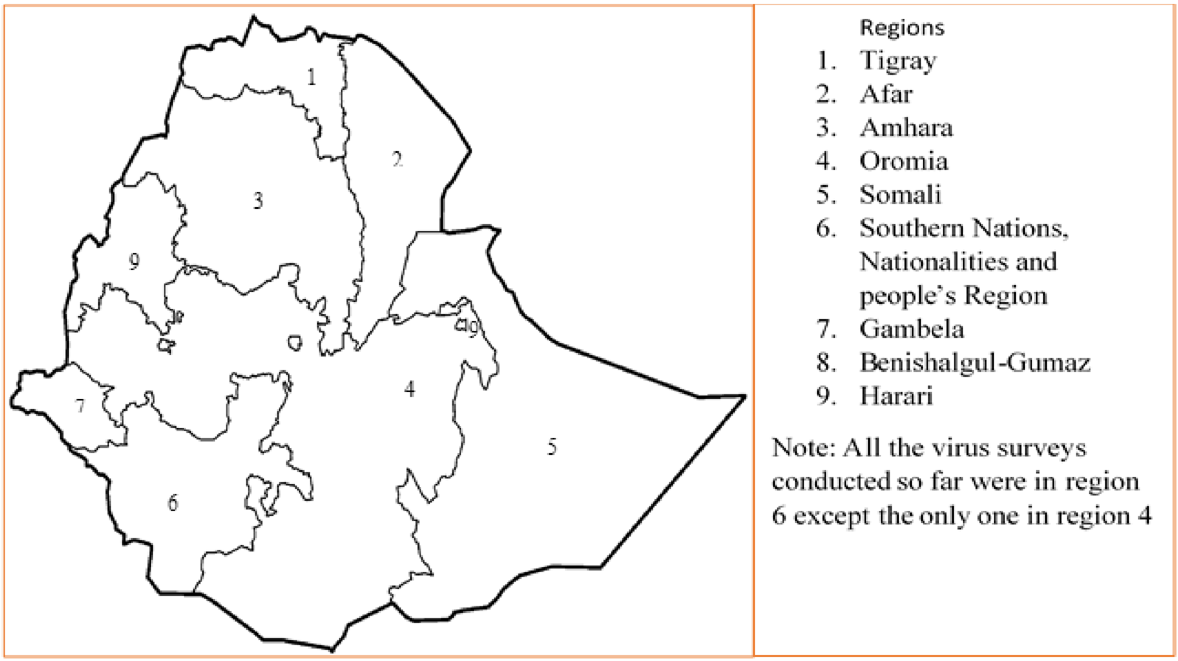

Figure 1. The nine regions in Ethiopia.

sweet potato production regions are required to determine the current status of sweet potato viral diseases in each location to determine the appropriate preventive measures. Furthermore, it is also important to study virus incidences in wild relatives of sweet potato, since these can act as alternative hosts to viruses infecting cultivated sweet potato.

Disease incidence and yield reduction

The incidences of viruses in sweet potato research sites and in farmer's fields in the southern part of Ethiopia are summarized in Table 1. Sadly, research sites (germplasm collections and experimental stations) are more infected than the farmers' fields. Up to 80 and 100% virus incidences were reported in the samples collected from farmers' fields and germplasm collection sites, respectively (Adane, 2010). The author reported one or more viruses detected in those samples. Likewise, Tesfaye et al. (2013) reported incidences of 75% in the samples from farmer's fields and 100% in the experimental stations. The relatively higher incidences SPCSV and SPFMV documented in samples collected from germplasm collections at research stations might be an indicator of the fact that the germplasms imported for adaptation trials were sources of virus infection. Exchange of germplasm between countries for adaptation trials has been a common practice in Ethiopia. However, farmers still grow few improved and mostly local cultivars. Free exchange of planting materials could also be one of the largest contributors to the spread and distribution of viruses in Ethiopia. Therefore, designing and establishing strong quarantine procedures is required to prevent introduction of infected materials. Moreover, any imported germplasm should be restricted from field planting for propagation and adaptation trials until confirmed free of pathogen and insect pest. The above findings confirmed the status of viral diseases incidence in germplasm in Ethiopia is similar to that of Uganda, which ranges from 86 to 100% (Aritua et al., 2007). However, it differs from that of Kenya 48% (Ateka et al., 2004) and Tanzania 17 to 33% (Ndunguru and Kapinga, 2007). Thus, the incidences and severity of sweet potato viral diseases in East Africa are variable.

SPVD is the main bottleneck of sweet potato production in many parts of the world as reviewed by (Gibson and Kreuze, 2015). As sated before, SPFMV and SPCSV incidences are at a high level and SPVD is widely spread in SNNPR, Ethiopia, (Adane, 2010; Tesfaye et al., 2011). SNNPR is the main source of germplasm for trials and planting materials for production to all production regions in the country. Hence, virus- infected materials distributed from SNNPR could be an important sweet potato production threat of the country. A general decline in sweet potato productivity per hectare was observed over the decades (FAO, 2017). However, no reliable studies have been conducted to estimate the extent of yield loss by virus infections.

As mentioned previously in this report, the incidence of viral diseases in Ethiopia is similar to that of Uganda. If the incidence of viral diseases correlates with the observed yield loss in Uganda, one could expect losses of up to 98% in Ethiopia (Gibson et al., 1998; Karyeija et al., 1998; Mukasa et al., 2003). A recent study that compared infected and healthy plants in screen house in Ethiopia showed up to 100% losses of yield, which depending on varieties and infecting virus(es) infection (Dereje, unpublished). However, yield losses are also dependent on the varieties grown, viral type present and climatic condition during the growth period. For example, the incidence of sweet potato viruses in China can be up to 90% (Wang et al., 2010), although the average yield loss due to viral diseases ranges between 20-30% (Feng et al., 2000). Nevertheless, the high incidences of viral diseases in Ethiopia, the lack of efficient diagnostic tools, and lack of virus-free planting materials are among the factors that continue to contribute to the dissemination of viruses within the country. Thus, there is a need to set up diagnostic laboratories and reliable detection methods.

Methods of virus detection

Virus testing employs different diagnostic methods. For virus detection, methods that range from the screening of disease symptoms in the fields to the use of more sophisticated molecular detection techniques can be applied (Boonham et al., 2014; Jeong et al., 2014). The available technological level, existing laboratory facilities and competent workforce to conduct the work influences the choice of any of the method. Assays based on the biological and serological properties of viruses is the commonly used method in developing countries. However, molecular detection methods are more rapidly emerging this days.

Many sweet potato infecting-viruses induces no or mild symptoms on infected plants. For example, SPFMV infected plants is mostly symptomless. Sweet potato Badnavirus causes no symptoms (Kreuze et al., 2017). In this circumstance, ELISA could not be a good testing method in regards due less virus titer in symptomless host. Therefore, grafting sweet potato to an indicator plant is very useful, especially when the virus titer concentration in the original host is below the detection limit of serological tests; Enzyme Linked Immunosorbent Assay (ELISA). However, grafting alone cannot decided the type of the infecting virus and needs other reliable method that target specific species. Unfortunately, grafting is a lengthy method (at least a month) and needs greenhouse space. In some cases, some viruses induce no symptoms even in the highly susceptible indicator plant *Ipomoea setosa* (Clark et al., 2012). Therefore, identification of viruses solely based on symptom expression on host plants is not recommended and should be combined with other testing methods.

ELISA is widely used in many laboratories across the globe. This method is very quick and sensitive for detecting plant viruses mostly that induces high virus titer in infected host, provided that antibodies are available. As described before, due to the low virus titer, an initial grafting to *Ipomoea setosa* or other hosts is required to increase virus titer to detectable levels in many cases. It also requires laboratory equipment, which makes ELISA less accessible, particularly in laboratories in developing countries. In recent years, a combination of serological and nucleic acid-based assays is common plant virus detection methods. Lack of proper laboratory facilities and technical capabilities, access to reagents have limited many developing countries from establishing these detection methods. The methods used to detect viruses in sweet potato plants grown in Ethiopia are summarized in Table 2.

Sources of virus infectious agents and possible means of dissemination

As previously stated, there is no clear evidence of when or how sweet potato viruses were introduced into Ethiopia. Nevertheless, it is believed that viruses were introduced after the 1980s, when the exchange of planting material for breeding purposes increased between many African countries. Many sweet potato cultivars were introduced to Ethiopia between 2001 and 2003 from African (Tofu et al., 2007). Consequently, viruses problem in southern Ethiopia was recognized during 2006 to 2009 (Shiferaw et al., 2014). For example, as presented in this paper, recently six new viruses not previously identified in farmer’s fields in Ethiopia were detected in sweet potato germplasm that was introduced from international sources (Shiferaw et al., 2017). This suggests that the exchange of materials within and between places can be one of the main sources for infecting new areas.

Even though breeding programs have been running since the late 1980s and sweet potato is the second most widely grown root crop in Ethiopia, there are no well-developed sweet potato certified seed production systems. Consequently, there is no established mechanism to generate and supply healthy planting materials to the farmers. Farmers obtain planting materials from many different sources none of them go through reliable phytosanitary control. Most farmers save their own sweet potato planting material from the previous year harvest, while others obtain it either through local exchange from neighboring farmers or buy it from nearby local markets (Dereje, unpublished). Such exchange of planting materials is done irrespective of the knowledge if the material is virus-free. For example, picture in Figure 2A is an example of farmer’s fields infected with virus and

Table 2. Virus detection methods used in Ethiopia.

Methods used for detection	References
Based on biological properties of the virus Biological (graft-inoculation)*	Alemu (2004)
Nucleic acid based (PCR, Sequencing of coat protein)*	Alemu (2004)
Based on viral proteins NCM-ELISA, DAS and TAS-ELISA	Alemu (2004); Adane (2010); Dugassa and Feyissa (2011); Tesfaye et al. (2011); Wondimu et al. (2012); Tesfaye et al. (2013)

*Performed in Germany on Ethiopian plant material. NCM-ELISA: Nitrocellulose membrane-Enzyme linked immunosorbent assay, DAS-ELISA: double antibody sandwich enzyme-linked immunosorbent assay, TAS-ELISA: Triple antibody sandwich enzyme-linked immunosorbent assay, PCR: polymerase chain reaction

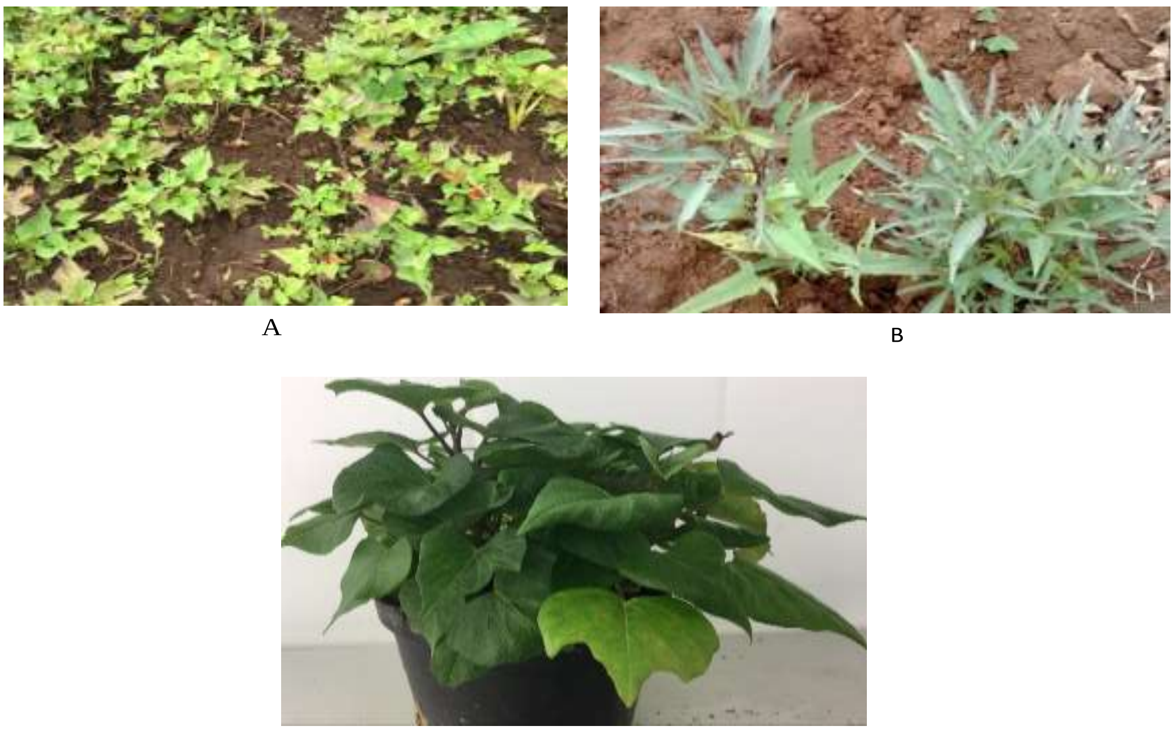

Figure 2. Sweet potato plants with viral disease symptoms in Wolayta zone, southern Ethiopia. (A) Virus like symptoms in farmers field, (B) Mild symptoms of SPFMV infected sweet potato plant ('Kulfo') in the farmer field, (C) Stunted sweet potato plant due to co-infection by SPFMV and SPCSV initially from fields (Photo: D.H. Buko).

show virus-like symptoms. Figure 2B and C respectively, show sweet potato cultivars in fields infected with SPFMV and double infection of SPFMV and SPCSV commonly called SPVD. Indeed, this is the main way in which viruses were introduced and spread from one area to another in African countries such as Uganda (Karyeija et al., 1998). In addition, farmers seldom renew their planting materials, but they keep it for many years by vegetative propagation. Therefore it builds up infection within their fields every year. This planting practice, combined with the fact that many sweet potato viruses are transmitted by aphids and whiteflies widely distributed in Ethiopia, (Table 3) increases the risk of more severe infections and the establishment of viral disease in neighboring virus-free fields.

Methods of virus elimination and virus-free planting material in Ethiopia

Different virus elimination methods have been developed and applied to produce disease-free clones of

Table 3. Symptoms of common sweet potato viral diseases and vectors involved in their transmission.

Virus	Symptom observed in sweet potato	Ways of transmission	Geographic distribution
SPFMV	Single infection: no clear observable symptoms when it infects alone or only mild circular spot on the older leaves or light green pattern along veins. Feathery, purple pattern in the leaves (Gibson et al., 1997; Ryu et al., 1998). It could vary based on cultivar infected and growth conditions	Via stylet of several aphid species in a non-persistent manner, (Stubbs and McLean, 1958)	Worldwide, Reported in Ethiopia
SPCSV	Single infection: causes slight stunting, purpling of lower leaves, mild chlorotic mottle and yellowing (Gibson et al., 1998; Gibson and Aritua, 2002).	Transmitted by whiteflies in Semi-persistently manner (Sheffield, 1957; Sim et al., 2000)	Worldwide, reported in Ethiopia
SPFMV + SPCSV (SPVD)	Dual infection: Infected plant became stunted and produce small-distorted edges, narrow crinkled, strap like leaves with chlorotic mosaic or vein clearing, purpling of older leaves, chlorosis along main leaf veins (Schaefers and Terry, 1976; Gibson et al., 1998)	See above for individual virus	Worldwide, but severe in Africa Reported in Ethiopia
SPVG	Ranges from symptomless to yellow spotting on the leaves	Aphids	Worldwide, reported in Ethiopia
SPMMV	Symptomless to mild leaf mottling and stunting	May be transmitted by whiteflies to sweet potato (Sheffield, 1957; Hollings et al., 1976)	Burundi, Kenya, Tanzania, Uganda, Philippines
SPVG + SPCSV	Symptomless to purple spots and inter-veinal yellow spots	Aphid (SPVG) and Whitefly (SPCSV)	
SPV2	No information for single infection	Transmitted by Aphid (Moyer et al., 1989)	Worldwide, reported in Ethiopia
SPVC	No information for single infection	Transmitted by Aphid (Moyer et al., 1989)	Worldwide, recently identified in Ethiopia (own unpublished)

SPFMV: Sweet potato feathery mottle virus, SPCSV: Sweet potato chlorotic stunt virus, SPVG: Sweet potato virus G, SPMMV: Sweet potato mild mottle virus, SPVC: Sweet potato virus C. SPVD: Sweet potato virus dieases, SPV2: Sweet potato virus 2

economically important crops around the world. Meristem tip culture and shoot tip culture alone and/or in combination with different therapeutic actions: heat treatment cryotherapy and chemotherapy have been used to eliminate virus from many crops, including sweet potato infecting virus in many countries (Spiegel et al., 1994; Panta et al., 2006; Wang and Valkonen, 2008; Panattoni et al., 2013). These methods have been applied to generate virus-free sweet potato in many countries of the world: Taiwan (Green et al., 1992), United States of America (Clark and Hoy, 1999), China (Feng et al., 2000), many countries in Europe (Wang and Valkonen, 2008) and Japan (Yamasaki et al., 2009). Virus from different plant species (root crops, ornamental crops, and tree) have been eliminated by heat-treating mother plants followed by meristem tip culture (Hakkaart and Quak, 1964).

In general, developing countries in East Africa, including Ethiopia, are seemingly left far behind in the adoption and application of tissue culture techniques for virus elimination. In Ethiopia, few attempts have been accomplished to develop *in vitro* propagation protocols and use of virus elimination techniques for sweet potato. However, meristem culture and heat treatment have been used and were able to eliminate viruses from three varieties of sweet potato in Ethiopia (Table 4) (Dugassa and Feyissa, 2011). These varieties cleaned of viruses were not made available may be they were not maintained or was done just for the master thesis study purpose. The efficiency of meristem culture and combined heat treatment have also been evaluated and compared (Dugassa and Feyissa, 2011). However, there is no schemes developed and in use to provide virus tested material.

Generating and providing ‘virus-free’ sweet potato planting materials increases yield per hectare, which improve human food security and livestock fodder. Virus elimination and explant-regeneration requires a good tissue culture protocol. Developing new or adopting and modifying existing protocols previously developed elsewhere in the world important. However, lack of and /or limited laboratory facilities, lack of practically trained

Table 4. List of sweet potato cultivars tested for virus and virus elimination methods used in Ethiopia.

Cultivar/access ions	Elimination methods	Number of clones tested	Elimination efficiency* (%)	Reference
'Hawassa 83'	Meristem culture	9	100	Dugassa and Feyissa, (2011)
	Shoot tip thermotherapy	9	88.9	
'Guntute'	Meristem culture	6	100	
'Hawassa local'	Meristem culture	8	100	
	Shoot tip thermotherapy	6	100	
'Bellela'	Meristem culture	24	99.9	Wondimu et al. (2012)
'Temesgen'	Meristem culture	24	100	
'LO-323'	Meristem culture	25	100	
'Zapallo'	Meristem culture	25	100	

*Efficiency of virus elimination methods were determined based on the percentage of virus-free plantlets obtained by each method.

workforce, less access to reagents, absence of functional greenhouses and insect proof screen houses are still considered to be the main challenges to adopt and use the existing techniques in Ethiopia.

Still virus infection high level, planting materials available to farmers are unreliable in terms of viral infection. Therefore, urgent attention and action is in need to increase the yield of sweet potato, mainly by providing clean planting material and measure to reduce re-infection rate. Implementation of integrated strategies that target the prevention of the introduction of virus, their vector, and their distribution into uninfected areas. Such strategies in turn, could enable resource-poor farmers to maintain healthy sweet potato planting materials.

Production challenge due to sweet potato virus call for intervention in Ethiopia

Strong quarantine restrictions

As presented in Table 1, five sweet potato infecting virus species were detected in southern Ethiopia. In addition, six more virus were reported from germplasm imported for improvement works (Shiferaw et al., 2017). To limit further spread of the existing viruses into new production regions, the different key players need to work in synergy. Before distributing planting materials from virus spot location of southern Ethiopia to new locations, healthy status must be first confirmed. In this regard, Research Centers must apply rigorous quarantine checks. The technical guidelines for the exchange of pathogen-free sweet potato plant materials should be followed (Moyer et al., 1989). Both exporting and importing bodies ought to abide by these guidelines.

Screening and breeding for resistance

In Ethiopia, cultivar diversity is getting lost due to low yielding and infection of viral diseases. This is true particularly in the virus-prone areas of SNNPR where one of the improved variety called Hawassaa-83 dominantly grown. It is possible to screen virus tolerant sweet potato cultivars from local cultivars and use them for resistance breeding. Experts in Agricultural Research Centers in Ethiopia have been trying for a long time to screen and use disease tolerant varieties. The effort to solve the problem appears not yet to be successful, thus, the yielding potential of sweet potato cultivars is declining. Though it needs further effort, Shiferaw et al. (2017) reported of promising accessions for virus resistance. As presented earlier in the paper, the efforts to screen for disease resistance and better yield in southern Ethiopia was not without the risk of introducing viruses along with germplasm. It is advisable to exploit local cultivar gene pools in the country instead of introducing infected material as quarantine restriction of the country is not strong enough to screen out.

Therefore, it appears that no adequate and appropriate interventions were made in screening and breeding for disease resistances in sweet potato and it needs a more coordinated effort of all stakeholders. Exploiting resistant genotypes from germplasm pools using both traditional and recent advanced molecular methods would be important.

Training

Sweet potato growing farmers and extension workers in Ethiopia have low perceptions of viral diseases (own, unpublished data). Inabilities to identify a virus-infected plant based on symptoms in the field and lacking basic know-how on its mechanisms of transmission affects proper selection of healthy looking plating materials. Moreover, it contributes to the continuous use and exchange of infected planting materials from season to season. Therefore, training basic practices on disease identification and management is very important. Moreover, training that enable farmers selecting and using of good planting materials and how to practice sanitation measures would be vital.

Naturally virus resistant/tolerant cultivars, show no symptom depending on many factors. If the farmers do not have access to virus-tested planting materials, and still have to grow it, training would help them to select the best mother plants from the existing symptomless plants (with possible low concentration of virus titers or healthy) in their farm. Farmers' training on removing weeds that may harbor virus-transmitting insects is vital. In additions, weeds may serve as an alternative host for the viruses, must be removed on time. Educating farmers how to identify and rouging out infected plants, proper and timely application of sanitation practices and crop management is very important.

Farmers' closer mentors have high impacts in improving agricultural practices. Study conducted in Ethiopia shows extension workers in the studied areas were less exposed to training on sweet potato diseases identification and management (own unpublished data). It is important to provide problem-solving practical training to those who work closely with farmers. Extension workers should get awareness and training on the sources and choice of good planting materials (diseases free, high yielding), the negative effects of sweet potato viral diseases, practical virus identification in the field and knowhow of appropriate disease management principles. In general, training will greatly contribute to proper virus management that results in the higher chance of reducing the infection of new areas and improves the yield of crops. Training should include practicing sanitation measures in the field, removing infected plants timely to avoid virus spreads within plants and avoiding contamination of pathogen-tested planting materials.

Technical capacity building and laboratory facility

Expertise and basic laboratories are required for diagnosis, identification, and elimination of viruses. Without proper knowledge, it is more difficult to manage virus diseases. There are limited numbers of professionals and poor laboratory facilities in developing countries in general, both for virus diagnosis and elimination. In Ethiopia, there are very limited numbers of experienced plant virologists. Moreover, they have limited access to practically oriented training on identification and elimination of plant viruses, mainly because of a lack of access to properly equipped laboratories and reagents, both at the regional and national levels. This may have Even though virus elimination techniques have been developed and largely utilized across the world, they are less used in Ethiopia. Because of poor facility and technical problem, farmers in Ethiopia have no access to virus-tested planting materials. As a result, farmers continue to use virus-infected vegetatively propagated sweet potato planting materials that could build up over years. Therefore, availability of basic facilities and technically skilled professionals is important to develop/adopt effective methods and establish programs to develop and maintain pathogen tested propagation stocks of farmers preferred root crop cultivars.

This calls for collective and individual roles of all key-players including the government, Non-Governmental Organisation (NGOs), private sectors, research institutions, Ministry of Agriculture and universities in funding for laboratories and capacity building. Universities and research institutions should be more involved in training extension workers, farmers and other stakeholders. The government should play a major role in allocating funds for laboratory facilities and research activities. Researchers are expected to conduct studies and know the virus species associated with farmers preferred varieties in all the production regions. They should also work to design methods adapted to local conditions and evaluate the best virus elimination and subsequent management methods for the respective viruses.

Provision of virus-tested planting materials

Availability of disease-tested planting materials with desired agronomic traits is key to increase production and thereby improving the life of the farmers. Virus-tested materials can be obtained either through screening naturally existing plant materials or by eliminating viruses from mother plants. Very little progress has been made to identify and eliminate virus from vegetatively propagated materials in Ethiopia. There are no big companies certified to supply virus tested sweet potato planting materials, except for some recent practices of using tissue-cultured plants as a starter. Further multiplication in open fields makes the plants prone to re-infection before reaching the farmers. Moreover, these small-scale multipliers are not getting basic clean starting materials and have no rigorous follow up. In multiplier fields, viruses can also get multiplied and when distributed to farmers, it transmitted to the susceptible host in the nearby field and infect sweet potato landraces on farmer's hands. Therefore, a short-term solution to tackle the problem is to intervene through the provision of vines of pathogen-tested sweet potato plants to the farmers and giving awareness on subsequent management practices to reduce the infestation rate. The use of clean and virus-tested planting materials is economically viable if there is an effective and efficient system for production, multiplication, and distribution of planting materials (Carey et al., 1997; Feng et al., 2000).

What intervention is needed? Providing clean planting materials of root and tuber crops boosts yield and farmer's income. Therefore, all stakeholders (Government, NGOs, Research centers and Universities) are advised to give attention and acknowledge the necessity to provide resources for virus assessment and elimination. The private sector should be encouraged to collaborate with the universities and research centres and invest on tissue culture facilities for commercial production of healthy and quality vines. Initiating new ideas of investment in tissue culture and strengthening existing institutions and farmers' associations to propagate virus-tested plant is a priority. In addition, extension officers should contribute to demonstrate that the use of clean/symptomless planting materials would consistently produce higher storage root yield than the naturally infected farmers' planting materials.

CONCLUSION AND RECOMMENDATIONS

Surveys on sweet potato viral diseases in Ethiopia revealed that viral disease incidence and severity is a critical issue for sweet potato production in the southern region of Ethiopia. National germplasm collection and farmers' fields are contaminated with the most common sweet potato viruses; SPFMV, and SPCSV. As a result, the rate of spread and its negative impact on the yield is discouraging farmers who grow and use sweet potato as a main food security crop. The Southern Nations Nationalities and Peoples Regional State is the National Center and the source for a further introduction of sweet potato plant materials to other parts of the country. Therefore, this current incidence of sweet potato virus in this region will be a potential threat to sweet potato production in the whole country. Collectively, this demands intervention at all levels (that is, both at institutions and farmers' levels). Moreover, new viruses are being introduced with germplasm from international sources. Therefore, in order to reduce the negative impacts of viruses on yield of sweet potato in Ethiopia, the following points are recommended and need attention.

(i) Organizing and the strengthening of the quarantine systems during importation to the country and certification of planting materials movement between regions is very important.
(ii) Germplasm introduction should be regulated and new materials should be inspected prior to introduction and multiplication in the open fields.
(iii) Standardized method for large scale virus detection in Ethiopia.
(iv) Future virus surveys should address more production regions in the country and use appropriate testing methods.
(v) Increase awareness of viruses to farmers and extension workers.
(vi) Supplying virus-tested planting materials and establishing a system of distribution would enhance the farmers' ability to increase production and productivity of sweet potato.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Impact of soil management and irrigation techniques on water use efficiency in cauliflower cultivation

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The objective of this research is to evaluate the impact of soil management and localized irrigation techniques on water use efficiency in the cultivation of cauliflower. The treatments consisted of 4 different types of soil management: Direct seeding using crotalaria and brachiaria cover crops, a treatment used both in conjunction, and another that employed a conventional system. Two different irrigation techniques were used: Surface and subsurface. The experimental design was completely random in a factorial arrangement (4x2) with four repetitions. Each experimental unit was composed of 20 cauliflower plants, with a density of 1.8 m between rows and 0.5 m between plants. The experiment was carried out in two stages; the first stage consisted of the establishment of the cover crops and the second stage of the planting of cauliflower cv. Avenger in the different treatments. The cover species were cut at ground level 108 days after they had been sown. The cauliflower seedlings were grown in a protected environment and transplanted after 21 days. Irrigation was managed through the use of tensiometry. Water use efficiency was the variable that was analyzed; the Tukey Test at 5% level of probability was applied to the data that was obtained. A combined use of crotalaria/brachiaria cover crops with subsurface drip irrigation is recommended; this was the combination that most efficiently saved water.

Key words: *Brassica oleracea* cv. botrytis, *Crotalaria ochroleuca*, *Brachiaria ruziziensis*, soil management, irrigation.

INTRODUCTION

At present, the problem of poor water use as a consequence of inadequate soil management techniques is prevalent. These techniques are mainly characterized by the excessive clearing of the soil through ploughing or harrowing; this represents an entirely insufficient practice that causes the degradation of the topsoil. For this

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reason, a variety of techniques and strategies are turned to in an effort to increase the productivity of soils and improve water use efficiency.

One of these techniques is the use of vegetation cover such as brachiaria (*Brachiaria ruziziensis*), crotalaria (*Crotalaria ochroleuca*) and/or a combination of both species. Depending on its use, this technique has the capacity to improve the physical, chemical and biological properties of the soil. These plant species also allow for better infiltration and retention of water. Another strategy that is used to improve water use is the deployment of more efficient irrigation systems such as subsurface irrigation, which involves burying irrigation tubing. The main advantage of this technique is the reduction of the volume of water needed, given that water is delivered directly to the radicular system.

Sousa et al. (2015) conclude that the use of mulch leads to better water use in the irrigated cultivation of *Phaseolus vulgaris.* It also aids the soil-water-plant-atmosphere relationship by reducing the temperature and the rate of evaporation and increasing retention of humidity in the soil profile. Furthermore, they mention that the differing rate of crop growth that was seen under the conditions evaluated is due to the water retention achieved by placing mulch on the surface of the soil. Given these factors, the objective of this research was to evaluate the impact of soil management and localized irrigation techniques on water use efficiency in the cultivation of cauliflower.

METHODOLOGY

The experiment was conducted in the area for irrigation experiments of the Faculty of Agricultural Sciences of the Federal University of Grande Dourados. The experimental area is located in Dourados, Mato Grosso do Sul at geographical coordinates 22° 13' 16'' latitude south and 54° 48' 20'' longitude west. In accordance with the Köppen classification (1948), the region has a Cwa climate type (humid subtropical), with rainy summers and dry winters, and an average annual temperature of 22°C. The soil is described as dystrophic red latosol with a very argillaceous texture (EMBRAPA, 2009).

The design of the treatments used was a factorial arrangement (4x2) distributed in an entirely random experimental design. The variables that were evaluated were: soil management and drip irrigation techniques. There were 8 treatments and 4 repetitions, giving a total of 32 experimental units. There were 4 different types of soil management: crotalaria (*C. ochroleuca*), brachiaria (*B. ruziziensis*), a combination of crotalaria and brachiaria and a conventional soil preparation system. There were two different types of irrigation: a surface drip irrigation system and a subsurface drip irrigation system.

Drip irrigation systems were employed. In the first irrigation technique, the drip tapes were installed on the surface of the soil (surface drip). For the other method, they were buried at a depth of 0.20 m (subsurface drip). Each experimental unit held 20 plants with a distance of 0.8 m between rows and 0.5 m between plants; the plants were distributed in simple rows with a density of 25.000 plants/ha. The experiment covered a total area of 8 m^2, with each EU having a length of 3.2 m and a width of 2.5 m. The two central rows were considered to be the useful area. The two plants at each end of these rows were not evaluated in order to avoid the fringe effect, giving a total of 6 plants used to carry out determinations.

The experiment was carried out in two stages: the first consisted of the establishment of the cover crops and the second of the planting of cauliflower under the previously mentioned experimental conditions. 40 days before the sowing of the cover species, a soil dressing was applied at a dosage of 200 g m^2; this was done in accordance with the recommendations of the soil analysis. The cover crops were sown with spacing of 0.45 m between rows; rows contained approximately 40 plants per linear metre. The combination of the two species was achieved by interspersing them; the same distance between plants that is mentioned above was used. No chemical fertilization was employed at this stage. Weeding was carried out manually.

108 days after being sown, the cover species were cut at ground level using a mechanical cutter. This was done when the crotalaria plants began to flower and when the brachiaria was at the milky-grain phase. The biomass of the cover crops was evaluated when they were cut. Before being cut, the brachiaria and the mixed cover (B+C) were chemically dried using Roundup (200 ml of active ingredient per 20 l of water). The preparation of the soil in the conventional system consisted of the use of a subsoiler and a disk harrow; this was done on the same day that the cover crops were cut in the other treatments.

The cauliflower seedlings were grown in polystyrene trays with 128 cells, which had previously been filled with commercial substrate and kept in a protected atmosphere. The Incline variety was used, which is suitable for planting in the region throughout the whole year. 21 days after sowing, when the seedlings displayed the necessary criteria for transplantation (height of 10 cm + 4 to 5 real leaves), they were transplanted to the previously prepared experimental area. The transplant was carried out in holes with an approximate diameter of 0.2 m and an approximate depth of 0.1 m. Before transplantation, fertilization was carried out according to the recommendations of the soil analysis and following the suggestions made by Fontes (1999). A week before transplantation, 150 kg ha^{-1} of N was applied in the form of urea, 300 kg ha^{-1} of P_2O_5 was applied using simple superphosphates, and 180 kg ha^{-1} de K_2O was applied in the form of potassium chloride. As well as chemical fertilization, organic fertilization was carried out using 350 g of commercial substrate per hole. Cover fertilization was carried out at 15, 30 and 45 days after the transplant, with 30, 40 and 30% of the total quantity of coverage fertilizer (100 and 200 kg ha^{-1} of N and K_2O respectively) being applied in the first, second and third period respectively. Foliar fertilization was carried out every seven days from the second week after transplantation onwards; this was done in order to prevent symptoms of nutritional deficiencies. Heringer®'s FHHF Fruit and Vegetable fertilizer was employed; its composition is as follows: 11% N; 11% P; 11% K; 2% Mg; 10% S; 0,15% B; 0,30% Cu; 0,11% Fe; 0,26% Mn; 0,04% Mo and 0,50 % Zn; at a dosage of 1.0 g.L^{-1}.

Weed control was carried out in the furrows using a hoe and manually between the cauliflower plants in rows. A notable difference in the amount of weeding carried out was observed; the conventional system was weeded four times during the cultivation cycle, whilst the different systems employing mulch were weeded just once. Given that there were some strong winds that could have hindered the growth of the crop during the experimental, hilling was carried out in order to improve the establishment of the plants.

Chemical control of pests was carried out initially 10 days after transplantation (DAT). Benzoylurea (Nomolt® 150, at a concentration of 25 ml 100 L^{-1} of water), was applied to control thrips (*Thrips tabaci*) and cucurbit beetles (*Diabrotica speciosa*). Caterpillars such as *Spodptera* sp., *Tricop lusiani* and *Pseudo plusiaincludens* appeared 30 and 45 DAT; these were controlled using benzoylurea (Nomolt® 150, at a concentration of 25 ml 100 L^{-1} of water) and thiamethoxam (Engeo Pleno® 247 SC, at a concentration of 50 ml 100 L^{-1} of water). The irrigation management of the cauliflower crop was carried out independently for each treatment. Irrigations were carried out based on readings of soil water tension measured using tensiometers installed at 50% of the effective depth of the radicular system, which is 0.40 m. A set of four tensiometers was installed in each treatment: three at a depth of 0.20 m (decision tensiometers) and one at a depth of 0.40 m (control tensiometer). These were installed in crop rows between two plants with a distance of 0.50 m between tensiometers. The readings were carried out using a puncture tensiometer twice a day: at 09:00 and 15:00. Irrigation depth was determined using equations 01 and 02 in order to restore soil humidity to values corresponding to field capacity. Irrigation was carried out when at least two readings obtained by the sensors installed at a depth of 0.20 m (decision tensiometers) were higher than 15 kPa,

$$\mathrm{NIWR} = \frac{\mathrm{GIWR}}{\mathrm{ae}} \quad (1)$$

Where: GIWR: Gross irrigation water requirement (mm); NIWR*:* Net irrigation water requirement (mm); *ae:* Application efficiency.

$$\mathrm{IA} = \frac{\mathrm{Q}}{\mathrm{A}} \quad (2)$$

Where: *IA:* Intensity of application of the irrigation system in each treatment (mm), *Q:* Total volume of water (L h^{-1}), *A*: Area occupied per plant (m^2).

$$\mathrm{T} = \frac{\mathrm{GIWR}}{\mathrm{IA}} \quad (3)$$

Where: *T:* Duration of operating time of irrigation system in each treatment; GIWR: Gross irrigation water requirement (mm); *IA:* Intensity of application of the irrigation system in each treatment (mm).

Over the first twelve DAT, micro-irrigation was employed using Santeno® tapes; 4 irrigations took place over this period. In this period, watering was carried out for a duration of one hour divided into four applications. This corresponded to an irrigation depth of 15.03 mm h^{-1} in each irrigation. All of the treatments were irrigated with the same quantity of water in order to maintain an adequate microclimate and to favour the establishment of the seedlings.

Two types of irrigation system were used: a surface drip system on the soil surface, and a subsurface drip system buried at a depth of 0.10 m. In-line, self-compensating drips were used, with an output of 3 L hm, DN 16 mm. They were inserted into the tube upon extrusion with a distance of 0.50 m between drips; this was done in order to generate a strip of consistently watered soil. The drip tapes were placed in such a fashion that there was one drip per plant. There was an operating pressure of 10 mca, which was controlled using a regulatory pressure valve inserted at the end of the control head.

The cauliflower plants in the experimental units were harvested manually at 82 DAT. Following the harvest of the inflorescences, evaluations were carried out. The determination water use efficiency (WUE) was obtained according to Equation 04. The commercial product is equivalent (Y_C) and the volume of water applied per hectare (AL) was estimated from the necessary total irrigation applied in each treatment during the growing cycle.

$$\mathrm{WUE} = \frac{\mathrm{Y_C}}{\mathrm{AL}} \quad (4)$$

Where: WUE: Water use efficiency (kg m^3); Y_C: Commercial product (kg planta); AL: volume of water applied per hectare (mm planta). An analysis of variance was undertaken using the F test and afterwards, a comparison of averages was carried out using the Tukey test at 5% level of probability.

RESULTS AND DISCUSSION

Characterization of climatic conditions

Figure 1 shows values related to the maximum, average and minimum air temperatures observed during the period in which the experiment was carried out. Temperatures during the experimental period varied between 6 to 31.7°C, with an average temperature of 21.24°C (Guia Clima-embrapa, 2015). Assuming that frosts occur at temperatures below 4°C, there were no frosts during the course of the experiment.

The maximum average temperature was 27.33°C and the minimum average temperature was 16.38°C. The temperatures that were observed during the experiment provide ideal conditions for the germination, and growth of the crop as for the majority of the cultivation period the temperature was between 13 and 28°C (Strange et al., 2010).

Figure 1 shows the maximum, minimum and average relative air humidity (RH) during the experimental period. The RH during the experiment was 80.8%, the maximum average RH was 95.23% and the minimum average was 56.28%. The RH during the experimental period oscillated between 97 and 28% (GUIA CLIMA-EMBRAPA, 2015).

Figure 2 shows the readings of the decision tensiometers installed in each surface-irrigation treatment. Tension values approaching zero are observed on rainy days, indicating saturation. No large differences in the readings from the different treatments were seen; this could be due to the frequent watering (on average, every two days). In general, the tension readings were higher in the conventional treatments, where they quickly reached higher tension values after a rainy period. After periods of rain, the mulch treatments took longer to show tensiometer readings indicating the need to use the irrigation system. This was due to higher retention and higher availability of water in those treatments; they showed higher microporosity. The higher biomass in these treatments also provided the advantage of lower rates of water evaporation.

Water use efficiency

The number of irrigations (N.I) varied in relation to the different cover species used. Lower irrigation volumes were required when using the crotalaria mulch and the combination of crotalaria and brachiaria; 24 irrigations were carried out using the surface drip irrigation system and the subsurface system. The brachiaria mulch was irrigated 26 times. The conventional system received 32 irrigations, meaning that it showed the highest level of water consumption of the different treatments. Equally,

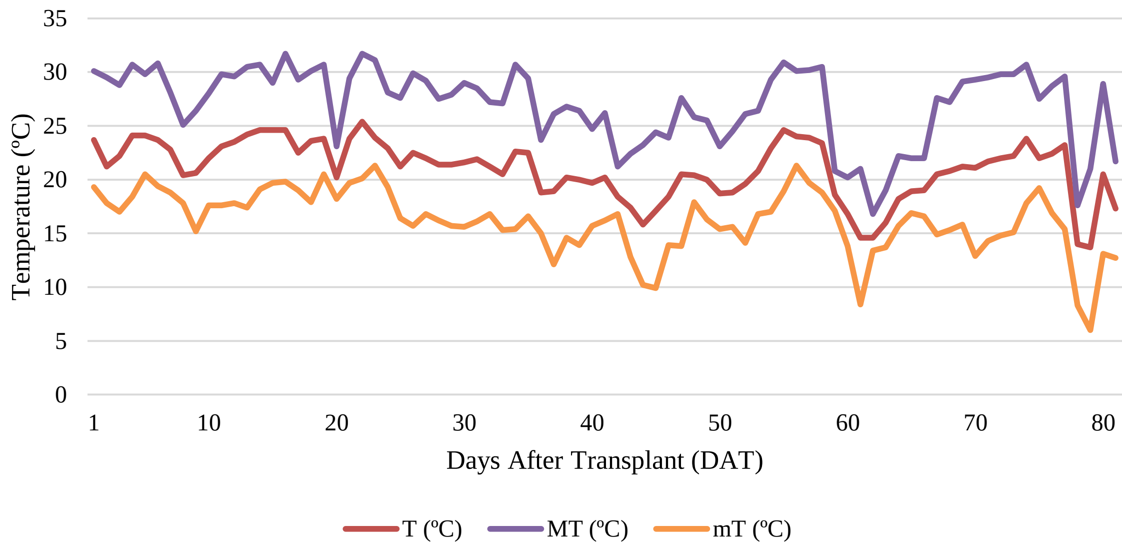

Figure 1. Daily air temperatures during the experimental period: maximum (MT), average (T) and minimum (mT).

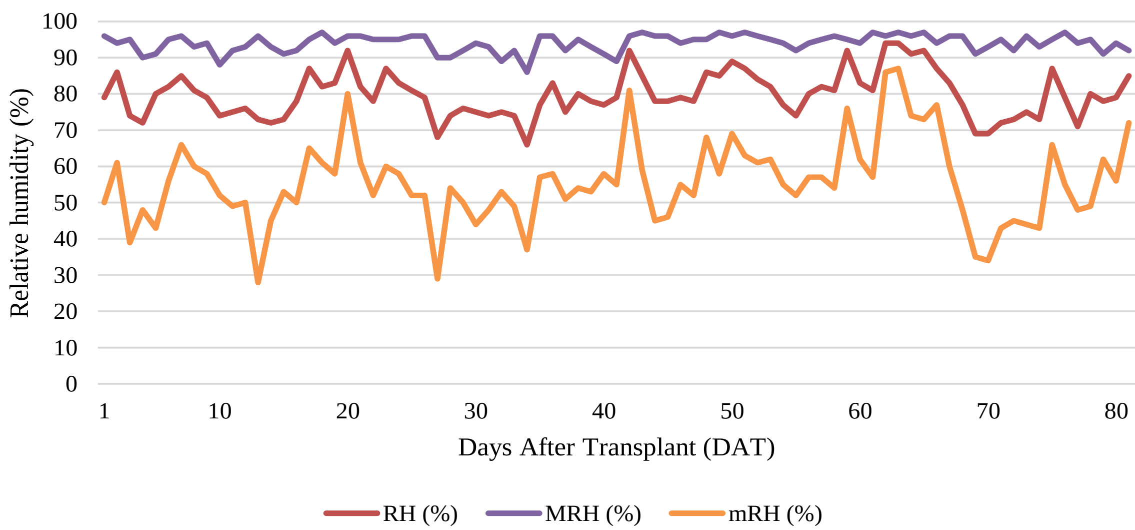

Figure 2. Maximum (MRH), average (RH) and minimum (mRH) relative daily air humidity during the experimental period.

lower total irrigation (T.I) was registered when employing mulches that reduced the number of irrigations (Table 1).

Results that are similar to those obtained in this experiment were observed by Marouelli et al. (2010) when studying water use efficiency in the production of cabbage using different quantities of straw in direct seeding. They observed that the treatments with vegetation cover received between 26 and 28 irrigations during the crop cycle. The conventional system received 32 irrigations. Pereira et al. (2002) also observed a decrease in the number of irrigations in treatments where mulch covered more than 50% of the soil surface, indicating a higher water retention in the soil. This allowed for the application of a lower quantity of water during the crop cycle, favouring the reduction of operational costs related to watering.

Reduction in water use in the treatments with mulch and surface irrigation varied between 5.72 and 15.7%; the brachiaria showed lower levels of water conservation whilst the combination of the two occupied showed higher levels of water conservation; the crotalaria a middle point, with 20.4% reduction in water use in comparison to the quantity of water applied to the treatment with a conventional system. Results with similar parameters to

Table 1. Depth of irrigation applied, number of irrigations in the different treatments, Dourados-MS 2015.

Treatment	Description	Depth of irrigation (mm)				N.I
		I.I	D.I	T.I	api	
CvS	Conventional with surface drip.	66.04	129.54	195.58	6.1	32
CvE	Conventional with subsurface drip.	66.04	122.24	188.28	5.9	32
BrS	Brachiaria with surface drip.	66.04	118.34	184.38	6.4	29
BrE	Brachiaria with subsurface drip.	66.04	107.89	173.93	6	29
CrS	Crotalaria with surface drip.	66.04	89.64	155.68	6.5	24
CrE	Crotalaria with subsurface drip.	66.04	85.94	151.98	6.3	24
CoS	Combination with surface drip.	66.04	98.64	164.68	6.9	24
CoE	Combination with subsurface drip.	66.04	95.15	161.19	6.7	24

I.I: Initial irrigation, D.I: Drip irrigation, T.I: Total irrigation, api: Average per irrigation, N.I: Number of irrigations.

Table 2. Depth of irrigation applied, production and efficiency of water use in the treatments (Dourados-MS, 2015).

Treatment	Description	Irrigation depth applied (mm)	Production (Mg ha^{-1})	WUE (kg m^{3})
CvS	Conventional with surface drip.	195.58	14.25	2.9
CvE	Conventional with subsurface drip.	188.28	14.81	3.1
BrS	Brachiaria with surface drip.	184.38	16.86	3.7
BrE	Brachiaria with subsurface drip.	173.93	16.55	3.8
CrS	Crotalaria with surface drip.	155.68	17.42	4.5
CrE	Crotalaria with subsurface drip.	151.98	18.86	5.0
CoS	Combination with surface drip.	164.68	17.13	4.2
CoE	Combination with subsurface drip.	161.19	18.35	4.6

WUE: Water use efficiency.

those that were studied in this experiment were found when working with direct and conventional seeding systems for melon cultivation. The system that employed mulch increased the efficiency of water use by 23% in comparison to the uncovered soil (Teófilo et al., 2012). The subsurface irrigation method in conjunction with soil management using of brachiaria, crotalaria and a combination of both (B+C) produced a lower water use of 7.6, 19.27 and 14.3% respectively compared to the conventional system.

Table 2 shows the water use efficiency of the different treatments. The crotalaria mulch was observed to be more effective regarding the reduction of water applied in comparison to the brachiaria mulch and the conventional system, which were the least effective treatments. The irrigation system used was a determining factor as differences were seen between the systems. The analysis of averages related to the reduction of water use which was carried out using the Tukey test at 5% level of probability (Table 3) shows that the treatment consisting of the combination of the two cover crops was statistically different from the other treatments. It was superior in relation to the variable of water use efficiency with 5.15 g mm. The treatment using crotalaria was statistically superior (4.11) to the treatment using brachiaria (3.25) and the conventional system (3.01). These last two were statistically similar. Regarding the variable of irrigation systems, the subsurface drip (5.32)–which produced the biggest benefits–and the surface drip (4.38) were statistically different.

One of the factors that allowed for a higher WUE by the crotalaria and the combination of crotalaria and brachiaria was the higher quantity of vegetation cover; 11,2 and 8,9 Mg ha^{-1} of dry material was registered by the crotalaria and the combination respectively. The brachiaria registered 8 Mg ha^{-1}. A higher quantity of dry material led to an increase in productivity and a reduction in the consumption of water. Similar results were obtained in other experiments in which 11,76 Mg ha^{-1} of *C. ochroleuca* dry material was produced (Cesar et al., 2011), and 4,46 Mg ha^{-1} of *B. ruziziensis* dry material in the dry season (Zago et al., 2010).

Another factor that influenced water use efficiency was the root system of the cover crops. Crotalaria has very aggressive, vigorous root system with a taproot. This reduced macroporosity, increased the microporosity of

Table 3. Analysis of averages of impact of treatments on water usage efficiency (WUE) in (g mm). Dourados-MS, 2015.

Soil management	WUE (kg m[3])
Combination	5.15[a]
Crotalaria	4.11[b]
Brachiaria	3.25[c]
Conventional	3.01[c]
Irrigation system	
Subsurface drip	5.32[a]
Surface drip	4.38[b]
C.V.: 12.05%	

Averages followed by the same letter are not shown to be different by the Tukey Test at 5% level of probability. C.V.: Coefficient of variation.

the soil and provided conditions that were more favourable for the retention of water in the soil, thus, allowing for more efficient irrigation. Covering the soil prevented the formation of a hard layer which would have promoted the sealing of the surface (Andrade et al., 2009). The subsurface drip irrigation system was shown to be more efficient; water was supplied directly to the radicular zone of the cauliflower plants, allowing for better use and a reduction of the rate of evaporation compared to the surface drip system. Geisenhoff et al. (2015) saw similar results to those found in this experiment. Whilst studying irrigation systems in cauliflower crops, they obtained comparable differences between subsurface and surface systems; the subsurface system produced advantages for production.

Conclusions

The covering of the soil surface allowed for an increase in water use efficiency. The most promising treatment was the combination of *C. ochroleuca* and *B. ruziziensis*, which, in comparison to the conventional system, allowed for a reduction in water use in the cultivation of *Brassica oleracea* cv. Italica. Additionally, the best results were obtained with the subsurface drip irrigation system; the necessary amount of water for the crop was provided with minimal losses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Global analysis of millet-based household farms: Characterization of the Senegalese production system of Niayes and Groundnut basin areas

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Niayes and Groundnut basin areas are among the largest strongholds of rainfed agriculture in Senegal. In the agricultural holdings of both agro-ecological areas, main speculations are far from optimizing their agronomic production potential. In order to analyze the organizational capacity and decision-making processes of producers, a global agronomic diagnostic work was undertaken out on the scale of 180 agricultural holdings through socio-economic and technical descriptions, while taking into account endogenous and exogenous factors of the agrarian environment. The results revealed technical and biophysical failures of the resources in their availability and/or use, but also a plethora of constraints which hinder the increase of agricultural activity and the dynamical transition of the unit. These constraints are justified by a precarious technical framework (-15%), a modest size of the agricultural unit (7.45 ha) and a small to medium crop rotation of which 42% of UAA for the first speculation, pearl millet. For the dry cereal, 19% N.P.K phosphate fertilizers use is rated with 26.05 kg ha^{-1}, and 0.46 t ha^{-1} of background manure for an average yield of 0.59 t ha^{-1}. These conjunctures point to a system of peasant production with low market capital, limited to the satisfaction of family needs.

Key words: Family farms, farming practices, pearl millet, Niayes, groundnut basin, Senegal.

INTRODUCTION

Senegalese agricultural sub-sector contributes 7.2% to Global Domestic Product formation (ANSD, 2015). However, it remains a serious pillar of the economy and indirectly employs 51% of the working age people labour force. Through its multiple interactions, it generates currencies for other sectors and sub-sectors (e.g. trade, transport and agro-industry, etc.). The agricultural sub-sector relies on several industrial or export and food-producing speculations, managed according to certain eco-potentialities. The latter largely influence the agricultural vocation of the natural regions through the types of speculations and sectors of activity and lead to the identification of six ecological entities including the Niayes and the Groundnut basin areas – covering 58% of

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the country's arable land and eight administrative regions.

The Niayes area and the Groundnut areas respectively provide 80% of horticultural production and two-thirds of local groundnut and pearl millet harvests, respectively. Located Situated between the 300 and 500 mm isohyets and in a rather heterogeneous pedological context, the Niayes are characterized stand out to 70% by slightly leached tropical ferruginous soils or “dior”. In adjacency, the northern Groundnut basin is also covered by “dior” soils and by brown calciform soils or “deck”. Also, in the southern Groundnut basin is covered by tropical ferruginous leached soils (“baqala”), and where annual rainfall can reach 800 mm. In these areas as in the rest of the country, *Pennisetum glaucum* L., a dry cereal, is a strategic crop for rural and urban households with a high protein content (Amadou et al., 2002). It is the second most common consumption after fairly imported rice (*Oryza sativa* L.) and covers part of the nutritional intake of the population. In 2013, pearl millet seedlings were valued at 52.5% of harvested agricultural lands.

Despite economic as well as food importance, the dry cereal is subject to coercion in traditional agro-systems. In recent years, episodes saw-tooth production has been observed. The ANSD (2016) estimates the decline in pearl millet production in 2013 at 22.2%. These production rebates may be due to agro-climatic, agronomic and socioeconomic crises. Indeed, the local agricultural context is marked by the reduction of set-aside time – and thus the over-exploitation of land – and the virtual absence of fertilization. From an agronomic point of view, the solution of the problems of pearl millet cultivation in the Niayes and the Groundnut basin areas must be done first and foremost by an inclusive and critical analysis of the real conditions of production.

The logic, the decisions-making methods, the production objectives and the adaptations of farmers to the social and environmental problems lead to reconsider as cultivated ecosystems and the holding unit, as a whole, a complex system. Thus the purpose of this study is therefore to analyze the production system of the Niayes and Groundnut basin areas holding unit through its social system, its operating system, its input flows and its cultivation processes, and in particular pearl millet. This research in this context is able to determine the constraints around agricultural activity and to uncover deficiencies in the technical route of pearl millet.

MATERIALS AND METHODS

The prospecting route was established on the basis of the distribution of pearl millet production and the availability of farm managers. Following a concerted effort by the members of the research team, six municipalities were selected for prospecting. For the smooth running and to facilitate the practical arrangements of the survey with farmers, contacts were made with the agricultural and rural advisers of these municipalities. With their diligence, a simple random sampling made it possible to obtain a good representation. As a result, 180 producers participated in the assessment and 30 per municipality. The meshing was therefore carried out between December 2013 and January 2016 and in the municipalities of Kab Gaye and Ngueune Sarr (Louga region), Meouane and Sessene (Thiès region), Keur Saloum Diane (Fatick region) and Paoskoto (Kaolack region) (Figure 1). To this end, no requirements were required needed for the participation of farmers in the study. He could or could not be a member of a peasant organization. The only determining factor was that he was active in pearl millet cultivation.

Once the contact was made and the unit was identified, an interview with the farmer was made. Inclusive, semi-structured interview maintenance was carried around the set of socio-ecosystem factors related to agricultural activity. The data collected over three growing seasons covered the chief holder, the labour force, the structure of the agricultural land and its tenure, crop rotation and practices, and the rate of production. The information thus collected was captured, translated into quantitative and qualitative data.

RESULTS AND DISCUSSION

The human capital of the agricultural holding

The chief farmer of the Niayes and Groundnut basin is 52 (9.79) years old. The 55-60 age group is dominant with 17% of the workforce, those aged 30 and under do at most 2% and those aged 70 and over was 4%. In 3% of cases, it is a 46-year-old woman. Less than one farmer in two is a member of a farmers' organization, with annual membership ranging varying from 1,000 to 7,500 CFA francs per year. Less than one in six producers benefit from technical guidance from rural advisors or sometimes from relay staff persons. To assist him in the field tasks, the support of other assets is required. In the Niayes and the Groundnut basin areas, the bulk of the agricultural tasks are generally carried out in general by the working family. 6.18 Human Work Unit (HWU) who evoke both a social unit of production and consumption. The evolution continuation of work by gender has moved to a level where both male and female workers from the family group are present. Thus 30.5% of agricultural workers are women. In case of overload of the works and if the financial resources means allow in 7.0% of cases, the producer rents the services of seasonals called “surga” or “nawetane”. Thus, he can simply supervise and organize the work within his property. On average, this external labour force is 1.5 seasonal for a fee of 100,000 to 150,000 CFA francs per growing season. In rare cases, the farmer producer can use day labourers who are paid on the job.

Factors and means of agricultural production

The family farms of the both two areas are small units

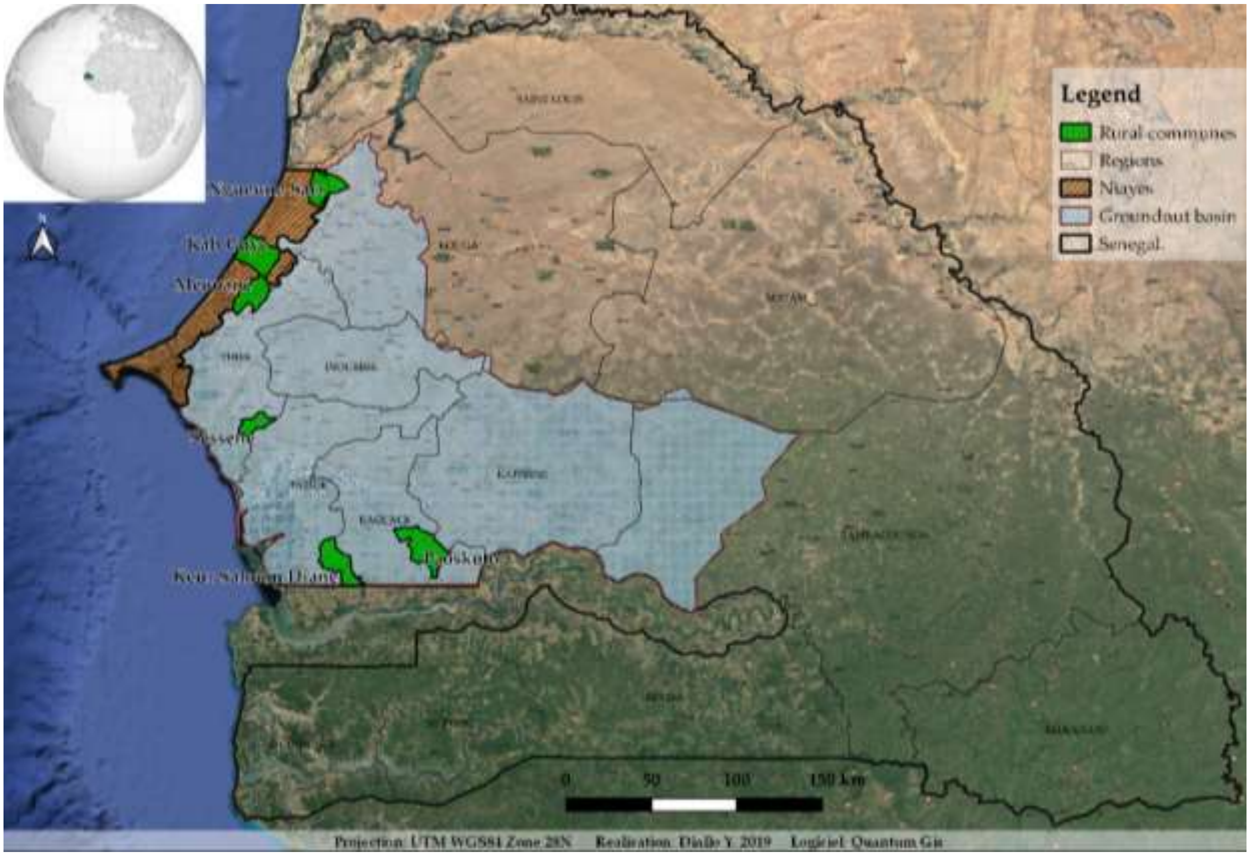

Figure 1. Study area: Municipalities surveyed in the Senegalese Niayes and Groundnut basin.

farms, often fragmented into three parcels of land. On average, the useful Utilized Agricultural Area (UAA) is 7.45 ha but can range from 2 to 21 ha. Small units of 2 to 7 ha represent 69 % of farms and large units of 14 to 21 ha make up 9%. The farms are the result of the dismantling of larger family estates domains used in owner-occupation. The difficult access to larger estates areas, and the overload of co-operators lead some producers (15.6%) to acquire land. This acquisition by lending, renting or sharecropping ("bey seedo") represents approximately 24.2% of the total Utilized Agricultural Area (UAA).

On these farms, a cell livestock (e.g. cattle, sheep, goats, equines and asins) is formed and for a density index of ruminants about 0.31 livestock Unit per hectare (LU ha^{-1}). Sheep make up 35% of ruminants, cattle 33%, and goats 32%. The farmer has more than 1.76 traction-coupling animals, 64% of which are equines. Cattle count for are a very small part of draught animals. In terms of availability compared to the diverse working tools of work, the results show near autonomy in the realization of the cultural, pre- and post-cultural operations. Agricultural equipment is often made up of a carts used for locomotion and the transport of heavy loads to the field, disc drills, tracted tool (e.g. "sine" or western hoe), and handle-type tool (e.g. "iler", "daba"). The thresher is the least available equipment and is often privately owned.

Agricultural production

Land the use of agricultural land shows a standardization of uniform the agrarian landscape. Pearl millet and groundnut [(*Arachis hypogea* L.)] are the two main crops, accounting for less than 80% of the seeded area planted. Secondary crops are subject to some sectorization, probably because a function of soil predisposition (Table 1). Millet is used with varieties: *Souna*, *Thialack*, *Souna 3*, *Sosat C88*, *Thialack 2*, *IBMV 8402*, *Gawane* and *ICTP 8203* at very low-use. Groundnuts are found in the forms *28-206*, *55-437*, *73-33*, *PC 79-79*, *GH 119-20* and *Fleur 11*. *Early Thai*, *Pan 12*, *Camara I* and *Synth C* are the crop types of corn grown. The various varieties of cowpea (*Vigna unguiculata* (L.)] sown are *66-35*, *Yacine*, *Melakh* and *Mougne*. Cassava (*Manihot esculentum* (L.)] is present there with *Soya*, *Kombo 1* and *2* and sorghum

Table 1. Rotation and main crops grown in the Niayes and the Groundnut basin.

Municipalities	UAA (ha)	Millet UAA (ha)	Groundnut UAA (ha)	Secondary crops UAA (ha)
Kab Gaye	8.50 ± 4.78[ab]	3.20 ± 2.21[a]	3.08 ± 1.36[a]	Cassava (2.22 ± 1.98)[ab]
Ngueune Sarr	6.50 ± 3.81[b]	3.20 ± 2.51[a]	2.80 ± 1.66[ab]	Cowpea (0.50 ± 0.51)[c]
Meouane	9.35 ± 4.04[a]	2.87 ± 1.12[a]	2.80 ± 2.06[ab]	Cassava (2.73 ± 2.75)[a]; Cowpea (0.95 ± 1.09)[b]
Sessene	6.35 ± 3.58[b]	3.65 ± 2.68[a]	1.96 ± 1.72[b]	Sorghum (0.74 ± 0.77)[c]
Keur Saloum Diane	7.00 ± 3.59[ab]	2.70 ± 1.58[a]	3.00 ± 2.05[ab]	Maize (1.30 ± 0.82)[abc]
Paoskoto	7.00 ± 3.82[ab]	2.96 ± 1.41[a]	2.88 ± 2.01[ab]	Maize (1.16 ± 1.18)[abc]
Mean ± SD	7.45 ± 1.20[ab]	3.10 ± 0.33[a]	2.75 ± 0.40[ab]	1.60 ± 1.18[abc]

Averages with the same alphabetic letters (a, b and c) are not significantly different (P > 0.05) according to the Kruskal-Wallis test.

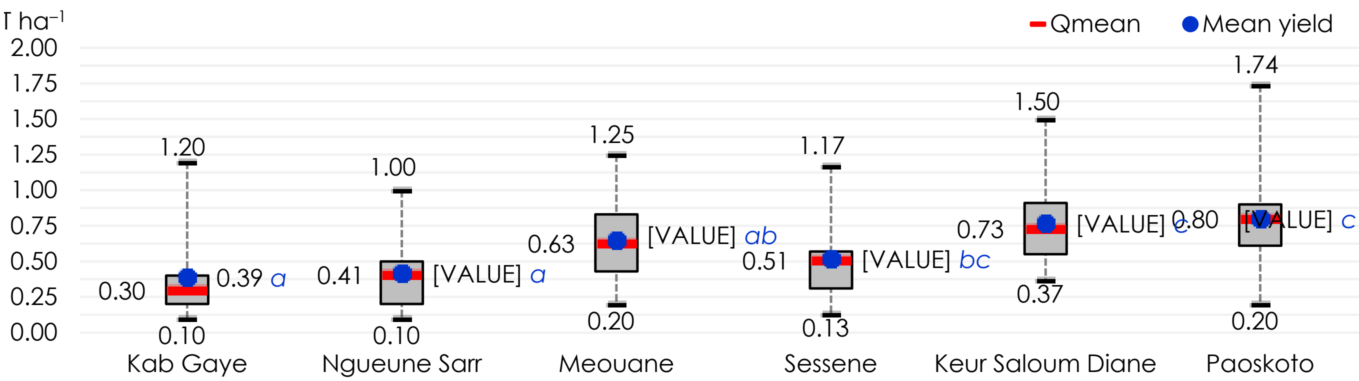

Figure 2. Yields of millet observed (t ha^{-1}) in the Niayes and the groundnut basin averages with the same alphabetic letters (a, b and c) are not significantly different (P > 0.05) according to the Kruskal-Wallis test.

([*Sorghum bicolor* (L.)] with *Nguinth* and *Darou*.

Pearl millet is grown on plots located around a 2 km radius of the concessions and covering 42% of the UAA. 60% of the crop is planted on sandy soils, while the bottom soils are present on 7% of the millet plots. The annual balance sheet in the two zones shows generally fairly low and highly very variable yields of millet grain (from 0.10 to 1.74 t ha^{-1}). The average yield recorded is 0.59 t ha^{-1} (Figure 2).

Technical itinerary for pearl millet cultivation

Taking into account the specificities of each culture crop and the dynamics of rotation place the development blossoming of subsequent speculation. The study of rotary crop rotation systems shows that in the six municipalities, a biennial sequence rotation with groundnut, a *fabaceae*, is practiced in agricultural units to the tune of 73%. Apart from groundnut, the precedents of millet are diverse. Among these previous crops, pearl millet is renewed in 11% of fields, cowpea 6%, unprocessed annual fallow 5%, maize and sorghum 2%, and cassava 1%. These various precedents have immediate effects on millet cultivation but are contrasting, both in terms of nitrogen residues and on the plant health aspect.

Clearing or slash of the seedbed ("routhie") in this traditional environment exists in two ways: the simple or without burning and the one by burning or slash and burn. However, simple clearing was not noticed. In the pre-cultural cleaning of the millet plots, the producer of this both areas the Niayes and the Groundnut basin gathers the stumps and other residues to for burning them, in order to clear the seedbed. This practice, which poses a high risk of degradation of organic matter, helps to eliminate good part of would contribute to the removal of much of the weed seeds from the surface layers of the soil, and to the destruction destroy of a good number of subservient parasites, especially after a previous cereal.

In the agro-pastoral zones of the Niayes and the Groundnut basin, the basic manure is in the form of livestock manure and household waste. Based on the survey results, the amendment on the pearl millet plot is practiced by 92% of producers with an average of 0.46 t ha^{-1}. Local background manure with doses range varying

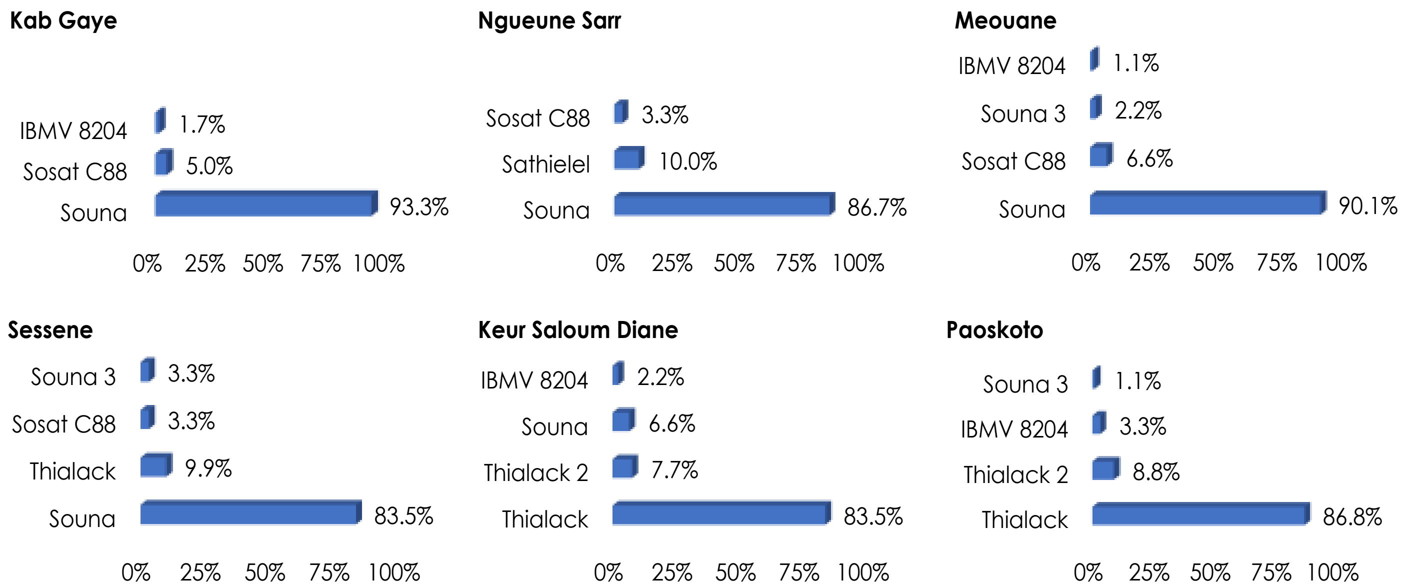

Figure 3. Distribution of the main varieties of pearl millet grown in the Niayes and the Groundnut basin.

locally from 0.14 to 0.90 t ha^{-1}. These quantities of background manure do little to meet the needs. If the practice improves the yield, it seems more advantageous to associate it with the effects of ploughing for an improved seedbed. Yet this superficial tillage or scratching is practised by only 22% of producers.

Despite the availability of improved seeds (for example Thialack 2, Sosat C88, Souna 3 and IBMV 8204), the utilization rate remains very low (Figure 3). Thus, Thialack 2 and Sosat C88 are adopted, respectively, at rates of 2.8 and 3.8%. Widely present on plots (91.73%), landraces (Souna and Thialack) are more appreciated for their adaptation to the diversity of traditional millet cropping farming systems of the Niayes and the Groundnut basin areas, despite their problems of low productivity issues. 75% of the cultivated millet varieties come from producers' personal reserves, 28% are purchased on the local market and 7% come from extension.

The recommended seeding densities for pearl millet are between 3.50 and 4.00 kg ha^{-1}, with spacing of 0.90 and 0.80 m between the lines and 0.80 m between the hills seed pockets. In practice, these densities go beyond or below these differences, depending on whether the crop(s) are pure or associated. The recorded doses are quite good, with a mean of 3.80 kg ha^{-1}, although between farmers it varies from 3.00 to 6.00 kg ha^{-1}. The date of sowing depends on the type of crop rotation (types and number of crops). 70% of producers proceed with a use dry seeding to alleviate lighten the growing calendar.

According to the results, only 29% of the producers practice a binary crop association (pearl millet and cowpea). The choice of species and varieties to be combined, the date of sowing and densities are above all essential to avoid any competition due to allelopathic effects. In the light of the study, millet is sown first and for the technical choice of a spatial organization, cowpea pokes patches are placed alternately between the lines of the cereal to allow mutual and enhanced production of crops and facilitate weeding operations.

Hoeing operations are carried out in two phases and rarely in three. The first maintenance phase, or "baxao", is usually carried out in the first week by 47% of producers and 53% in the second week following the millet surge. During this step, the thinning demarcation or wolli is done at three millet plants per poke. The second weeding or "bayaat" coincides with the run-up phase and is done between the 15th and 20th days following the first weeding by 67% of the farmers in the agro-systems or between the 25th and 30th days by the other farmers. A third weeding or "balarci" optimizes the phytosanitary aspect and is performed by more than 30% of farmers within a fortnight of the second weeding.

In the areas studied, only 19% of farmers acquired NPK phosphate fertilizers of type N.P.K. This ratio goes from 17 (Kab Gaye and Sessene) to 43% (Paoskoto). The fertilizer dose applied to the plot is 26.05 kg ha^{-1}. From one municipality to another, the dose ranges from 15.80 (Sessene) to 38.50 kg ha^{-1} (Paoskoto). The various formulas of N.P.K identified are 15-15-15, 15-10-10, 10-10-20 and 6-20-10. Fractionation is usually done based on the amount of fertilizer available. Thus, cover fertilization, for 35% of the plots was carried out in two phases (sowing-thinning demarcation or thinning-running-up rigging) and thus 65% in one step (sowing or thinning singling).

Following a millet harvest of millet ears, two threshing options are possible. In the first case, the ears are piled up packed in bundles of 8 to 15 kg and kept in granaries attics. Manual threshing is done carried out as needed and in small quantities to cover the daily food ration diet. It is carried out in 14% of the units households. Less restrictive, the second option is practised by 86% of operators. The use of time threshers is one of the major constraints. The producers waiting, store their crops in the field. Once motorized threshing is completed, the grain is placed in bags and placed in a storehouse room in the dealership.

Senegalese agricultural social system

The farmer of the Niayes and Groundnut basin areas is a senior-aged leader with more than a dozen valid arms, mostly from the domestic square. The high labour costs explain very little the contribution to the salary activity of the family unit. It is more justified by the form of reciprocity or loyalty within the Senegalese family, which makes its agriculture a family activity (Gafsi, 2014). More than one in two managers is over 51 years old. The total tenacity of the elderly at the head of the units is partly explained by the hierarchical basis of the traditional Senegalese society. The inheritance of land is hereditary and is done according to a legitimate lineage (Bosse-Platière, 2007). The son who has the right to inherit, simply supports his father within the unit, by organizing the family support caregiver. These present circumstances, combined with the by the children of farmers from the sector, are placing putting more and more women in charge of family farms. Cultural and religious burdens being unfavorable to them, especially the access to labour land; little was done to make them responsible for the productive work of the units. The number of women who have their own farm is still relatively insignificant. The general observation is that they seem to be more perceived as surplus labor (Bessière and Gollac 2014). Otherwise, his the lack of crude adherence of the producer to peasant development networks on the one hand and its precarious supervision by the advisory structures on the other hand, justify its weak cooperative work, its precarious capacity in the logic of production and marketing but also in the management of its production unit.

Senegalese agricultural operating system

If the UAA of the farm and the resources mobilized return to a small family farm, the social dimension of productive purposes makes the farmer of both Senegalese agro-systems, a subsistence farmer (Sourisseau et al., 2012). Furthermore, inheritance of the family property to the heirs frequently leads to the nuclearization of agricultural land, and has establish in Niayes and Groundnut basin to the phenomenon of peasant landless and the land market. The understanding of the system of primary production of goods involves the regionalization of crops, linked either to the quality and availability of land, and to local activities, thus to the district economic base fabric. Beyond the natural features, the intended productive goals relate mainly to the life cycle of the agricultural holding unit, its social structure and the professional singularities of the farm manager (Madelrieux et al., 2012; Pradel and de Gervillier 2011). These production goals generally revolve around major crops such as groundnuts and pearl millet and secondary or minor speculations – occupying less than 5% of the agricultural area, often planted as infill or field borders. However, the adoption of a productive system must meet the obligation to finance the agricultural unit and the primary needs in order to ensure the social cohesion of the family. Thus the chosen medium field must have suitability for cultivation to sustain a certain production.

"Dior" soils guarantee a good root respiration, nevertheless its carbon, nitrogen, in useful water and exchangeable bases, make the crop production systems of the Niayes and the Groundnut basin are uncertain. Short-term fallow becomes an obligation to restore the productive land base of land (Boli and Roose, 2000). However, its inadequate local practice leads to reconsider a the land pressure as well as a and the changes of actors – with new production objectives – in the transmission of farming land. By socioeconomic and technical-economic parameters, the frequency of biennial rotation of the majority biennial is explained, besides an agronomic interest, by the surplus-value of the groundnuts in the 1960s. This priority development for cash crops often allows for hoarding in livestock production, diversifying activities and cash receipts. In spite of this, the lack of pasture roads in the areas and the insecurity in rural areas zones can only allow in the majority of the production units, a case small breeding.

Input flows and millet cultivation processes

The technical itinerary of pearl millet cultivation reveals peasant practices based on knowledge and perceptions related to the socio-economic environment. The producer, thus, becomes rational in his decision and is right to do what he does. The family farm unit of the Niayes and the Groundnut basin areas is confronted to the difficulty of using human and animal energy to conduct the crop (Gafsi et al., 2007). The primary tillage of the soil is insufficient and its non-performance cannot be justified by the reduction of the organic base but by the heavy work for the draught animal. The amended

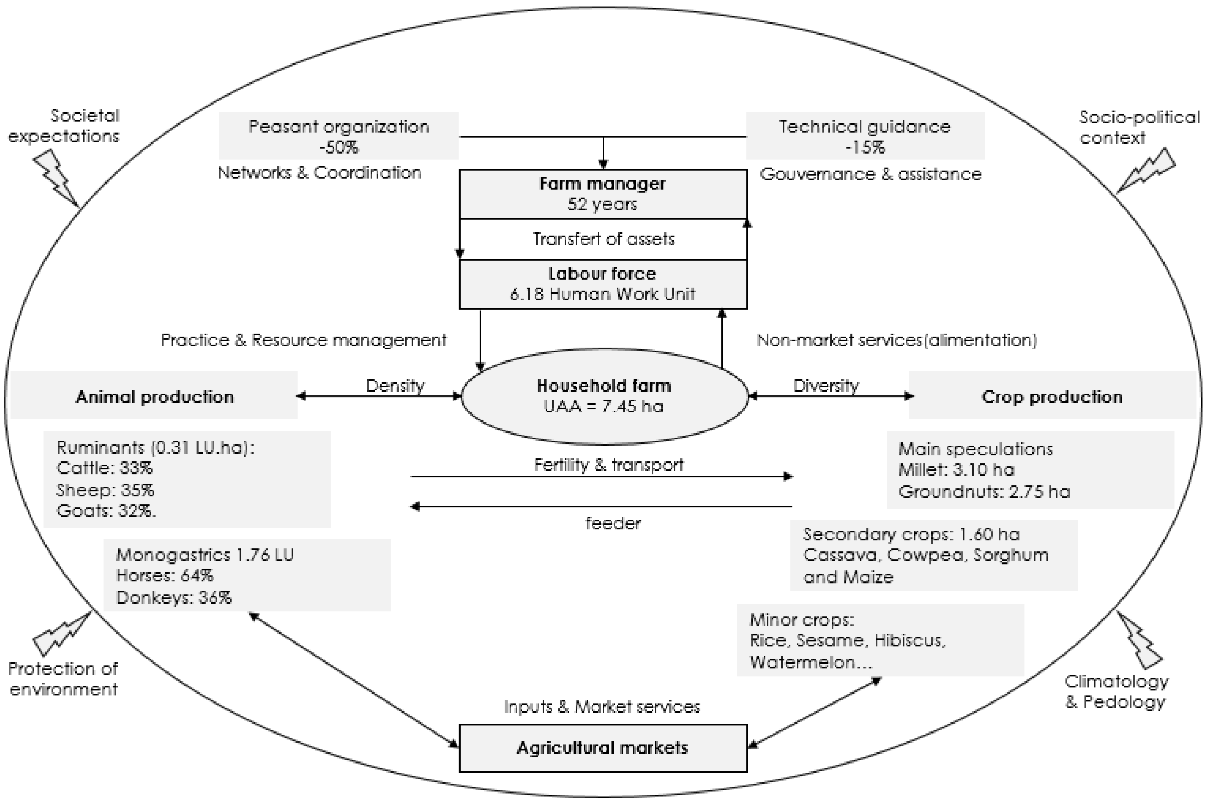

Figure 4. Systemic vision of the household farm of Senegalese Niayes and groundnut basin.

quantities do not allow a significant gain in pearl millet productivity in these agro-systems. These doses are relevant to the size of the farm. Crop systems are globally, without or with low fertilizers because of the input cost and value ratio of the millet crop, poorly commercial, and because of the lack of subsidy policies. But the low uptake of improved varieties is partly related to the delegate aspect of extension and not to a the thorny question of purchasing power, hence a regular and adaptive renewal of seed capital. In view of Compared to the rainy regime, the first rains are of crucial decisive importance for the lifting, the start-up of the grain and its competition against weeds (Fox and Rockstrom, 2003). In both agro-systems, the heaviness of manual labour and the obsolescence of agricultural equipment constitute an obstacle to good harvests of crops. However, weed pressure can be reduced by a cultural association with cowpea (Lawane et al., 2010). Although and the poor means of crop conservation remain the major problem of pearl millet culture in Senegal.

Conclusions

The agronomic analysis undertaken at the scale of the Niayes and the Groundnut basin suggests an interesting diversity of productive logics underlying technical skills the economic and organizational aspects of the agricultural unit and its social links to the activity (Figure 4).

Cleary the methodology of the overall analysis of the agricultural holding reveals many failures and adaptive processes of the local production system in phase with the sociopolitical and climatological injunctions through its exploiting population, its farming land, its management and its relations with other actors. These failures are materialize at the level of the agricultural holding by a weak alternation crop rotation and precarious cultivation activities. These conjunctures deficiencies are exported on the millet plot by a crop route and farming practices defective. Overall, it emerges, a Senegalese family and food agriculture, with weak policy of support and financial capacity for production, extensive and low level of efficiency that is rather unstable and subject to the hazards of the climate and the vagaries of domestic markets.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Retrospective study of the contamination of exported sesame by *Salmonella* species from 2007 to 2017 in Burkina Faso

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Sesame (*Sesamum indicum*) is one of the oldest and most traditional oilseed crops, valued for its high-quality seed oil. This culture was introduced in Burkina Faso at the beginning of the 20th century where it occupies a place of cash crop. The majority of sesame produced in Burkina Faso is export oriented. However, the contamination by *Salmonella* remains an unsolved problem. This retrospective study was carried out in order to assess the microbiological safety of sesame samples received at the Laboratoire National de Santé Publique between 2007 and 2017. Out of 359 samples unevenly distributed according to months, 26.46% showed the presence of *Salmonella* species. The persistence of this food borne pathogen in sesame is a strong signal that new strategies of growing, harvesting and postharvest and special attention and emphasis on control measures must be given to the chain of production of this commodity in a view of its economic and medical impact.

Key words: *Salmonella*, *Sesamum indicum*, Burkina Faso.

INTRODUCTION

The worldwide food poisoning pathogen, *Salmonella* first came into prominence in the 1880s, soon after the isolation of the "hog cholera bacillus" by Salmon and Smith (Steele, 1969). Since, this pathogen has been recognized as responsible for a wide range of outbreak (Sir William, 1956; Puglisi and Maida, 1969; Small and Sharp, 1979; Unicomb et al., 2005; Angulo et al., 2006; Kunwar et al., 2013). While *Salmonella* remains the leading cause of bacterial gastroenteritis, it is also one of the most extensively studied and well characterized bacterial species (Chami and Bao, 2009). However, despite a vastly greater understanding of their structure, relationships and natural history, *Salmonella* remains what they were in 1900: an unresolved conundrum in microbiology, epidemiology, and public health (Hardy, 2004). *Salmonella* acts as primary reservoir for foods

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such as chicken meat, milk and milk products, eggs and meat products (Birol Özkalp, 2012). Nowadays, *Salmonella* has been detected in a wide range of agricultural products such as sesame and *Salmonella* outbreaks associated with sesame seed-based products have been reported (Unicomb et al., 2005). In Burkina Faso, studies have shown that *Salmonella enterica* is circulating species. Bonkoungou et al. (2013) find out 15 different serotypes of *S. enterica* subsp. enterica in childhood diarrhea samples: *Salmonella* Typhimurium, *Salmonella* Cubana, *Salmonella* Muenster, *Salmonella* Angers, *Salmonella* Banana, *Salmonella* Dublin, *Salmonella* Kentucky, *Salmonella* Montevideo, *Salmonella* Ouakam, *Salmonella* Soerenga, *Salmonella* Poona, *Salmonella* Stanley, *Salmonella* Tamberma, *Salmonella* Vilvoorde, and *Salmonella* Typhi. On the other hand, some other published papers report the presence of *S. enterica* in raw meat (Kagambèga et al., 2011), in milk (Touwendsida et al., 2014) and water, fish, lettuce (Traoré et al., 2015).

Sesamum indicum is an extraordinary plant that can be grown on almost all types of land. It is a survivor crop known around the world. For 5,000 years, it has been planted by subsistence farmers in areas that will not support the growth of other crops or under very difficult conditions with drought and/or high heat (Langham, 2007). *S. indicum* is an erect annual plant of variable size, with mature plants ranging from 40 to over 200 cm in height. Its stems are obtusely square with grooves on their faces, yellow-green but often splashed with varying amounts of a striking deep eggplant-purple color (Bedigian, 2004). This plant is adaptable to a range of soil types, although it performs well in well-drained, fertile soils of medium texture (typically sandy loam) at neutral pH (Islam et al., 2016). Sesame is thought to have originated in Africa and later taken to India at a very early date (Alegbejo et al., 2003). Its origin is still controverted and requires further deep genetics research.

Although, dried fruits such as sesame are too dry to allow bacterial growth, pathogens such as *Salmonella* can become sheltered and cause diseases. There are few reports on the presence of *Salmonella* in dry products, and most of these studies were focused on pre- and postharvest practices for processing of ready-to-eat products. Sesame contamination by *Salmonella* contributes to negative economic impacts due to the cost of surveillance investigation, treatment, prevention of illness and worse, contribute to an international pathogen's exchanges.

Burkina Faso is a land lock country located in West Africa and neighbored by six countries (Mali, Côte d'Ivoire, Ghana, Togo, Benin and Niger). Its economy remains essentially agricultural. More than 86% of the active population derives its income from agriculture (MAAH, 2010). Sesame has been cultivated since the beginning of the 20th century in Burkina Faso and entered crop rotations before the development of the industrial cotton crop. Burkina Faso produces mostly mixed (mixed) sesame sold mainly in China, Japan and Turkey (Rongead, 2013). The majority of the national production is export oriented. Besides, the international trade command sesame to be in a best quality as possible that mean free of bacteria such as *Salmonella* and other chemicals such as pesticides. However, in Burkina Faso, the international sesame demand is far higher than global supply, particularly in the conventional market. This exacerbates the pressure on producers and traders throughout the sesame commodity chain and has fostered some structural changes over the last decade (Glin et al., 2013).

Performing sesame quality in Burkina Faso supposes casting a glance at the sesame quality background. In response, a retrospective study was undertaken on sesame samples, received at the Laboratoire National de Santé Publique from 2007 to 2017 with the aim of assessing the microbiological safety of sesame submitted to exportation, with a particular focus on the detection of *Salmonella* species.

MATERIALS AND METHODS

Study site and sampling

This study was conducted in Burkina Faso, West African country which covers 273,800 km^2 of land and 400 km^2 of water. Its economy is closely linked to agriculture. These sesame samples submitted to this study (359 sesame samples) were obtained by the National Public Health Laboratory for foods quality control before exportation. The locality of the country from which these seed samples were harvested remains unknown but their common point is that they have been cultivated in Burkina Faso and are all intended for export.

Salmonella detection

Conventional cultivation technique was used for the isolation of *Salmonella* spp. Detection of the presence of *Salmonella* is carried out according to the ISO 6579:2002 (updated in 2007) standard - Horizontal method for detection of *Salmonella* spp. This includes four stages of the detection process as described by Fricker (1987) and Zadernowska and Chaj (2012).

Pre-enrichment in non-selective liquid medium broth

For the first stage, 25 g of each sesame sample were preenriched in 225 ml of non-selective buffered peptone water (Liofilchem diagnostic, Italy) and incubated for 18 to 20 h at 37°C. Pre-enrichment culture allows the number of cells of interest to increase and to repair any lesions of damaged cells and thus regain their resistance to selective agents, prior to enrichment.

Selective enrichment in liquid media

After the non-selective pre-enrichment stage, 1 and 0.1 ml of each sample suspension was transferred into 10 ml of selective media Tetrathionate broth Müller-Kauffman (Liofilchem diagnostic, Italy)

Table 1. Sesame distribution over years and *Salmonella* presence.

Year	Number of analyzed samples	***Salmonella* detected cases [n (%)]**
2007	83	22 (26.5)
2008	113	34 (30)
2009	27	6 (22.2)
2010	26	6 (23)
2011	8	3 (37.5)
2012	2	1 (50)
2013	1	1 (100)
2014	2	1 (50)
2015[a]	31	12 (38.7)
2016	35	7 (20)
2017[b]	31	2 (6.5)
Total	359	95 (26.46)

[a]Year of the largest percentage of contaminated probes. [b]Year of the smallest percentage of contaminated probes. Years 2011 to 2014 were not consider.

and 10 ml of selective media Rappaport Vassiliadis Soy (Difco laboratories), respectively. A brilliant green at 0.95% was added to the selective media Tetrathionate broth in order to inhibit the growth of Gram-positive bacteria and then incubated for 18 to 20 h at 37 ± 1°C. The selective media Rappaport Vassiliadis was incubated for 18 to 20 h at 42 ± 1°C. Selective enrichment procedures involve inhibitory substances or procedures to impede the growth of most organisms but permit, though not necessarily encourage, the growth of the desired organisms (Fricker, 1987).

Plating on selective media

During the third stage, a loopful of cultures suspension from selective media was placed on two selective media, so as to receive individual colonies. The first of them was the Xylose Lysine Deoxycholate (HiMedia Laboaratories, India) agar and the second was *Salmonella-Shigella* (HiMedia Laboaratories, India) agar. The choice of selective plating media must receive special attention. They must support the growth of a very wide range of strains of the particular type required and should, wherever possible, inhibit the growth of other bacteria. Typical *Salmonella* colonies onto Xylose Lysine Deoxycholate agar can be colorless, very light, slightly shiny and transparent (color of the medium) with a dark tinted center, surrounded by a light red area and yellow edge, or of pink to red color, with or without a black center. Onto the *Salmonella-Shigella* agar, typical *Salmonella* colonies are colorless or very light pink, opaque or semi-transparent with a black center or not.

Biochemical and serological identification of characteristic colonies

At least five colonies suspicious for *Salmonella* were picked per plate and purified onto nutrient agar for 24 h. Then, colonies were sowed onto triple sugar iron agar (Difco laboratories) to access sugar utilization, MR-VP broth for Voges Proskauer reaction, Christensen agar for urea utilization and peptone water broth for indole production. *Salmonella typhimurium* (ATCC 14028) and *Salmonella Enteritidis* (ATCC 13076) were used as positive control. Suspected colonies were purified on nutrient agar and then submitted to API 20E (BioMérieux) test for biochemical confirmation. The key biochemical tests including the fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H_2S production, and fermentation of dulcitol (Odumeru and León-velarde, 2000).

RESULTS

The results showed that *Salmonella* was detected in 95 (26.46%) sesame samples out of a total of 359 samples examined. The annual distribution of *Salmonella* from 2007 to 2017 varies from 6.5 to 100% (Table 1).

The distribution of sesame samples was not uniform over the years, the number varied from 01 sample in 2013 to 113 sample in 2008. Surprisingly, *Salmonella* was still detected in each year even if sample number were low. The highest number of samples was 113 in 2008 with a contamination rate of 30%.

From the year 2011 to 2014 (4 years), few samples (13 samples) were received with the lowest number in 2013 where only one sample was received but shown the presence of *Salmonella.*

Figure 1 shows the percentage of *Salmonella* detected cases compare to the number of samples received per year for statistical matter, we expressly remove the sample of the years where the number is below 30% of average samples taken per year (2011, 2012, 2013 and 2014).

While trying to understand if there is a link between the number of sesames analyzed at the National Public Health Laboratory and the national sesame exportation, we used the INSD data stat (2010 and 2018). Figure 2 shows the evolution of sesame exportation in tons and the income it generates in CFA franc during the study period.

DISCUSSION

This was the first and unique retrospective study about

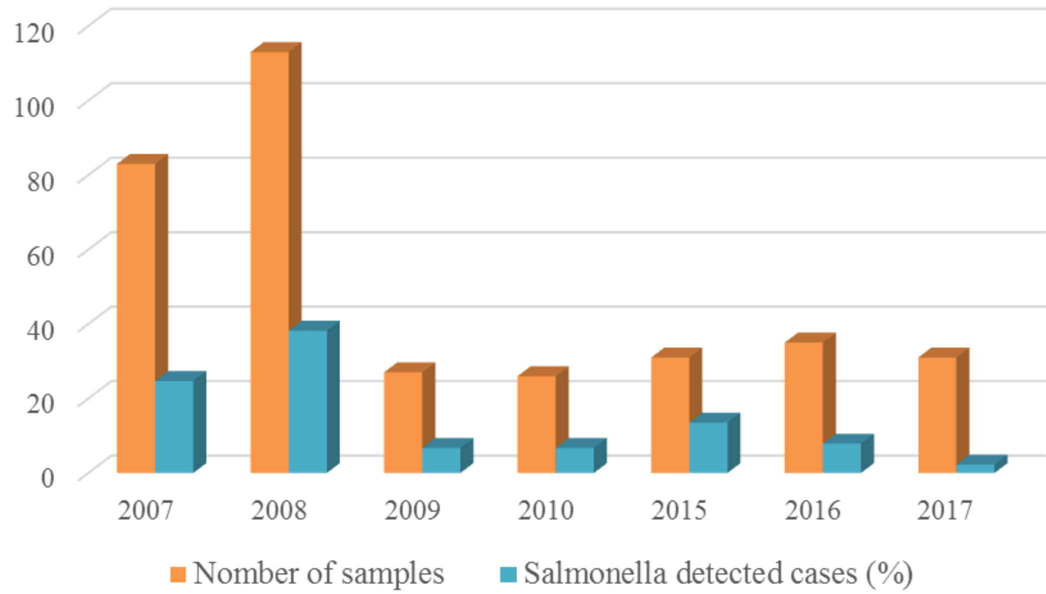

Figure 1. Distribution of samples and the presence of *Salmonella* per year (years 2011 to 2014 were removed).

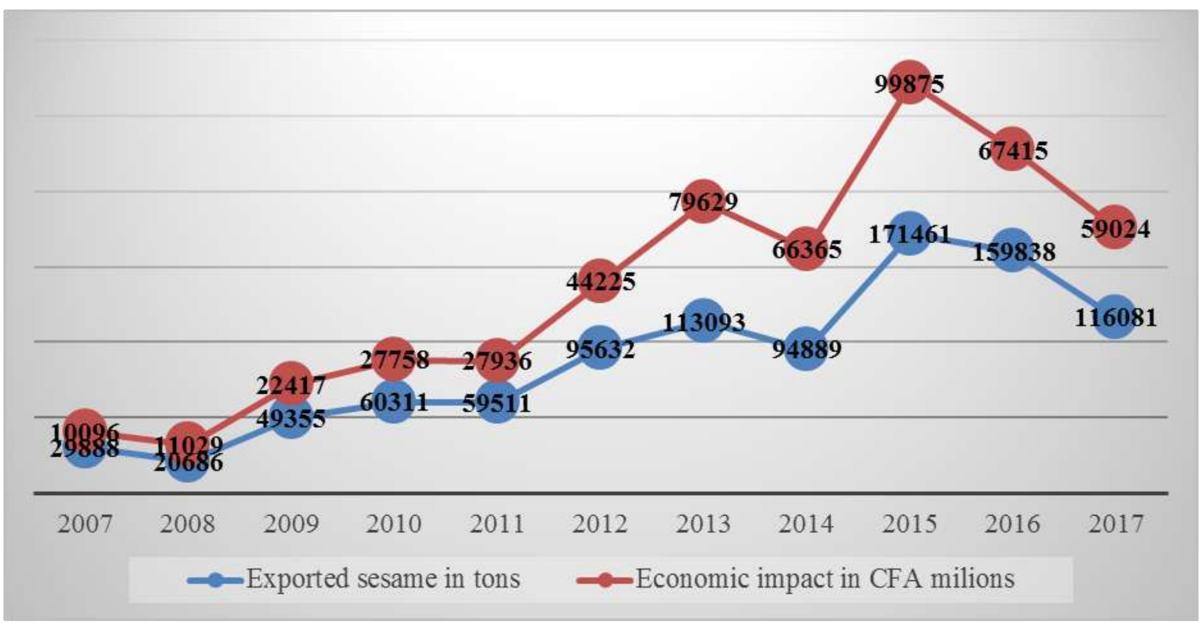

Figure 2. Sesame exportation from 2007 to 2017.
Source: INSD (2010, 2018).

sesame contamination by the worldwide food poisoning pathogen *Salmonella* in Burkina Faso. A compilation of the results of sesame analyzed from 2007 to 2017 were analyzed. These sesame samples were received at the National Public Health Laboratory for *Salmonella* research. Out of 359 samples received unevenly according to months, the results showed the mean prevalence of 26.4% sesame probes contaminations by *Salmonella* between 2007 and 2017. This rate is high but seems to have its origins in the traditional cultural practices of sesame post-harvest treatment. Alaouie et al. (2017) find out a 17% prevalence of *Salmonella* spp. while assessing the microbial quality of Tahini (Sesame Paste) in Lebanon. In fact, sesame harvesting involves a very important step, namely drying. Sesame is thus exposed to all kinds of physical, chemical and biological contaminations. Sesame seeds are sometimes known to harbor various molds, fungi and bacteria relatively (Angulo et al., 2006) because postharvest treatments are usually done at room temperature in open. Contamination can occur due to poor hygiene around the space reserved for sesame drying. Most of the time, the feces of poultry and birds helped by the wind are responsible. Good agricultural practices on the farm and protection of the product from animals and birds during the drying process will help mitigate the risk of *Salmonella* contamination of sesame seeds (FSANZ, 2013). *Salmonella* spp. can remain viable for months in soil. The organism may also be dispersed in dust and aerosols generated during the handling and processing of animals (Lake et al., 2010). According to Brockmann et al. (2004), sesame seed can be contaminated with *Salmonella* during growth, further storage, or processing and irrigation and fertilization with manure and sludge, plant irrigation with surface water from streams, and animal droppings are potential sources of contamination. Further studies are needed to specify the circulating species in Burkina Faso sesame.

The sesame of Burkina Faso at the rate of 86% is exported oriented (Compaore, 2014). Local use of sesame is mainly for traditional dishes and transformation into sweet backers and salted or natural seed. So, even the low rate of local use of sesame, in regard of *Salmonella* prevalence can cause a public health issue if adequate treatments are not done. Sesame seed are consumed worldwide and has been linked to some international outbreaks (Sir William, 1956; Puglisi and Maida, 1969; Small and Sharp, 1979; Unicomb et al., 2005; Angulo et al., 2006; Kunwar et al., 2013). This increases the surveillance level in imported countries. More, *Salmonella* outbreaks linked to sesame products have heightened alerts and product control for the EU and Asian countries. We suppose that the high number of samples received at the National Public Health Laboratory for *Salmonella* research in 2007 and 2008 might be linked to the international trade control and some posted outbreak alerts due to *Salmonella* in sesame products (Unicomb et al., 2005). In February 2006, organic sesame seed from Burkina Faso were blocked at the entrance of the European Union (EU) because of *Salmonella* contaminated (Ouedraogo, 2006). That was a very important issue for sesame exporters. The operators concerned, with the support of NGOs organized a round table with all stakeholders in the sesame sector, with a view to implement a series of measures to face the risks of contamination. One of the measures is the systematic quality control upstream before export.

Therefore, the quality control was required in the exported country to avoid important lost from exporters by setting up a pretreatment. Sesame contamination by *Salmonella* spp. might be linked to collection system that consisted, sometimes to mix different sesame seed received from elsewhere. Seeds are mixed together without any precaution or treatment. The consequence is that a quality of an entire stock can be easily compromised causing an important lost for exporters. Sesame seeds collected elsewhere by, posed a serious problem of its heterogeneity and at the same time setup difficulties of sampling for *Salmonella* detection. Besides, the standard method of *Salmonella* spp. detections in food products (ISO 6579:2003) are still in use by many labs, especially by regulatory agencies and well accepted. Assessing to the microbiological quality of the entire batch by examining only one 25-g sample seems unacceptable. In general, the larger the sample examined, the greater the chance of isolating the desired organisms (Fricker, 1987). The sampling system will have to be reviewed to better guide and protect producers and exporters.

Sesame is more an important source of income and economic factor growth for Burkina Faso. From 2007 to 20013, sesame exportation jumped from 29887.5 to 113092.9 tons, respectively (Figure 2) (INSD, 2010, 2018). This generated a very important income for all the sesame actors. The drastic drop of sesame exportation in 2014 might be linked to the socio-political conditions that the country was facing. It was notably the popular insurrection which stunned the economy of the country especially the flow of imports and export. The year 2015 was the highest year that a very important quantity of sesame seeds was exported. It was believed that this large quantity of sesame exported is the sum of the sesame not exported from the 2014 campaign and that of 2015. Unfortunately, the following two years registered a decrease of exportation. We did not find any relation between the quantity of exported sesame seeds and the quantity of sesame sample brought at the laboratory for control. *Salmonella* presence in sesame seeds is a very big issue, specific methods for the prevention and control of salmonellosis have been undertaken with the support of several associations and farmers' cooperatives, government and NGOs. In order to break the contamination chain specifically, the way *Salmonella* came to be carried into sesame seed. We believed that it is necessary for all sesame growers, collectors and exporters, at different levels, to be trained in food safety and postharvest treatment. Training is essential to ensure that actors understand their responsibilities in terms of sesame seed handling. Fortunately, over the past decades, several associations and farmers' cooperatives supported by the government and NGOs have been working to this end.

From 2011 to 2014, 8, 2, 1 and 2 sesames samples were received successively in four months. Surprisingly, all samples showed at least a positive *Salmonella* cases even if the sample number were low. It is then, imperative to setup an easier and clear traceability that can be understood by all sesame actors in order to solve the issue at the root. In many cases, bacteria contamination could be avoided with the proper food safety procedures put into place. Interestingly, the *Salmonella* cases decreased notably the last three years and dropped from 38.7 to 6.5%, giving an evidence that a background work were done. On the other hand, while quality is improving these last three years, exported sesame quantities are decreasing because of the important fluctuation of sesame seed kilogram price in the local and international market.

The market for sesame in Asia and Europe is growing at a very high rate in the last decade because the products from sesame readily meets the health requirements for food in the developed world and the popular cuisine in the oriental world (Olowe et al., 2005). This makes the overall international demand of sesame seed steadily growing. In Burkina Faso, this demand is far higher than global supply despite the increase of arable land. From 2007 to 2017, exports of Burkina Faso sesame rose from 29887.5 to 116081.2 tons, with a peak of 171461.1 in 2015, and generated at the same period around 1941.5 USD billion (INSD, 2010, 2018). This production ranked the country among the top ten worldwide countries' producing sesame (Gamené, 2016). On the contrary and according to our knowledge, the increase in production is linked to an improvement of quality as the contamination rate dropped from 26.5 to 6.5%.

Conclusion

In view of this strong growth and the economic income it generates, new strategies of quality improvement should be developed to setup the country in the best place in the international market. This retrospective study about the sesame contamination by *Salmonella* spp. over eleven years showed that contamination rate is high but it also decreases drastically over years. It is believed that our study would contribute and serve as a starting point for more accurate knowledge of the quality of the sesame of Burkina Faso.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Inhibition of root growth as mode of action of two rice yellow mottle virus pathotypes isolated in Mali

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In Mali, the economy is mainly based on agriculture. Rice contributes about 5% of the GDP and constitutes a strategic sector with enormous potential. Despite its strategic importance, rice cultivation faces multiple biotic constraints among which rice yellow mottle virus disease is the most important one with an incidence varying from 60 to 100%. To reduce yield loses due to this disease, we determined the mode of action of two rice yellow Mottle virus (RYMV) pathotypes previously isolated in rice fields in Mali, and identified by Reverse transcription polymerase chain reaction (RT-PCR). Both types significantly decrease rice height with a more pronounced effect with the type A, which showed more aggressivity even on the SK20-28 genotype recognized as RYMV disease-tolerant variety. Root dry weight, grain production and tiller number were the most affected by the disease. Except for tiller number, the virus type A was always more virulent than the type B. Significant positive correlation was found between disease severity and % reduction in tiller number, percentage reduction in plant height, and grain production. The data presented root inhibition as a mode of action of the two RYMV pathotypes isolated in Mali. This information should be used in order to improve the management strategies for these pathogens on field rice in Mali.

Key words: Rice yellow mottle virus, root inhibition, rice, Mali.

INTRODUCTION

Rice yellow mottle virus is a disease of rice causing high losses to rice production (Onwughalu et al., 2010; Traoré et al., 2009). Rice plants affected by this virus show yellow or orange leaves at the early stage of the crop, and leaves roll up and dry in severe cases (Kouassi et al., 2005; Odedera et al., 2016). Stunting, reduced tillering and poor panicle filling are other possible symptoms (Onwughalu et al., 2010; Suvi et al., 2018). The effects of

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the virus on rice plants result in low or no seed production if affected at booting stage and poor grain quality (Onwughalu et al., 2010). Rice yield losses caused by field infestation by rice yellow Mottle virus (RYMV) were estimated between 17 and 100% (Onwughalu et al., 2011) with significant socio-economic impacts on smallholder farmers (Abo et al., 1998; Onwughalu et al., 2010; Asante et al., 2013; Sereme et al., 2016).

Characterization of rice varieties using the lack of expression of symptoms and the un-detectability of the virus content in plant tissues by ELISA test as resistance factor has made it possible to identify rice (*Oryza sativa*) varieties (Gigante and Bekarosaka) as well as *Oryza glaberrima* species (Tog5681, Tog5672, Tog5674 and Tog7291) as very resistant to RYMV (Oludare et al., 2016). For that, the most widely strategy currently used for disease control is genetic resistance, through the use of resistant varieties against pathogens (Samaké et al., 2018; Suvi et al., 2018).

However, the ability of pathogens to overcome resistant genes in crops compromised the durability of these resistances (Ochola and Tusiime, 2011). West and Central Africa have a high pathogenic diversity (Abo et al., 1998; N'Guessan et al., 2001; Oludare et al., 2016; Odedara et al., 2016) and this genetic diversity occurring among RYMV populations seems to present a big challenge for breeding rice for durable resistance to plant virus (Munganyinka et al., 2016). An hypervirulent pathotype of Rice Yellow Mottle Virus was identified in West and Central Africa (Hebrad et al., 2018; Pidon et al., 2017). This pathotype named pathotype T, is able to overcome all type known sources of high resistance and appears to be more abundant than suspected (Hebrad et al., 2018).

Despite the production losses, socio-economic impacts are caused by the disease across Africa, and the ever-increasing resistance breakdown (Sereme et al., 2016); research activities continue mainly in the search for resistant varieties (Ndjiondjop et al., 1999; Albar et al., 2006; Thiémélé et al., 2010; Suvi et al., 2018), and few studies have been conducted to study how the virus affects the growth of the different parts of the plant to impact its production (Opalka et al., 1998; Abebrese et al., 2019).

The studies carried out in this direction have shown that partial digestion of pit membranes resulting from programmed cell death may permit virus migration through them, concomitant with autolysis (Opalka et al., 1998). In addition, the displacement of the Ca^{2+} from pit membranes to virus particles may contribute to the disruption of the pit membranes and facilitate systemic virus transport (Opalka et al., 1998). It was also shown that the virus negatively impacts plant water and mineral nutrition by initiating an imbalanced growth between roots and leaves (Guinagui et al., 2018). In Mali, these modes of action were not detected by the works of the researcher. This suggests that the rice yellow mottle virus genotypes affecting rice varieties in Mali use different modes of action. To be able to efficiently control the RYMV disease in Mali, we must know how the virus cause damages to rice plants. In this study, we firstly identified the type of RYMV in rice fields in Mali (Ohasanya et al., 2006). Secondly, we determined the mode of action of the different types of the identified virus using different rice varieties cultivated in Mali (Mogga et al., 2012).

MATERIALS AND METHODS

Study area

To determine the pathogenicity of two types of Rice Yellow Mottle Virus newly isolated in Mali, an experiment was conducted in a greenhouse under artificial conditions at the Laboratory of Research in Microbiology and Microbial Biotechnology (LaboREM-Biotech), Faculty of Sciences and Techniques; University of Sciences, Techniques and Technologies of Bamako in Mali. This experiment was conducted between June and October 2017.

Rice varieties

In this study, nine rice varieties most cultivated in Mali (Table 1) were used. These rice varieties were obtained from the Institute of Rural Economy of Mali (IER).

Isolates of rice yellow mottle virus

Two types of the RYMV isolated from leaves of infected rice plants collected from farmers' field at Niono, Baguineda and Selingué (Samaké et al., 2018) were used in this study. These two isolates were identified by molecular typing following the method described by Uke et al. (2016), and propagated in greenhouse using the highly susceptible cultivar Kogoni 91-1 by mechanical inoculation of two-week-old seedlings using finger-rub technique (Samake et al., 2018). Four weeks after inoculation, rice leaves showing typical yellow mottle symptoms were harvested to prepare the inoculum used for inoculating rice genotypes in the greenhouse experiment.

Inoculum preparation

The inoculum used was prepared according to the method by Munganyinka et al. (2016). Briefly, one gram of infected leaf tissue was first crushed in a drop of doubly-distilled water using sterile mortars and pestles until 80% of the leaf tissue material was macerated. The resultant leaf extract was diluted 10 times by adding10 ml of doubly-distilled water and kept in a refrigerator.

Experimental design and treatments

In this experiment, we used a complete randomized bloc design (CBRD). The RYMV isolates type A and type B, and the non-inoculated represent the three blocs studied. Rice varieties represent the twenty-one treatments studied. Each treatment was replicated four times. Five seeds of each tested rice variety were seeded directly in a plastic pot filled with 10 kg of soil. After one week the seedlings were thinned to two plants per pot. The cultivar Kogoni 91-1 was used as the highly susceptible check while Gigante was the highly resistant check. Each pot was constantly

Table 1. Analysis of variance for rice tiller number, plant height, grain weight and dry root weight.

SOV	df	Tiller number	Plant height	Grain weight	Dry root weight
Rice varieties	8	96.67***	1707.04***	3193.25***	4000.27***
Repetitions	2	0.48^{NS}	0.65^{NS}	34.26^{NS}	23.83^{NS}
Rice varieties*Repetitions	18	0.37^{NS}	1.10^{NS}	32.03^{NS}	9.60^{NS}
Treatments (Virus + control)	2	1009.91***	13817.97***	507.33***	14872.73***
Treatments*Varieties	18	16.57***	409.67***	272.19***	165.54***
Treatments*Repetitions	4	0.04^{NS}	1.43^{NS}	28.89^{NS}	15.68^{NS}
Error	16	0.34	0.89	30.79	9.67

*** Significant at $P<0.001$ and NS: Not significant at $P<0.05$.

supplied with tap water in the morning. To avoid confusion between the yellowing associated with disease development and that due to malnutrition, 2 g of NPK fertilizer was applied to the plants at 28 days after planting followed by application of 2 g urea at 45 days after planting and at early flowering stage of the plants (Onwughalu et al., 2010).

Inoculation of rice varieties

The inoculation which started 14 days after transplanting using a mixture of carborundum powder to aid the infection, was performed by rubbing the mixture onto the leaves from the base to the top using pieces of cotton wool (Munganyinka et al., 2016). Care was taken to ensure maximum wetting and formation of mild bruises that acted as infection passageways, according to Ochola and Tusiime (2011).

Data collection

Disease severity

Appearance of symptoms and disease progress were monitored for each inoculated genotype from 7 to 32 days post inoculation. Disease severity was scored with a version of the scale developed by John and Thottapilly (1987) and modified by Ochala et al. (2011), where 0 was equivalent to non- infection and 9 was 100% infection.

Components of the yield

Data on important agronomic traits were collected according to Munganyinka et al. (2016). The data include the plant height of inoculated and uninoculated plants (measured from the soil surface to the tip of the shoot in cm); root architecture and length were determined and measured after carefully washing the roots; the number of tillers per plant was recorded for each hill, and the seed weight was determined. These data were used to assess the impact of the disease on the growth of the rice (Munganyinka et al., 2016). In each case, the impact of the disease was assessed using the following formula:

Impact (%) = (Ni-I) × 100/Ni (Zouzou et al., 2008)

Where: Ni = mean values on the seedlings not inoculated and I= mean values on the seedlings inoculated.

Data analysis

Analysis of variance was performed for each parameter (Rice genotypes and Virus types) according to the General Linear Model (GLM) procedures using SAS software (version 9.2). Whenever the Fisher test indicates a significant effect at a probability of 0.05, Fisher's Protected Least Significant Difference (LSD) test was used to compare the means.

RESULTS AND DISCUSSION

Effect of different types of RYMV on rice aerial and root growth

Effect of two RYMV pathotypes isolated in Mali on rice aerial growth

The effect of two types RYMV on the growth of the aerial part of the rice is presented in Figure 1. No significant effect was observed on the growth of the aerial part of the two rice genotypes after inoculation with water in the absence or presence of carburundum (Figure 1). However, inoculation with RYMV shows a decrease in plant size with a stronger effect of the two types of RYMV in Kogoni 91-1 (Figure 1). Indeed, both types of RYMV significantly decrease the hard rice size with a more pronounced effect with the RYMV type A on Kogoni 91-1. RYMV type A was more aggressive even on the SK20-28 genotype, which is recognized as disease-tolerant, than type B (Figure 1).

Effect of RYMV on rice root growth

The effect of two RYMV types on rice root growth is shown in Figure 2. No significant effects of inocution with water were observed on root growth. However, inoculation with RYMV showed a decrease in root size in the Kogoni91-1 and BG 90-2 genotypes with a strong effect of both RYMV types in Kogoni 91-1 (Figure 2). The more root growth is inhibited, the less the aerial part of the rice grows (Figures 1 and 2).

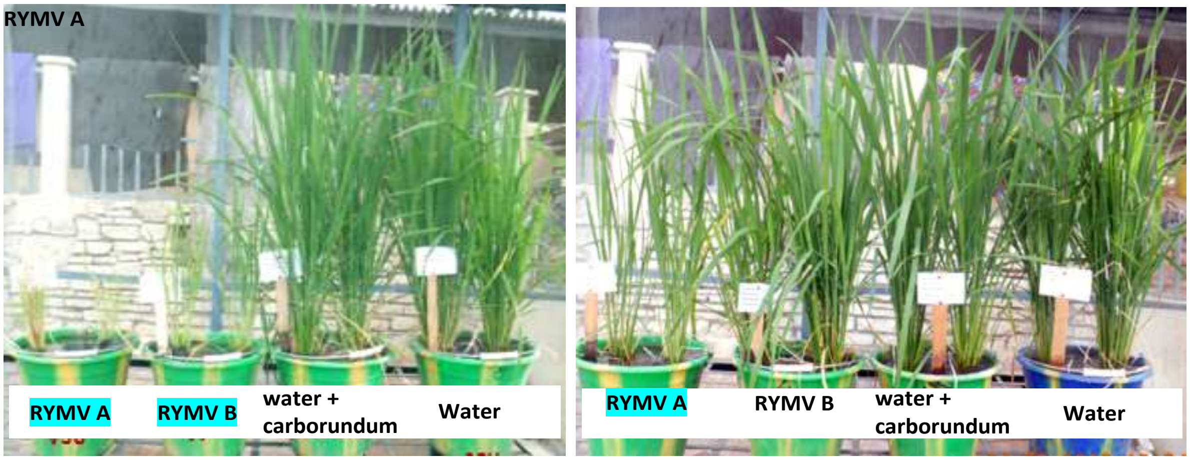

Figure 1. Effect of two RYMV pathotypes (RYMV A and RYMV B) isolated in Mali on the aerial growth of the rice genotypes Kogoni 91-1 and SK20-28.

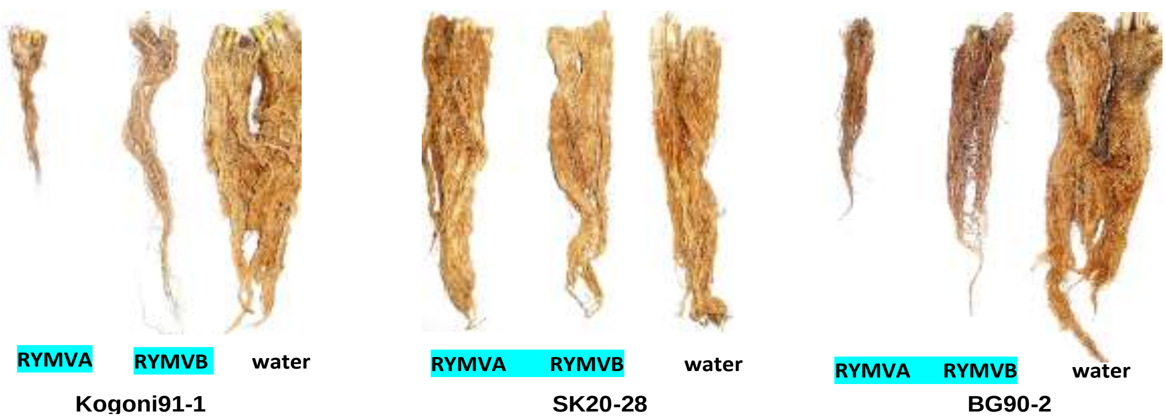

Figure 2. Effect of two RYMV types isolated in Mali on the root growth of the rice genotypes Kogoni91-1, SK20-28 and BG90-2.

Table 2. Effect of inoculation of two RYMV genotypes on tiller number, plant height, grain weight and root dry weight of 9 rice (*O. sativa* L.) gentoytes.

Virus type	Tiller number	Plant height (cm)	Grain production	Root dry weight (g)
Control	7.10^{a}	17.29^{a}	13.20^{a}	14.43^{a}
Type A	4.57^{b}	11.02^{b}	4.02^{b}	3.13^{b}
Type B	3.42^{b}	15.03^{a}	5.59^{a}	4.67^{c}

Effect of the different types of RYMV isolated in Mali on rice yield components

The mean squares for the effect of RYMV disease on rice yield components are presented in Table 1. Rice varieties, treatments (virus pathotypes and an uninoculated control) and their interactions significantly ($P \leq 0.001$) affected rice tiller number, plant height, grain weight and dry root weight (Table 1). Contrariwise, no significant effect was observed with repetions and their interaction with rice varieties or treatments.

Inoculation with RYMV reduced the yield parameters studied as compared to the non-inoculated treatments (Table 2). Root dry weight, grain production and tiller number were the most affected by the disease. Except for tiller number, the virus type A was always more virulent than the type B (Table 2). Results presented in Table 3 show the disease effect on yield components.

Table 3. Effect of RYMV disease on the components of the yield for 9 rice genotypes after artificial infection under greenhouse condition during the season 2016-2017.

Rice genotype	Disease severity scores	Impact			
		Tiller reduction (%)	Height reduction (%)	Grain production reduction (%)	Root dry weight reduction (%)
Kogoni 91-1	4	37.5	34.88	53.09	51.23
BG90-2	6	15.04	22.95	21	21.48
Adny11	5	23.23	25.86	23.43	29.1
Fan	5	31.68	30.86	31.04	40.61
NERICA1	7	13.11	7.22	13.1	19.4
NERICA2	7	15.02	9.18	12.13	18.78
Wassa	4	44.25	41.95	56.99	58.93
SK20-28	8	6.39	2.88	4.2	8.91
Sim2-Simadel	7	12.8	11.88	9.52	16.11

Table 4. Mean squares from analysis of variance for regression of reduction in plant number of tilers, plant height and grain weight on RYMV disease severity.

SOV	df	Tiler number reduction	Plant height reduction	Grain production reduction
Regression	1	1325.95***	1336.89***	2761.24***
Residual	7	8.97	160.21	85.61
Total	8			
″b″		0.016	-1.34	-6.94
R^2		0.99	0.89	0.97
S.E.		1.13	4.78	3.50

*** Significant at 0.001 probability level.

Results showed that RMYV infection reduced rice production between 4.2 to 56.99% depending on the genotypes whereas reduction in tiller number per plant varied from 6.39 to 44.25%, plant height from 2.88 to 41.95% and plant root dry weight from 8.91 to 51.23% (Table 3). The rice genotype Sim2-Simadel showed a small reduction in grain production compared to the tolerant check SK20-28. The susceptible check varieties, Kogogni 91-1 lost 53.09% of its production. The variety Wassa which appears to be more succeptible than Kogoni 91-1, lost 56.99% of its production in contrast to NERICA 2, NERICA 1 and BG90-2, which lost 12.13, 13.1 and 21% respectively (Table 3). Significant positive correlation was found between disease severity and % reduction in tiller number (R^2=0.99), percentage reduction in plant height (R^2=0.89), and grain production (R^2=0.97) (Table 4).

DISCUSSION

Root growth and plant height of rice genotypes Kogoni 91-1 and BG90-2 (*Oryza sativa* L.) were strongly inhibited by the two types of RYMV isolated from Mali. These results confirmed that the intraspecific lines (*Indica* species) were highly susceptible (Munganyinka et al., 2016). Guinagui et al. (2018) showed that RYMV infected leaves contain less minerals (P, K, Ca and Mg) and chlorophyll than those of the non-infected rice plants. The early inhibition of rice root growth may affect water and mineral absorption and can explain the reduction in grain yield in suceptible rice varieties, mainly in *O. sativa* L. intraspecific lines. Contrary, to succeptible rice lines, in the presence of these two types of RYMV the rice genotypes SK20-28 and Sim-simadel showed no significant inhibition in root growth. Abebrese et al. (2019) indicated that some rice hybrids decrease the incidence of the RYMV disease and improve rice yield. So, they recommaded the introduction of these rice hybrids.

In this work the effect of RYMV disease on yield components differed among varieties and the type of virus. Results were confirmed by a highly significant regression of disease score on % reduction in tiller number, plant height, grain production and dry root yield. Results from this study confirm the findings of Munganyinka et al. (2016) in which they showed that RMYV infection reduced plant height between 2.0 to 20.3% depending on genotype; whereas tiller number per plant varied from 4.84 to 30.6%. Onwughalu et al. (2010) stated that the behavior of the rice varieties they tested varies by variety and the parameter selected, and revealed negative corrections with RYMV infection.

Likewase, Guinagui et al. (2018) obtained a high yield loss of 94.4% in Bouake 189 at seedling and booting infection stages. These results establish the fact that yield losses to RYMV are strongly influenced by host cultivars as well as time of virus infection, and revealed that the period from seedling and booting represents the most vulnerable phase to RYMV infection in rice growth stages (Onwughalu et al., 2010).

Conclusion

The rice varieties Kogoni91.1 and BG90-2 were very susceptible to rice yellow mottle virus. These rice varieties were the most appreciated by consummers and smallholder farmers in Mali. Two RYMVs were identified in rice fields in Mali. It appeared through this reseach results that: these two RYMV genotypes impact negatively the tested rice varieties' growth and yield, by strongly inhibiting root sizes (length, width and volume). Thus, improving rice root growth in the presence of RYMV disease could improve growth and yield of susceptible rice varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Constraints limiting the improvement of manure management as climate smart technology for smallholder dairy farmers

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The global quest for a sustainable bio-economy has brought to the fore importance of engaging agricultural systems in the production and in practice change. There have been issues limiting farmers from improving the practice of manure management as smart climate technology. The objective of this paper was to highlight the constraints, type, and valuation of manure types and information sources that smallholder dairy farmers find it useful to change practices regarding manure management. In this present study, 336 smallholder dairy farmers were surveyed on various constraints the farmers faced and, on the type, and value of different manure types and information on manure management received by the farmers. The study used descriptive statistics for the variables and compared them using frequency tables. The key findings from this study would support information to stakeholders in inducing climate-smart manure management practices as a climate adaptation practice. The study highlights the type of information systems that determine areas for further investigation as drivers of practice change for smallholder dairy farmers. The paper focuses on these constraints and synthesizes them into factors that determine practice change on manure management by smallholder dairy farmers in order to improve manure management.

Key words: Manure management, agricultural information, smallholder dairy farmers, practice change, information value.

INTRODUCTION

The global quest for a sustainable bio-economy has brought to the fore importance of engaging agricultural systems in production and in practice change by farmers (Adeyemo et al., 2019; Ribaudo et al., 2014; Tanner et al., 2001). Climate-smart agriculture (CSA) has been named as a new approach to guide farmers to the needed changes in agricultural systems that can address food security and climate change (FAO, 2013). This use of CSA's approach has been realized partly through targeted farmer information to the extension workers, subsequently reaching the targeted farmers (Ndambi et al., 2019; Snapp et al., 2002; Staal et al., 2002). In Sub-

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Saharan agricultural areas, smallholder farmers being the majority, have a need for agricultural information with them facing many challenges in production (Almagro and Martínez-Mena, 2014; Tanner et al., 2001). These smallholder farmers have been described as mixed farmers due to their wide variety of economic activities (Chagunda et al., 2016; Marenya et al., 2012). There is the use of manure from the dairy livestock on farms in smallholder mixed crop-livestock systems as a source of crop nutrients (Castellanos-Navarrete et al., 2015; Delve, 2001; Zake et al., 2010). Farm size and farm uses have been used to define smallholder farmers with few studies looking for other definition areas (Cohn et al., 2017; Herrero et al., 2014; Samberg et al., 2016). The common characteristic that smallholder farmers use manure leads to improved manure management as a key recommendation in many studies on smallholders (Paul et al., 2013; Rufino et al., 2006). The focus on improved manure management has also been observed as the potential to minimize greenhouse gasses emissions as well as also minimizing nutrient losses (Markewich et al., 2010; Pelster et al., 2016; Rosenstock et al., 2016).

The practice changes for various smallholder farmers have been observed to have constraints (Ongeri, 2014; Ouédraogo et al., 2017). The focus on the constraints is due to the realization that for smallholder farmers, climate change has and is causing them to experience challenges due to these demographic being highly susceptible to climate change impacts on weather patterns (Ara Parvin and Reazul Ahsan, 2013; Bellarby et al., 2014). There have been issues limiting smallholder farmers from improving the practice of manure management as smart climate technology. Few studies focus on these constraints as there is more research on ways to improve practices that lead to increased production by smallholder farmers (Gibbons et al., 2014; Zingore et al., 2007). The key drivers affecting smallholders farmers ability to manage and use manure on own farms as fertilizer that have been observed from studies to be land, labour, number of livestock, lack of knowledge, inadequate funds and level of education (Ilukor et al., 2019; Jolliffe, 2004; Lekasi et al., 2001; Mutoko et al., 2015; VanLeeuwen et al., 2012). This, however, has led to the observation of the need to use this and other variables to observe the key information drivers in terms of constraints that affect smallholder farmers from improving their manure management. Information is needed on community practices and perceptions to manure management, and this specifically needs to be focused on manure removal from livestock housing systems, barriers to manure handling, and source of awareness of farm practices (Lekasi et al., 2001; Waithaka et al., 2007). Different information on barriers to improvements in practices by smallholder farmers, aside from manure management practices and livestock housing need be assessed (Mutoko et al., 2015). Further studies have also been needed on community constraints and drivers of practice change for manure management by smallholder dairy farmers. Besides, just the knowledge of the constraints key should be to determine the value of information sources to these farmers.

This study seeks to derive the constraints to improved manure management and describe through analyses information sources to improved manure management by the smallholder dairy farmers in Nandi County. Therefore, the specific objectives were (i) to determine the constraints to improving manure management by smallholder dairy farmers, (ii) to determine the sources and value of these sources of information on manure management to the smallholder dairy farmers of Nandi County.

MATERIALS AND METHODS

Study area

The field study was conducted on smallholder dairy farmers within Nandi County, Kenya (0.565°N, 34.736°E, 0.565°N, 35.437°E, 35.437°E, 0.118°S, 34.736°E, 0.118°S). The mean annual temperatures range from 18- 22°C, with temperatures at lower elevations (<1400 m) going as high as 26°C. Altitude ranges from approximately 600 m a.s.l. in the South to over 2200 m a.s.l. in the Northeast of the county. The highlands are recognized for their high agricultural potential (GOK, 2015; Mudavadi et al., 2001). However, livestock and crop farming are mainly subsistence, with average land sizes of approximately 4.5 ha per household. Dairy production is common throughout the county, with tea as a major cash crop, and maize as the primary staple crop (GOK, 2015).

Field survey

This study utilized a household survey that was done using a questionnaire tool customized from the Integrated Modelling Platform for mixed Animal Crop systems (IMPACTlite). IMPACTlite was modified from IMPACT to collect household-level data detailed enough to capture within-site variability on key indicators of technical, socio-economic, and institutional constraints as well as evaluating the value of the different types of manure and information sources received and found effective by smallholder dairy farmers. The household questionnaire was completed through face-to-face interviews using the Open Data Kit (ODK) platform (ODK, 2017). In case of absence of the household head, the most senior member available or the household member responsible for the farm was interviewed. In the course of the actual household survey, after interviewing the first household, skipped the third, and interviewed the fourth household. This continued until the computed sample size was done. This was done to ensure quality data collection. The study population was the community in Nandi County, while the target population was the dairy cattle farmers households. The unit of analysis was the household, and in order to get a good representative of the targeted population, the procedure and formula below were employed. The sample size was computed using the simple random sampling technique to draw a sample size of 400 respondents for the survey using Fischer's formula as described by Mugenda and Mugenda (2003).

$$n = \frac{N}{1+Ne^2} \quad (1)$$

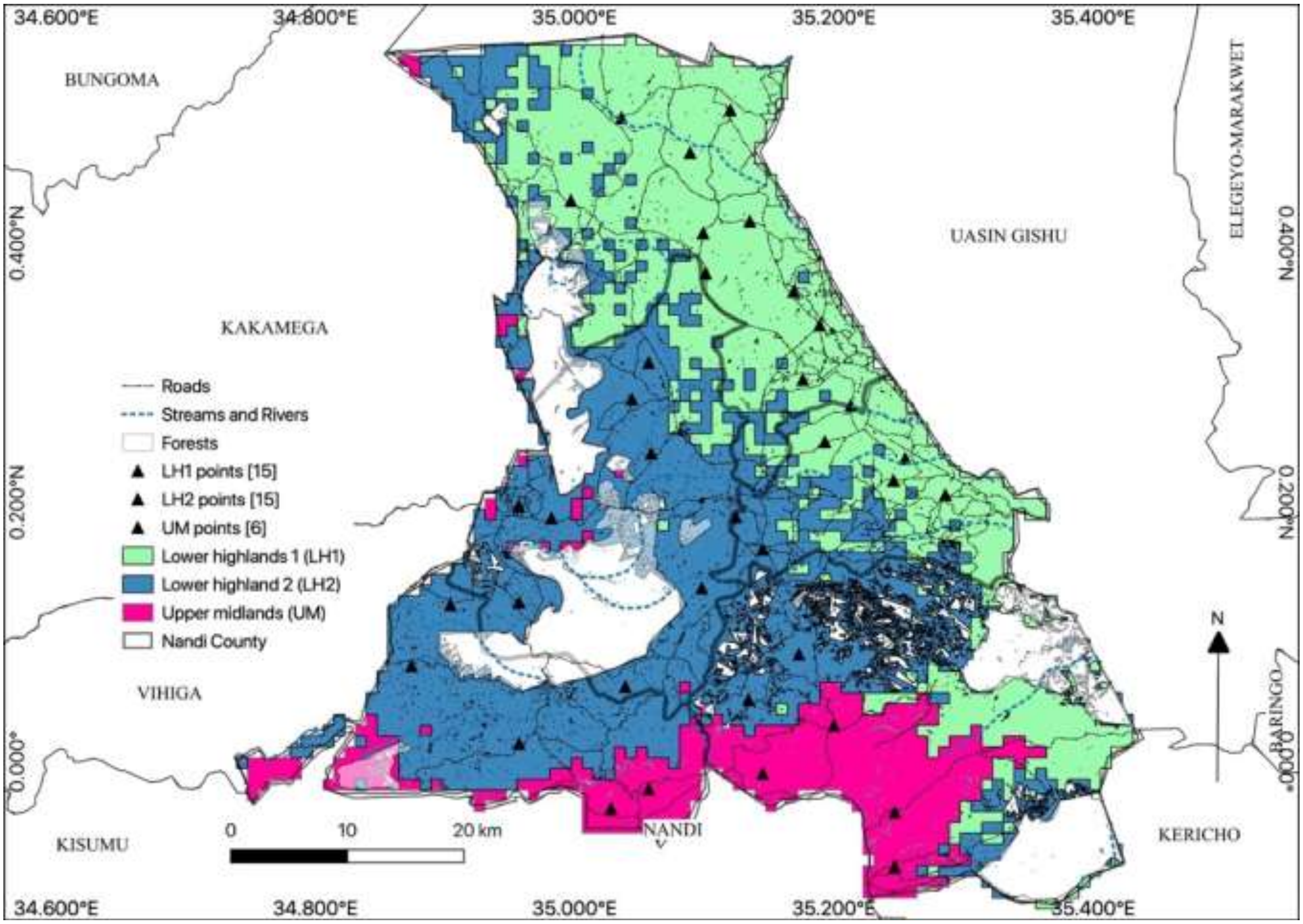

Figure 1. Sampling map showing Nandi County with the 36 sampling points in the derived biophysical zones (Lower highlands 1, Lower Highlands 2, and Upper Midlands).

Where n is the sample size, N is the targeted population (N= 41311 ≈7- adult population of Nandi County removing youth below 15 years 45% of total population (KNBS, 2010; NCPD, 2017) and e the desired confidence level (e=5%) of the sample population of 336 households.

The population of Nandi County, which is 751129 (KNBS, 2010), removing 45% who are youth below the age of 15 (NCPD, 2017), gives 413117, which was considered the study population in the computation of the sample size. The confidence level was taken to be a 5% level of significance with the calculation shown. Random sampling points were generated using QGIS in the three Ago-Ecological Zones of Nandi County and distributed by a fraction of the land area (Figure 1). At each of the 36 random points, about 11 farmers were targeted and interviewed to generate a sample size of 396 households, of which 336 were interviewed. The actual study response rate was 84.8%. The data was found to be sufficient for analysis since it was above 80%, according to Babbie (2013), who considers a response rate above 70% to be very good. The dataset comprised household survey results where both descriptive and inferential statistics were performed using Base R Package (RStudio V 1.1.442) (Rstudio Team, 2016). The variables on constraints, awareness, and source of information on manure management were then analyzed using frequency tables and descriptive statistics.

RESULTS AND DISCUSSION

Technical, socio-economic and institutional constraints

Technical and socio-economic constraints to farmers that hinder improvement to manure management were analyzed and shown in Table 1. The results of these constraints show that the lack of farm labour had a majority (52%) citing it as a very important factor limiting improved manure management. The constraint with the least important was the view that manure had too low benefits when used as fertilizer, compared to the benefits when used as a fuel (dung cakes) with 99% thinking it is not important/ irrelevant. An assessment of institutional constraints that affect the smallholder dairy farmers in Nandi County from improving manure management is tabulated in Table 2. The majority view lack of information to improve manure from institutions (85%), lack of access to available information (86%), lack of access to loans (63%), and lack of access to required equipment (53%) as major constraints.

Table 1. Frequency of technical and socio-economic constraints to smallholder dairy farmers to improve manure management in Nandi County by Agro-Ecological Zones (Lower Highland 1, Lower Highland 2 and Upper Midlands) and gender (percent per constraint is 100%).

Issue and importance	LH1_Male (%)	LH1_Female (%)	LH2_Male (%)	LH2_Female (%)	UM_Male (%)	UM_Female (%)	Total
Lack of farm labour							
Very /important	12.90	9.30	15.00	8.10	5.40	1.80	52.30
Not so important	9.60	8.10	13.40	8.40	6.30	2.10	47.80
Lack of manure collection capacity							
Very /important	12.90	10.50	12.60	8.70	3.30	1.50	49.20
Not so important	9.60	6.90	15.80	7.80	8.40	2.40	50.70
Lack of manure storage capacity							
Very /important	6.00	3.90	6.00	4.80	3.00	0.60	24.20
Not so important	16.40	13.40	22.40	11.60	8.70	3.30	75.80
Lack of manure treatment capacity							
Very /important	2.70	1.20	4.80	3.00	1.20	0.90	13.70
Not so important	19.70	16.10	23.60	13.40	10.40	3.00	86.30
Lack of manure transport capacity							
Very /important	3.60	2.10	7.20	3.90	3.00	0.60	20.30
Not so important	18.80	15.20	21.20	12.50	8.70	3.30	79.70
Lack of suitable equipment to apply manure							
Very /important	3.30	2.70	3.30	1.80	1.50	0.00	12.50
Not so important	19.10	14.60	25.10	14.60	10.10	3.90	87.50
Lack of land to apply manure, because there is none available							
Very /important	1.20	1.20	0.30	0.30	0.90	0.00	3.90
Not so important	21.20	16.10	28.10	16.10	10.70	3.90	96.10
Lack of land to apply manure, because the prices of land are too high							
Very /important	1.20	0.90	0.30	0.00	0.30	0.00	2.70
Not so important	21.20	16.40	28.10	16.40	11.30	3.90	97.30
Not enough collateral to get credit for investments?							
Very /important	3.90	3.00	6.90	4.20	0.60	0.00	18.50
Not so important	18.50	14.30	21.50	12.20	11.00	3.90	81.50

Table 1. Contd.

Too high transport costs, compared to the use of mineral fertilisers								
Very /important		1.50	1.20	2.70	0.60	0.00	0.00	6.00
Not so important		20.90	16.10	25.70	15.80	11.60	3.90	94.00
Too high labour costs, compared to the use of mineral fertilisers								
Very /important	6.60		5.40	8.70	4.20	2.10	1.20	28.10
Not so important	15.80		11.90	19.70	12.20	9.60	2.70	71.90
Too low benefits when used as fertiliser, compared to the benefits when used as a fuel (dung cakes)								
Very /important	0.90		0.30	0.30	0.00	0.00	0.00	1.50
Not so important	21.50		17.00	28.10	16.40	11.60	3.90	98.50

The study found similar observations to Mutoko et al. (2015) and Mwirigi et al. (2014) who all found the availability of funds to farmers as being major limiting factors to the improvement of farm practices. The results of this current study show that farmers, including smallholder farmers, do have an opinion on the constraints that affect their practice. That aside from lack of finances, these farmers is aware of related concerns to finance and that there are other constraints that have been highlighted. This observation is also alluded to in other studies to be a solution space (Greenhalgh et al., 2019; Ouédraogo et al., 2017). The expectation that institutions are the best way to offer solutions and extension, and this current study offers opportunities for the private sector to fill such gaps on information dissemination especially if backed up with financial support and incentives (Abebe et al., 2013; Jensen et al., 2019).

Smallholder farmers perception of the value of the type of manure as a fertilizer on own farm

Smallholder dairy farmers were assessed for the value of slurry from dairy cattle and from other animals. Slurry from dairy cattle (16%) was perceived very/important than from other livestock (8%) (Table 3). A similar analysis of farmers' perception of the importance of solid manure from dairy livestock and compared to other livestock is shown in Table 3. The majority (94%) thought solid storage from dairy cattle was very important. The farmers found solid manure from dairy cattle and other livestock very important with higher margins compared to slurry from similar livestock. The smallholder dairy farmers responded to being asked if they had spent any time/money in the last five years to improve manure management at their households. These results were tabulated in Table 4 below, where the majority (60%) of the smallholder dairy farmers spent time and money to improve manure treatment. Analysis of areas that these farmers spent time/money on in terms of manure collection, storage, treatment, transport, and the application was done and tabulated in Table 4. This table showed that the majority (96%) made improvements in terms of manure treatment with the same farmers' transportation and storage (both at 76%), manure collection (61%), and least was an application (57%).

The importance of manure to farmers has been highlighted in many studies, especially the use of own farm manure to return nutrients (Diogo et al., 2013; van Wijk et al., 2009). Such studies also have shown how to improve productivity. This present study shows the focus of smallholder farmers on improving manure thus highlighting key gaps that can be filled in regard to improved manure management (Jensen et al., 2019; Van Der Wolf et al., 2019). The show that the farmers

Table 2. Frequency institutional constraints to smallholder dairy farmers to improve manure management in Nandi County by Agro-Ecological Zones (Lower Highland 1,Lower Highland 2 and Upper Midlands) and gender (percent per issue is 100%).

Issue and importance	LH1_Male (%)	LH1_Female (%)	LH2_Male (%)	LH2_Female (%)	UM_Male (%)	UM_Female (%)	Total
Lack of information to improve the manure management							
Very /important	17.60	13.20	23.20	15.60	11.40	3.90	84.90
Not so important	4.80	4.20	5.10	0.90	0.30		15.30
Lack of access to available information							
Very /important	17.90	13.80	23.50	15.90	11.40	3.90	86.40
Not so important	4.50	3.60	4.80	0.60	0.30		13.80
Lack of access to loans for the required investments							
Very /important	14.10	9.30	17.70	13.20	7.50	1.20	63.00
Not so important	8.40	8.10	10.70	3.30	4.20	2.70	37.40
Lack of access to required equipment and machines							
Very /important	10.80	7.20	17.60	10.50	6.00	0.90	53.00
Not so important	11.60	10.10	10.70	6.00	5.70	3.00	47.10
Lack of trading infrastructure							
Very /important	8.70	6.90	16.10	11.10	3.60	0.90	47.30
Not so important	13.70	10.40	12.20	5.40	8.10	3.00	52.80
Lack of regulations, leading to possible privileging of groups							
Very /important	4.20	2.70	9.90	6.00	4.20	1.80	28.80
Not so important	18.20	14.60	18.50	10.40	7.50	2.10	71.30
Spatial separation of livestock farms and arable farms due to specialization							
Very /important	3.00	2.10	2.40	1.50	0.60	0.00	9.60
Not so important	19.40	15.20	26.00	14.90	11.00	3.90	90.40

Table 3. Frequency of the value of slurry and solid manure to smallholder dairy farmers in Nandi County by Agro-Ecological Zones (Lower Highland 1, Lower Highland 2 and Upper Midlands) and gender (percent per manure type is 100%).

Issue and importance	LH1_Male (%)	LH1_Female (%)	LH2_Male (%)	LH2_Female (%)	UM_Male (%)	UM_Female (%)	Total
Slurry from dairy cattle							
Very/ Important	3.30	1.80	6.60	2.10	2.10	0.30	16.20
Not so important	19.10	15.50	21.80	14.30	9.60	3.60	83.90
Slurry from other livestock							
Very/ Important	1.20	0.90	2.40	0.90	0.30		5.70
Not so important	21.20	16.40	26.00	15.50	11.30	3.90	94.40
Solid manure from dairy cattle							
Very/ Important	21.20	17.00	24.20	16.10	11.10	3.90	93.50
Not so important	1.20	0.30	4.20	0.30	0.60	0.00	6.60
Solid manure from other cattle							
Very/ Important	17.00	15.30	19.40	14.10	9.00	2.40	77.20
Not so important	5.40	2.10	9.00	2.40	2.70	1.50	23.10

in this current study have spent money on improving their practices does show the willingness to pay and also awareness of challenges that these farmers go through.

Considerations to improve manure management

The reasons for these farmers' improving manure management were asked to the farmers, and the results categorized and tabulated in Table 5. The results focused on on-farm hygiene, water quality, nutrition to crops (Navaratne et al., 2019; Odendo et al., 2009).

Information to improve manure management

The number of smallholder dairy farmers whoreceived the information within the last five years on improving manure management were analysed and tabulated in Table 6. The table showed that the majority (80%) received information. Table 7 looked at the perception in the value of the information source on improving manure management. The table showed the smallholder farmers' value for other farmers' information led (12%) terms of the source of information to improve manure management. Table 8 analysed the media that influence farmers' behaviors in terms of manure management, and these were tabulated by gender of the farmer and the AEZ that the farmer has. Local radio (75%) was very/ important for manure management information for smallholder dairy farmers in Nandi County.

Billboards/posters were found to be least effective as a source of information on manure management. These findings were in concurrence with Mutoko et al. (2015) whose observations included increasing and motivating farmer trainers to train more groups and using diverse techniques lie field days and learning tours. Studies on information systems to farmers are varied in their focus, with most agreeing on the importance of agricultural information to farmers (Hochman et al., 2017; Kante et al., 2017; Makawia, 2018; Moglia et al., 2018). This study evaluation of the value of information and sources also agrees with such studies and also focuses on the smallholder farmers' perception of the sources of agricultural information and its value to them. The movement beyond just climate change as a key constraint is best demonstrated with knowledge of the value of farmers give to various sources of information (Maguire-Rajpaul et al., 2020). The findings from this present study disagree with other recent

Table 4. Frequency of smallholder dairy farmers in Nandi County investment of either Time or Money to improve manure management and aspects of improvement of manure management within the last five years.

Issue	LH1_Male (%)	LH1_Female (%)	LH2_Male (%)	LH2_Female (%)	UM_Male (%)	UM_Female (%)	Total
Invested							
Yes	15.50	11.60	14.60	8.70	6.60	3.30	60.30
No	6.90	5.70	13.70	7.80	5.10	0.60	39.80
Manure collection							
Yes	15.50	11.90	14.60	8.70	6.60	3.30	60.60
No	6.90	5.40	13.70	7.80	5.10	0.60	39.50
Manure storage							
Yes	17.90	14.60	19.10	11.90	9.30	3.30	76.10
No	4.50	2.70	9.30	4.50	2.40	0.60	24.00
Manure treatment							
Yes	21.20	17.00	26.00	16.10	11.60	3.90	95.80
No	1.20	0.30	2.40	0.30	0.00	0.00	4.20
Manure transport							
Yes	19.10	14.60	20.00	12.20	6.90	3.60	76.40
No	3.30	2.70	8.40	4.20	4.80	0.30	23.70
Manure application							
Yes	14.30	17.60	10.70	7.20	3.60	3.60	57.00
No	3.90	3.00	10.80	5.70	4.50	0.30	28.20

This is aggregated by Agro-Ecological Zones (Lower Highland 1, Lower Highland 2, and Upper Midlands and gender (Total for all is 100%).

studies focusing on ICT as the next frontiers for farmer information being most beneficial, this study shows that smallholder farmers prefer medium that is more local thus these technologies would need to be localised (Hartmann et al., 2020; Mereu et al., 2018).

This study revealed that access to information on manure management was a major constraint to improving manure management. Those farmers sited lack of labour and lack of manure collection capacity as major technical and socio-economic constraints. The study also found a lack of information from institutions on manure management and lack of access to information on manure management as major important institutional constraints. The farmers also cited a lack of loans in terms of capital and lack of access to equipment and services for manure management in relation to installing biogas systems as major institutional constraints. These findings agreed with Chibanda et al. (2009); Mudavadi et al. (2001) and Waithaka et al. (2007) who also found that smallholders in these areas were initially major cash crop producers and by moving to milk the commercialization was of key importance with lack of institutions focussing on manure management, they also found labour availability is a constraint.

Table 5. Frequency of smallholder dairy farmers in Nandi County consideration to improve manure management within the last five years.

Issue and importance	LH1_Male (%)	LH1_Female (%)	LH2_Male (%)	LH2_Female (%)	UM_Male (%)	UM_Female (%)	Total
Improve on-farm hygiene, considering human health							
Very/ Important	6.30	4.80	13.80	7.80	4.50	0.60	37.80
Not important/irrelevant	16.10	12.50	14.60	8.70	7.20	3.30	62.40
Improve on-farm hygiene, considering animal health							
Very/ Important	5.70	5.10	13.50	7.80	5.10	0.60	37.80
Not important/irrelevant	16.70	12.20	14.90	8.70	6.60	3.30	62.40
Improving on water quality, from the point of view of human health							
Very/ Important	6.30	4.80	12.60	7.80	4.50	0.60	36.60
Not important/irrelevant	16.10	12.50	15.80	8.70	7.20	3.30	63.60
Improving on water quality, from the point of view of animal health							
Very/ Important	6.30	5.10	11.70	7.80	4.50	0.30	35.70
Not important/irrelevant	16.10	12.20	16.70	8.70	7.20	3.60	64.50
Abatement of odour problems, also for neighbours							
Very/ Important	4.50	4.50	10.50	6.00	4.20	0.60	30.30
Not important/irrelevant	17.90	12.80	17.90	10.50	7.50	3.30	69.90
Improving fertiliser value (nutrients) for their own crops							
Very/ Important	3.90	4.20	9.90	4.80	3.90	0.60	27.30
Not important/irrelevant	18.50	13.10	18.50	11.70	7.80	3.30	72.90
Improving fertiliser selling value (income) when sold to other farms							
Very/ Important	3.90	4.20	9.90	4.80	3.90	0.60	27.30
Not important/irrelevant	21.80	16.40	26.30	15.60	11.70	3.90	95.70
Incentive measures by the government and/or other institutions							
Very/ Important	0.30	0.00	0.30	0.00	0.00	0.00	0.60

Not important/irrelevant	22.10	17.30	28.10	16.40	11.70	3.90	99.50
Restrictive measures by the government and/or other institutions							
Very/ Important	0.30	0.00	1.20	0.00	1.20	0.00	2.70
Not important/irrelevant	22.10	17.30	27.20	16.40	10.50	3.90	97.40

This is aggregated by Agro-Ecological Zones, Gender and confinement systems (Total for each issue is 100).

Table 6. Frequency of smallholder dairy farmers who have received information on manure management in the last 5 years aggregated by Agro-Ecological zone and gender (Total is 100%).

Response	LH1_Male	LH1_Female	LH2_Male	LH2_Female	UM_Male	UM_Female	Total
Yes	19.40	13.10	20.90	13.40	9.60	3.30	79.70
No	3.00	4.20	7.50	3.00	2.10	0.60	20.40

Table 7. Frequency of smallholder dairy farmers in Nandi County value of information sources on manure improvement aggregated by agro-ecological zone and gender.

Issue and importance	LH1_Male (%)	LH1_Female (%)	LH2_Male (%)	LH2_Female (%)	UM_Male (%)	UM_Female (%)	Total
Value of another farmers information							
Very/ important	2.7	2.4	3.9	2.4	0.6	0.3	12.3
Not important/irrelevant	19.7	14.9	24.6	14.0	11.0	3.6	87.8
Value of government extension workers							
Very/ important	0.9	1.2	3.0	1.5	0.3	0.0	6.9
Not important/irrelevant	21.5	16.1	25.4	14.9	11.3	3.9	93.10
Value of non-commercial advisors							
Very/ Important	0.3	0.9	0.6	0.0	0.0	0.0	1.80
Not important/irrelevant	22.1	16.4	27.8	16.4	11.6	3.9	98.20
Value of commercial/private advisors							
Very/ important	1.8	1.2	3.0	1.5	0.3	0.3	8.10
Not important/irrelevant	20.6	16.1	25.4	14.9	11.3	3.6	91.90
Value of local teachers and trainers							
Very/ important	2.1	1.2	3.6	1.5	0.9	0.0	9.30
Not important/irrelevant	20.3	16.1	24.8	14.9	10.7	3.9	90.70

Value of any other actor							
Very/ important	2.7	2.1	3.6	0.6	0.3	0.3	9.60
Not important/irrelevant	19.7	15.2	24.8	15.8	11.3	3.6	90.40

Table 8. Frequency of smallholder dairy farmers sources of information about manure management aggregated by Agro-Ecological Zones, gender and Livestock confinement systems.

Issue and importance	LH1_Male (%)	LH1_Female (%)	LH2_Male (%)	LH2_Female (%)	UM_Male (%)	UM_Female (%)	Total
National television							
Very/ important	5.40	3.90	11.10	4.50	3.60	1.20	29.70
Not important/irrelevant	17.10	13.50	17.40	12.00	8.10	2.70	70.80
Local television							
Very/ important	7.80	4.80	9.90	3.90	3.30	1.20	30.90
Not important/irrelevant	14.70	12.60	18.60	12.60	8.40	2.70	69.60
National radio							
Very/ important	6.3	3.9	13.1	6.9	6.0	1.8	38.00
Not important/irrelevant	16.20	13.50	15.20	9.60	5.70	2.10	62.30
Local radio							
Very/ important	16.80	13.20	21.20	10.80	9.00	3.60	74.60
Not important/irrelevant	5.70	4.20	7.20	5.70	2.70	0.30	25.80
National newspaper							
Very/ important	5.10	2.40	7.50	2.10	1.20	0.60	18.90
Not important/irrelevant	17.40	15.00	20.90	14.40	10.50	3.30	81.50
Local newspaper							
Very/ important	3.00	1.20	3.60	1.20	1.20	0.00	10.20
Not important/irrelevant	19.40	16.10	24.80	15.20	10.50	3.90	89.90
Farmers' magazines							
Very/ Important	2.40	1.50	3.90	1.80	0.90	0.30	10.80
Not important/irrelevant	20.00	15.80	24.50	14.60	10.80	3.60	89.30
Farmers' group meetings							
Very/ important	10.50	7.20	8.40	3.30	2.40	1.20	33.00
Not important/irrelevant	12.00	10.20	20.10	13.20	9.30	2.70	67.50
Field excursions/farm visits/open days							
Very/ important	7.80	6.30	10.50	5.40	3.30	0.60	33.90
Not important/irrelevant	14.70	11.10	18.00	11.10	8.40	3.30	66.60

Table 8. Contd.

Individual meetings							
Very/ important	8.40	5.70	13.10	5.10	4.50	0.60	37.40
Not important/irrelevant	14.10	11.70	15.30	11.40	7.20	3.30	63.00
Billboards/posters							
Very/ important	0.30	0.30	0.30	0.00	0.30	0.00	1.20
Not important/irrelevant	22.10	17.00	28.10	16.40	11.30	3.90	98.80
Pamphlets/leaflets/brochures							
Very/ important	1.80	0.90	3.60	0.30	0.30	0.00	6.90
Not important/irrelevant	20.60	16.50	24.80	16.10	11.40	3.90	93.30
Videos							
Very/ important	0.60	0.30	0.30	0.30	0.60	0.00	2.10
Not important/irrelevant	21.80	17.00	28.10	16.10	11.00	3.90	97.90
Internet							
Very/ important	1.80	0.30	3.30	1.20	1.20	0.30	8.10
Not important/irrelevant	20.60	17.00	25.10	15.20	10.50	3.60	92.00
Social media							
Very/ important	0.90	0.00	2.40	0.00	0.60	0.30	4.20
Not important/irrelevant	21.50	17.30	26.00	16.40	11.00	3.60	95.80

CONCLUSION AND RECOMMENDATIONS

The study demonstrated key constraints as access to information on manure management and manure collection capacity, which are subject to labour availability and financial capital for smallholder dairy farmers. The institutional constraints that matter most to these farmers were access to information on manure management, access to financial capital, and equipment and services for manure management. This creates a mix of agricultural and financial information that was preferred by smallholder dairy farmers. These farmers felt that such information would enable them to manage their manure better, thereby mitigating Greenhouse gas emissions while minimizing nutrient losses through managed manure.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

A literature review on yield gaps of various root, tuber and banana crops as a background for assessing banana yield reductions due to pests and diseases at a field site in western Burundi

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Banana pests (corm weevil and root nematodes) and diseases (Xanthomonas wilt of banana, banana bunchy top disease and fusarium wilt) are major constraints to banana production in Central Africa. The pests cause various degrees of yield reduction, while plants affected by three of the diseases eventually die before producing an edible bunch. Studies on yield gaps for most of these constraints are currently limited. This paper reviews yield gap studies of some root, tuber and banana crops broadly and with a specific focus on biotic constraints. It also presents an initial case study conducted in Burundi to understand yield gaps due to various banana pests and diseases. Bunch weights of banana varied widely at production zones in western Burundi due to biotic constraints. Boundary line analysis revealed large yield gaps due to the various pests. The often sub-optimal, medium and small bunch sizes found in visibly healthy fields however indicate that in addition to mitigating effects of biotic constraints, significant improvements in bunch weights could be attained through the application of agronomic/field management practices that enhance soil fertility, soil moisture content and soil health. Simple and robust methods [such as the boundary line analysis] for estimating yield gaps caused by pests and diseases, and abiotic constraints on farm are crucial for informing/guiding on the need to apply agronomic and/or disease control efforts. In addition, continuous/sustained field monitoring, with the involvement of farmers, over time will be necessary for a more accurate assessment of yield gaps caused by diseases and pests.

Key words: Banana bunchy top disease, boundary line analysis, fusarium wilt, nematodes, weevils, Xanthomonas wilt.

INTRODUCTION

The Great Lakes region of east and central Africa, of which Burundi is part, is endowed with a broad

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diversity of banana cultivars spread across a wide range of altitudes and agro-ecological zones. This region also constitutes one of the secondary centers of *Musa* diversity, more specifically for the East African highland bananas, AAA-EAH (Karamura et al., 2004). Banana and plantain (*Musa* sp.) are an important staple and income-generating crop for rural communities in this region.

Banana pests and diseases however severely constrain banana production in Central Africa (Blomme et al., 2013, 2017; Ocimati et al., 2013). In Western Burundi, Xanthomonas wilt of banana, banana bunchy top disease and fusarium wilt occur together in production landscapes and farms ranging from the humid lowlands to the cooler high altitude hilly zones (Lepoint et al., 2012, 2013; Ocimati et al., 2013). In addition, weevil and nematode pests are present (Blomme et al., 2012; Ocimati et al., 2013). Xanthomonas wilt has been reported to cause yield losses of up to 100% in 'Bluggoe'/'Pisang Awak' [*Musa* ABB type]-dominated systems in Uganda (Blomme and Ocimati, 2018), while Fusarium wilt decimated the 'Sukari Ndizi' (AAB) production zones in Rwanda (Karangwa et al., 2016). Losses from banana bunchy top disease (BBTD) are significant in Cavendish production systems (e.g., in Malawi), while they are more moderate in plantain (AAB) and 'Bluggoe'/'Pisang Awak' dominated systems (Ngama Boloy et al., 2014; Mikwamba et al., 2020). This paper reviews yield gap studies of some root, tuber and banana crops broadly and with a specific focus on biotic constraints. It also presents an initial case study conducted in Burundi, using proven yield gap assessment methodologies, to understand yield gaps due to banana pests and diseases.

Banana yield gap review

What are yield gaps?

Though the concept of yield gaps is not new, and an increasing number of studies have calculated it for a variety of crops, particularly grain crops, a standard definition of a yield gap does not exist. Sumberg (2012) lists the following variable definitions of *yield gap* in his review paper:

(i) Becker et al. (2003): the difference between actual farmers' yield and calculated average potential yield.
(ii) Cassman et al. (2003): the "exploitable" yield gap is the difference between yield potential and the actual yield achieved by farmers.
(iii) de Bie (2004): the difference of the average production situation with the anticipated best one.
(iv) Zinck et al. (2004): the gap between the actual crop yield and the expected yield.
(v) de Bie (2004) and Waddington et al. (2010): best versus average yield.
(vi) Ortiz-Ferrara et al. (2007): the gap between farmers' and experimental yields.
(vii) Lobell et al. (2007): the difference between average and maximum yields.
(viii) Audebert and Fofana (2009): the difference between simulated yields and observed yields.

More recently, in their introduction to the special issue on Crop Yield Gap Analysis, van Ittersum and Cassman (2013) define yield gap as the *difference between the yield under optimum management and the average yield achieved by farmers*, while Sheehy et al. (2015) define it as the *difference between potential (and water-limited potential) yield and average farm or actual yield, under the same environment*.

With this relatively wide variety of definitions, not all of which are interchangeable, it is important to clearly define what is considered a yield gap and how to calculate it, particularly as the definition also determines which methods can or should be used to calculate the yield gap.

Generally, yield gaps (Yg) can be defined as the difference between a non-limited yield or potential yield (Yp)- e.g., the yield of a crop cultivar when grown without any water or nutrients limitations and with biotic stresses effectively controlled (Evans, 1993; van Ittersum and Rabbinge, 1997) – and actual yields (Ya). For rain-fed crops, van Ittersum et al. (2013) suggest that the water-limited yield (Yw), equivalent to the maximum potential yield when water is limited, is the most relevant benchmark. They define Yw as "similar to Yp, but crop growth is also limited by water supply, and hence influenced by soil type (water holding capacity and rooting depth) and field topography (runoff)".

Often, yields derived in yield experiments in agricultural research stations, under the best management conditions currently known and applicable for any given crop growth environment are defined as Yp, though Yengoh and Ardö (2014) refer to these as the *maximum attainable yield*. Based on Lobell et al. (2009) and van Ittersum et al. (2013) distinguish four methods to estimate yield gaps at the local level: (1) field experiments, (2) yield contests (where participating farmers compete against each other to achieve maximum yields for a certain crop in a given season), (3) maximum farmer yields based on surveys, and (4) crop model simulations. For a given crop, the first step in each method is to estimate potential yields (Yp and Yw) in a certain location or region. Yg is then calculated as the difference between farmer's actual yields (Ya) and estimated potential yields (Yp or Yw).

Alternatively, the Yp can be simulated using crop models. However, there are no published guidelines about standard sources and quality of data input for weather, soil, actual yields, and cropping-system context, or requirements for calibration of crop models used in such studies (Grassini et al., 2015). Nonetheless, a robust approach to simulate accurate crop yield potential and estimate Yg requires: (i) input data that meet minimum quality standards at the appropriate spatial scale, (ii) agronomic relevance with regard to cropping system context, (iii) proper calibration of crop models used, and (iv) flexibility and transparency to account for different scenarios of data availability and quality (Grassini et al., 2015).

Yield gaps in roots, tubers and banana (RTB) crops

Most of the yield gap studies have looked at the yields of grain crops (esp. wheat, maize or rice), while the yields and yield gaps of RTB crops have generally been given less attention. Recently, potato (*Solanum tuberosum* L.) yields and yield gaps have been studied in some depth (Haverkort et al., 2014; Haverkort and Struik, 2015; Svubure et al., 2015), and other RTB crops (cassava (Fremont et al., 2009) and banana (Wairegi et al., 2010)) have received some attention. In general, two types of yield gap studies have been done: very specific, looking at one constraint (e.g. % losses due to different levels of pests or diseases on a specific cultivar) or model based, looking at a variety of theoretical constraints and attributing them levels of loss (e.g., based on crop models to estimate potential yields).

Yield gaps in potato - simulated and actual yields

Haverkort et al. (2014) determined potato yield gaps across a range of agroecological zones in six distinct potato-growing areas in Chile using a crop simulation model. They defined a yield gap as being the difference between the potential simulated yield (Y_{pot}) and actual yield (Y_{act}). Actual yields were collected by surveying growers, whose cropping systems were also characterized with regard to field size (smaller or larger than 10 ha per season grown), technology usage levels and aim of potato production (seed, early or late potato). The potential yields were calculated using the LINTUL-POTATO simulation model into which monthly weather data (maximum and minimum temperatures, solar radiation, precipitation and evaporation) acquired from local meteorological stations, soil data and planting and harvesting dates were fed.

Actual and potential yields, as well as yield gaps varied among locations and the latter were generally larger on small farms than on “not so small farms”. The average yield across productions systems was 31 t ha^{-1} (range: approx. 15-50 t ha^{-1}), while the average potential yield was 74 t ha^{-1} (range: approx. 20-120 t ha^{-1}), leading to a yield gap of 43 t ha^{-1} or of 58%.

In areas with higher potential yields, actual average yields were about 40% of potential yields, that is, a 60% yield gap, while in areas with relatively low potential yields, actual yields approached potential ones, leading to a much smaller yield gaps. Individual contributing factors to the yield gaps were not identified (that is, diseases, low soil fertility, etc.), but the authors noted that daily growth rate explained almost 70% of the variation in reported actual yields; while the length of the growing season was not clearly related to tuber yields. Increasing levels of technology (e.g., application of fertilizers and/or irrigation water) helped to close the yield gap.

In this paper, yield gaps are graphically presented as a ratio of Y_{act}/Y_{pot}, which ranges from approximately 0.15 to 1.20, with 17 of the 20 systems analyzed ranging between 0.20 and 0.70. Haverkort and Struik (2015) consider a Y_{act}/Y_{pot} ratio of about 0.6 in potatoes to be close to an economic optimum (that is, where growers apply all inputs and carry out all necessary cultural practices in a balanced and optimal manner).

In the same vein, they define potential yields (Y_{pot}) as “those not necessarily economical but the highest yields when all inputs are supplied such that the crop faces no shortage” (Haverkort and Struik, 2015). So the potential yield of potato is the theoretical yield that can be calculated or modelled for a certain cultivar grown in a certain environment without any limiting or reducing factor being present. They list a number of papers in which this approach is described in greater detail, and highlight two models for potatoes: “the simple, robust LINTUL-POTATO model” (Kooman and Haverkort, 1995) – also used in Haverkort et al. (2014), and “the more complicated, but also more versatile model GECROS” (Khan et al., 2014).

Svubure et al. (2015) also used the LINTUL-POTATO model to simulate the yield of potato – both potential yield (Y_p) and water-limited yield (Y_w) – for different agro-ecological regions in Zimbabwe and calculate yield gaps. Actual tuber yields ranged from 8 to 35 t ha^{-1}, while the simulated potential yield ranged from 88 to 96 t ha^{-1} across the seven studied agro-ecological regions. Water-limited potential yield (Y_w) was simulated for four agro-ecological regions with different climates. A theoretical planting date (15th day of the month) was assumed in this simulation, and water-limited potential yields were compared to potential yields, revealing that Y_w followed the same pattern as Y_p but is dependent on the rainfall pattern in all the areas. The two simulated yields were very similar when planting took place during the rainy months of the year (September through January) while the crop completely failed ($Y_w = 0$ t ha^{-1}) in the dry winter plantings of April through July and rose with the summer rains thereafter. Irrigation as practiced is therefore essential for year-round potato production in Zimbabwe. Svubure et al. (2015) report yield gaps from 65 to 92% between simulated potential potato yield and actual yields reported by the growers, so there is a lot of room to increase yields, including measure to optimize planting dates and irrigation, use of IPM and improved/appropriate planting material. It seems that for potatoes, a number of well-developed crop simulation models have been developed, which can reliably predict potential yields; though they have not been recently used to investigate the impact of defined biotic constraints on yields, but rather to get a general idea of theoretical yield gaps and potential yields in relation to solar radiation and water availability, particular in respect to climate change.

Yield gaps in cassava - boundary line analysis

Another RTB crop whose yield gaps have been looked at recently is cassava, in Uganda and Kenya. According to Fremont et al. (2009), the "ideal cassava plant" should have a fresh root yield of 75-90 t ha^{-1}, while recorded survey yields ranged from 6.1 to 11.7 t ha^{-1} – translating to theoretical yield gaps of 84-93%. However, not only are the production conditions in Uganda and Kenya not necessarily ideal for cassava, the ideal cassava plants are also not necessarily available or planted, so the authors define the cassava yield gap as the gap between the actual and the attainable yield (y_{max}). The latter was defined as: "the maximum yield observed in a given agro-ecological zone with a given management intensity".

Actual yields were farmers' estimates of average cassava yield in their fields for past seasons. Attainable yields were defined as the greatest yields achieved in trial fields receiving "management level 3" (the use of improved crop establishment and genotypes) in two consecutive sets of on-farm cassava trials in Kenya and Uganda. Though this definition is straightforward, the authors did not simply subtract one yield from another, but used an adapted boundary line approach based on Webb (1972), van Asten et al. (2003) and Shatar and McBratney (2004) to identify in detail the contribution of individual abiotic, biotic and management factors to the yield gap.

This approach consisted in defining boundary lines that represented the maximum yield response (the dependent variable) to the various independent variables (e.g., rainfall), after having sorted the independent variables in ascending order and removed outliers. Boundary lines were fitted through selected boundary points (Schnug et al., 1996) following the model:

$$y_l = \frac{y_{\max}}{1 + (K \exp(-Rx))} \quad (1)$$

Where y_l was the boundary line, $y_{\max}$ the observed attainable yield level at management level 3 (improved crop establishment and genotypes), x the independent variable and K and R constants. For each field and each independent variable, individual boundary lines were used to calculate the maximum cassava yield that could have been obtained if production would only have been limited by the independent variable in question ($y\_\max_{ij}$). These individual boundary lines were then combined in order to create a multivariate model, assuming responses according to von Liebig's law of the minimum (von Liebig, 1863; Shatar and McBratney, 2004), and the model was used to predict yields for each field.

Finally, the yield gap caused by each independent variable in each field was determined by subtracting the $y\_\max_{ij}$ from the attainable cassava yield ($y_{\max}$). Using this approach, they established that using their "complete management package" – consisting of improved crop establishment, an improved genotype and application of NPK fertilizer, average yields on farmer fields more than doubled, from ca. 9 to 21 t ha^{-1}, and simulated attainable yields increased from ca. 18 to 37 t ha^{-1}, in both the Kenyan and Ugandan sites. Cassava yield gaps therefore ranged from 43-76% and are caused by a multitude of production constraints. Fremont et al. (2009) conclude that abiotic constraints and related crop management practices are far more important than perceived by farmers and scientists and that efforts to improve productivity should therefore be reappraised and be geared towards combining approaches to identify and overcome the most important constraints simultaneously, rather than focusing on single constraints, and particularly on specific pests and diseases.

Yields of Musa spp.

To analyze yield gaps in banana and plantains, we must first come to a standard definition of yield. For annual and perennial crops with a distinct harvest period, defining yield is not usually a problem: yield is the mass of harvested product from a defined area after a single harvest (season) (e.g., Mt or kg ha^{-1}). For banana and plantain (genus *Musa*), which are harvested throughout the year, but whose production is nonetheless affected by a number of factors, including planting time, the predominant perennial nature of the crop, seasonal rainfall, cultivar type and crop cycle, defining yields is not straightforward. In their 2010 paper, Hauser and van Asten note that "a standardized approach on harvesting banana yields and a common and comparable way to express them is important". Unfortunately, their advice has not filtered into many studies, and many publications on the subject fail to clearly define *Musa* yields.

In addition to including a unit of time in the calculation of *Musa* yields, Hauser and van Asten (2010) suggest:

(1) Weighing fresh bunches using a cultivar-specific peduncle cut-off point, and give two examples:
(i) Plantains (*Musa* AAB): peduncle to be cut off halfway between the first and second empty bract above the first fruit hand.
(ii) East African highland bananas (*Musa* AAA-EAHB): peduncle to be cut off where it enters the pseudostem at the point where the two youngest leaf petioles cross.
(2) Converting fresh bunch weight data into edible dry matter (conversion calculations are cultivar-/banana-type-dependent).
(3) Clearly defining plot areas, borders, and plant densities (incl. reporting non-producing plants).

(4) Avoid extrapolating survey yield findings from one time to longer time periods.

These considerations complicate the already challenging task of calculating yield gaps, but should not be ignored, as they form the basis for comparison.

Yield gaps in East African highland bananas (EAHB) - boundary line analysis

Wairegi et al. (2010) also used the boundary line approach to distinguish and identify the importance of different biotic and abiotic factors affecting the yields of East African highland bananas. To do so, they monitored fresh banana yields (t ha^{-1} yr^{-1}), as well as biotic and abiotic constraints on 159 plots on on-farm demonstrations and ordinary farmer fields in three distinct banana-growing regions in Uganda. Farmer yields ranged from 10 to 20 t ha^{-1} yr^{-1} in the regions. In the model calculations, the maximum yield ($y_{\max}$ in Fremont et al., 2009) was replaced with the attainable yield (Y_{att}), defined as "the highest yield observed in each region", which ranged from 31 to 37 t ha^{-1} yr^{-1}.

In addition, each boundary line function was used to predict the maximum yield possible (Y_{xi}) for each biophysical factor (i=1, 2, . . ., n) in each plot. For each biophysical factor and each plot, the gap between Y_{att} and Y_{xi} was calculated. The yield gap was then expressed as percentage of Y_{att} to allow for comparison among regions. For each plot, the minimum predicted yield (Y_{min} = Min (Yx_1, Yx_2, Yx_n)) was also identified. Two types of yield gaps were presented: the *explainable* yield gap was defined as the difference between the maximum attainable yield (Y_{att}) and the predicted minimum yield (Y_{min}) while the *unexplained* yield gap represented the difference between the predicted minimum yield (Y_{min}) and observed yield (Y_{obs}).

Average yield gaps (expressed as percentage of Y_{att}) corresponding to different factor by region combinations (factors: nematodes, weevils, weeds, mulch, fertilizer, N-total, K/(Ca +Mg), banana population, pH, SOM, N-total, clay and rainfall) were identified by plotting separate boundary lines for each factor and region. Depending on the region, there were only significant differences in average yield gaps between control and demonstration plots for the factors fertilizers (yield gap from 4.8 to 53.1%), weeds (4.6 to 36.1%) and mulch (10.4 to 28.0%). As the distribution of yield gaps varied among factors and regions, median and not average yields were used to graphically represent yield gaps associated with various factors for a typical plot in each region. The explained average yield gap ranged from 10.9 to 18.4 t ha^{-1} $year^{-1}$ and the unexplained yield gap averaged 1.9 to 5.0 t ha^{-1} yr^{-1}. Total yield gaps, that is, difference between attainable (Y_{att}) and observed (Y_{obs}) yields, ranged from 5.9 to 16.2 t ha^{-1} yr^{-1}, when the attainable yield was assumed to be the highest yield observed in each region (31 to 37 t ha^{-1} yr^{-1}).

The authors conclude that the boundary line approach is more appropriate for data confined to a single agro-ecological zone and less appropriate for data covering a wide geographical region. They suggested that management decisions should not be only based on visual assessment of single constraints but based on a comprehensive understanding of causes of yield reduction.

Wairegi et al. (2010) identify soil fertility as a major constraint in all regions, while pests (weevils and nematodes) are more important in some regions than in others. They state that in general, the low banana yields observed in Uganda are due to abiotic factors (soil fertility and moisture) to a greater extent than biotic factors (pests and diseases), though these can vary greatly from plot to plot. They highlight that although yield gaps attributed to root necrosis had a median of 7.9% in one region, the outliers suggested that in some farms, yield loss attributed to nematodes could be as high as 80%. This suggests the need to target units [of analysis] smaller than regions when diagnosing constraints and subsequently developing recommendations" (Wairegi et al., 2010). The effect of diseases was not analyzed in this paper, as they did not seem to be a major problem in the monitored plots. Nonetheless, Wairegi et al. (2010) do recognize the potential yield limiting effects of diseases and suggest that banana systems be continuously monitored to prevent potentially devastating disease outbreaks.

Yield gaps associated with specific pests and diseases of banana and plantains

Nematodes

Substantial yield increases (20-75%) in production areas where nematicides were applied revealed the extent of production losses due to nematodes (Broadley, 1979; McSorley and Parrado, 1986; Sarah, 1989; Gowen, 1994). However, yield gaps were not defined in these studies, though various levels of infestation, as revealed both by assessing % of root necrosis, the relative proportion of dead to functional roots and counting the number of plant parasitic nematodes per 100 g root tissue have been associated with general negative effects on yields (Gold et al., 1994; Speijer and De Waele, 1997).

A particular research focus has been given to the effects of nematodes on commercial banana plantations in lowland tropical areas, where the most damaging nematode is the burrowing nematode, *Radopholus similis*. In these plantations, nematode populations are monitored on a monthly basis, and a nematode threshold of 10,000 *R. similis*/100 g functional root was established by Tartré and Pinochet (1981). This threshold was used to determine the need for nematicide applications on plantations in Latin America (Chávez and Araya, 2001).

Nonetheless, despite years of research on plant parasitic nematodes that attack *Musa* spp., a direct relationship between the number of nematodes in roots or the % of necrotic roots and certain levels of yield losses has not been established for any particular banana cultivar nor for any particular nematode species. In addition to the multitude of different cultivars and species that would come into play, not to mention pathotypes of certain nematodes with variable levels of aggressiveness towards banana plants, levels of nematode resistance and tolerance to nematodes also differ from cultivar to cultivar. Environmental factors such as flooding, drought, and particularly temperature in relation to altitude, as well as plant density and agronomic practices, such as mulching, can also affect the health of banana plants in general, and subsequently their tolerance of and resistance to nematodes (Gaidashova et al., 2009).

Since even establishing a clear relationship between the presence and density of nematodes in roots and levels of root necrosis has at times been difficult, with positive plant growth conditions at times masking the negative effects of nematodes (Gaidashova et al., 2009), establishing a clear relationship between levels of yield loss and nematodes has never been a research priority and no such calculations have been published. While it is not disputed that nematodes cause both a decline in plant health and productivity – with weaker plants and root systems, longer production cycles and smaller fruit bunches all being cited as effects of nematode infestations, no clear equations have been published or proposed to quantify this relationship.

Insect pests

Among the various insects that directly attack *Musa* spp., the Banana Weevil, *Cosmopolites sordidus*, is most destructive, particularly in banana producing areas of Africa. This insect has been intensively studied over the years, and many attempts have been made to relate levels of field infestation and corm damage to yield losses, particularly by the group of researchers working with Clifford S. Gold (Gold et al., 1994; Rukazambuga et al., 1998; Kiggundu et al., 2003; Gold et al., 2004; Gold et al., 2005). Among the yield losses the group associates with *C. sordidus* damage are mat disappearance, plant loss and reductions in bunch weight.

Rukazambuga et al. (1998) calculated yield losses in East African Highland Bananas (*Musa* AAA-EAHB cv. 'Atwalira') to *C. sordidus* in field trials in Uganda. They concluded that the banana weevil is the leading cause of banana decline and even the disappearance of banana from parts of central Uganda. After having to slightly modify their experimental design (weevils invaded designated "weevil exclusion zones"), they determined that damage to the central cylinder (CC) (that is, inner section of a root, vascular cylinder including xylem and phloem bundles) had the most important effect on yields, and that high levels of damage in the plant crop will have negative effects on ratoon plants (smaller plants and bunches – that is, no recovery observed). Yield losses increased from 5% in the plant crop to 44% in the third ratoon, with yield losses attributed to both high levels of plant loss (up to 29% in the 3rd ratoon) and reductions in bunch weight. In another study by the same group, Gold et al. (2004) found that yield loss averaged 42% during the last four years of a 7-year yield loss trial.

In their review of assessment methods for evaluating damage *C. sordidus* on EAHB, Gold et al. (2005) concluded that damage estimates on the corm periphery are not useful parameters for assessing pest status of *C. sordidus*. They go on to state that internal damage revealed in cross sections of the central cylinder is the best of the available damage predictors for assessing *C. sordidus* pest status, though even this method was not very reliable for assessing yield losses, as the relationships between the different damage parameters, plant size and bunch weight were weak.

Nonetheless, Rukazambuga et al. (1998) estimated yield losses by comparing plants with high levels of *C. sordidus* damage with the controls (that is, plants with little or no weevil damage), using data collected from four generations of bananas (plant crop and 3 ratoon crops). Damage to the CC was assessed for each plant after harvest and plants of each crop cycle were grouped into damage categories. Yield losses were calculated by taking into account not only actual bunch weights, but also lost plants.

Though not explicitly named *expected yield* (Y_{exp}) in Rukazambuga et al. (1998), an *expected yield* was calculated by establishing the average bunch weight of plants in the lowest weevil damage category (0-5% weevil damage to CC) and multiplying this by the number of plants initially planted on the plot, to end up with an average expected yield of X kg ha^{-1} (e.g., 500 plants ha^{-1} x 10 kg bunch^{-1} = expected yield of 5000 kg ha^{-1}). Average bunch weights for each damage category were then also calculated. Yield losses were calculated by subtracting the actual yield (Y_{act}: product of the number of plants in each damage category multiplied by the average bunch weight in that category) from the expected yield (Y_{exp}). For example: 500 plants are planted on a 1 ha plot. At the end of the harvest of the plant crop:

(i) 100 plants: low damage and 10 kg bunch^{-1}
(ii) 100 plants: moderate damage and 8 kg bunch^{-1}
(iii) 100 plants: heavy damage and 6 kg bunch^{-1}
(iv) 100 plants: very heavy damage and 4 kg bunch^{-1}
(v) 100 plants: dead / failed to produce a bunch

The expected yield is 500 plants ha^{-1} × 10 kg bunch^{-1} = 5000 kg ha^{-1}, while the actual yield is:

$(100\times10) + (100 \times 8) + (100 \text{ x } 6) + (100\times4) + (100 \times 0)$
$= 1000 + 800 + 600 + 400 + 0$
$= 2800$ kg ha^{-1}

Yield gap is therefore: $Y_{exp} - Y_{act} = 5000 - 2800 = 2200$ kg ha^{-1} or 44%.

Though this method allows researchers to estimate yield losses due to banana weevil attack, the exhaustive monitoring necessary to calculate yield losses in this manner is not particularly adoptable to assessing yield losses or gaps under survey conditions, over large areas, and on fields with a patchy planting history.

Identifying yield gaps in Musa spp. using models to simulate yields

Haverkort and Struik (2015) defined the potential yield of potato as the theoretical yield that can be calculated or modelled for a certain cultivar grown in a certain environment without any limiting or reducing factor being present. In order to use simulated yields to calculate yield gaps for bananas, similar models would have to be developed and validated for different banana cultivars, however, as noted by Tixier at al. (2004):

"Banana crops represent a collection of individual plants that vegetatively propagate at their own rhythm, with stabilised but unsynchronised production of inflorescences over time. Such agrosystems cannot be simulated with existing crop models due to the unsynchronized behavior of individual plants."

Due to this problem, Tixier at al. (2004) first developed a model (SIMBA-POP) that can be used to predict harvest periods over cropping cycles and the dates of harvest peaks ($R^2 = 0.99$) and to simulate or compare new population management decision criteria, based on the cohort population concept, but not actual yields. They calibrated and validated the SIMBA-POP model with field data from the French West Indies (Guadeloupe and Martinique) for *Musa* AAA group, cv. Cavendish Grande Naine. The same team of researchers later developed various sub-modules for the SIMBA model to simulate additional variables, such as yield in Mg ha^{-1} y^{-1} (SIMBA-GROW; $R^2 = 0.55$; Tixier et al., 2008), weekly nematode populations (SIMBA-NEM; Tixier et al. 2006), weekly water potential (SIMBA-WAT; Tixier, unpublished data), pesticide risk rank (Rpest; R2 = 0.92; Tixier et al., 2007) and nitrogen dynamics (SIMBA-N; Dorel et al., 2008). SIMBA and all its modules run at a weekly time-step at the field scale. Unfortunately, no actual yields are presented in the paper, nor are they compared to actual yields of commercial plantations. While no yield gaps were calculated, such a model could help in identifying particular contributors to yield gaps in specific environments.

Chaves et al. (2009) published an article entitled "Modeling plantain (*Musa* AAB Simmonds) potential yield", but unfortunately did not present any yield data in their paper, except to say that the average plantain yields in traditional mixed systems are about 10 t ha^{-1}. They concentrated on modelling the yields of the plant and first ratoon crops, but state that their model could be applied to previously existing plantations or to second or third production cycles; the complexity they imply is out of the scope of their work. However, they do note that the design of their model permits the incorporation of water, nutrient and pest limitations, though none of these are included in the presented model.

Due to the problematic nature of *Musa* crops and their often poorly defined yields and disparate harvest times, modelling these crops is particularly challenging, and cultivar specific crop models for bananas have not yet been developed. This may be in part because a lot of information is available for some cultivars (Cavendish group), while much less is available on others. However, there should be enough information already available in the banana research community to populate a model which should also include data on climate and soil (abiotic conditions, if not necessarily constraints). These data could be fed into one of the already existing models to help make the models more versatile and adaptable to different cultivars and agroecologies.

How best to calculate yield gaps

In their review, van Ittersum et al. (2013) emphasize the need for accurate agronomic and current yield data together with calibrated and validated crop models and up-scaling methods to larger geographical units. The protocol, including the effects on Yg of uncertainties in weather, soil, cropping system management and crop growth simulation models, remains to be tested and refined, a process which is currently undertaken in the Global Yield Gap Atlas project (www.yieldgap.org) (van Ittersum et al., 2013).

In 2012, the first phase of the Global Yield Gap and Water Productivity Atlas (Global Yield Gap and Water Productivity Atlas, 2020; www.yieldgap.org) was developed and continues to expand. This platform "provides robust estimates of untapped crop production potential on existing farmland based on current climate and available soil and water resources", and proposes a "standard protocol for assessing yield potential (Yp), water-limited yield potential (Yw), yield gaps (Yg) and water productivity (WP)".

For fully irrigated crops, the yield potential is defined as the yield of a crop cultivar when grown with water and nutrients non-limiting and biotic stress effectively controlled. Therefore, crop growth is determined by solar radiation, temperature, atmospheric CO_2 concentration, and genetic characteristics, but, in theory, not dependent on soil characteristics. For rain-fed crops, water-limited yield potential (Yw) is similar to Yp, but crop growth is also limited by water supply, and hence influenced by soil type and field topography. It is the most relevant benchmark for rain-fed crops.

The protocol, which relies on the collaboration of agronomists with knowledge of production systems, soils, and climate governing crop performance in their countries, is applied for all crops and countries based on best available data, robust crop simulation models, and a bottom-up approach to upscale results from location to region and country.

Though the first phase of the project (2012-2015) focused on cereal crops, the crop list has extended to soybean, sugarcane and potato, and the GYGA aspires for global coverage of yield gaps for all major food crops and countries that produce them. To date, data on potato are only available for Jordan and Tunisia, which reveal a 32 and 34% yield gap in irrigated potato, respectively. Both potential and actual irrigated potato yields are higher in Jordan (47.5 and 32.2 t ha^{-1}, respectively) than in Tunisia (27.8 and 18.3 t ha^{-1}, respectively). All data on yield gaps, including information of data collection site (station), climate zone and crop and type of production system (rainfed or irrigated) can be downloaded from the site (www.yieldgap.org). Data on potential yields, actual yields and resulting yield gaps are presented for each station and averaged for each country.

This type of international cooperation is very encouraging, though the actual data that have been made available, particularly for RTB crops are still very scarce. Crop models based on solid data appear to be the most promising means of calculating attainable yields for RTB crops. This type of information can be collected from breeding programs, where they exist, and such data are made available. However, the necessary agronomic data are not available for most cultivars – especially landraces – which are not necessarily included in formal breeding programs. For these cultivars, data from specific field trials or surveys will be required to calculate potential yields.

Once potential yields can be estimated, actual yields can be collected, along with supporting data to assess individual contributing factors to yield gaps. Boundary line analyses appear very interesting in this regards, as the author who used this method (Fremont et al., 2009; Wairegi et al., 2010) on RTB crops seemed to have been able to generate a lot of information out of their results. However, the method in not very intuitive to follow, and requires a lot of computing and statistical skills to correctly interpret the results.

Nonetheless, before starting fieldwork to assess yield gaps and identify various contributing factors, it is necessary to clearly define the yield – simply weighing bunches is not sufficient, irrespective of the number of bunches collected, as a yield is calculated for an area, and, in the case of perennial crops like *Musa* spp., also for a specific amount of time. Ideally, a comparison should be made between plants of the same cultivar, of a similar age and development stage (plant crop, ratoon), under control and farmer field conditions – and all this over a certain harvest period and for a clearly defined area.

Bunch weight is not equal to yield. Particularly over time, when individual banana mats die, yields on a plot can drastically decrease, while individual bunch weights may increase (e.g., due to less competition between surviving banana plants for limited resources such as water, etc.). Nonetheless, a basis for comparison has to be made, though this depends on the aims of the work.

To gain a general idea of what portion of yields are lost to particular diseases and pests within a certain area or at specific altitude bands, first potential yields have to be estimated. This could be done either by using a model to estimate potential yields (where available) or identifying “ideal plots", where the plants are under no biotic stress, and abiotic stresses (water and nutrient availability, etc.) are taken into account. After surveying and collecting data on actual yields within those same areas, yield losses can be calculated – but strict monitoring is necessary to allow for this, as assessing yields for bananas cannot be done with a single farm visit.

As the relationships between nematode and weevil damage and yield losses are unfortunately weak, estimating yield gaps attributable to these pests based on visual assessments (% damage to CC or roots) is not as straightforward as hoped for. Nonetheless, these methods can give an indication of what factors are contributing to yield losses, and actual yield losses observed can then be “traced back” to these pests. This study could in fact actually help to better define the portion of yield loss that can be attributed to the different diseases and pests, and identify tolerant and or resistant cultivars, and agronomic practices that negate the detrimental effects of these biotic stresses – all depending on the type and quality of data collected.

Yield gaps due to banana diseases: a case study in western Burundi using yield gap assessment methods discussed in the literature review sections

This part of the paper describes a concrete field study that assessed yield levels and gaps due to selected biotic (pest and disease) constraints in a banana production landscape in Western Burundi.

MATERIALS AND METHODS

Small-scale farmers in western Burundi grow mixtures of various banana cultivars, with the dominant cultivars being ‘Igisahira’ and ‘Indarama’ (both East African Highland Banana (EAH); cooking type; *Musa* AAA genome), ‘Km5’ a AAA genome dessert banana, ‘Pisang Awak’ (ABB, beer) and to a lesser extent ‘FHIA 17’ (a tetraploid AAAA hybrid, multipurpose). Bunch weight data were collected on apparently healthy plants (that is, plants that showed no aboveground disease symptoms) in over 270 farms (with each farm having at least 50 banana mats) across 3 altitude bands (800-1,200 m asl, 1,201-1,600 m, 1,601-2,200 m). Zoning by altitude was

Table 1. Plant disease incidence (%) for Fusarium wilt, Xanthomonas wilt and banana bunchy top disease by altitude band.

Altitude band (masl*)	Fusarium wilt	Xanthomonas wilt	Banana bunchy top disease
800-1,200	0.012	0.022	0.027
1,201-1,600	0.018	0.021	0.027
1,601-2,200	0.003	0.053	0.026
Fpr	0.032	0.054	0.989
Lsd	0.008	0.024	0.020
Cv%	103	110	104

*meter above sea level. Plant disease incidence was calculated as the number of diseased plants over the total number of plants in an assessed field.

done to reduce within zone differences between farms. At least 10 disease-free 'Igisahira' 'Indarama', 'Km5' and 'FHIA 17' plants were assessed across various fields per altitude band. In addition, for at least 50 apparently healthy plants per cultivar, nematode root necrosis % (using the method described by Speijer and De Waele, 1997) and weevil larvae corm damage (Gold et al., 1994) were assessed. Xanthomonas wilt of banana (XW), banana bunchy top disease (BBTD) and Fusarium wilt plant incidences (that is, number of infected plants over total number of plants in an assessed field) were assessed at 35 farms per disease and per altitude band. These three banana diseases cause a total loss of the affected plants and yield gaps due to them were postulated to be proportional to plant disease incidence levels. Yield gaps due to banana pests were calculated using the boundary line analysis. The boundary line analysis procedures and concepts described by Fremont et al. (2009) and Wairegi et al. (2010) and as described in the sections above were used with a slight modification as described in the steps as follows:

(i) The most influential yield outliers were identified and dropped with the help of boxplots.
(ii) Pearson correlation analysis was then used to identify the relationship between the bunch weight and the biophysical constraints (that is, corm weevil damage and nematode root necrosis) for the different banana cultivars. Constraints either had a positive or negative influence on the bunch weight, and bunch weights were sorted, respectively, in an ascending or descending order.
(iii) This was followed by determination of the yield due to each biophysical factor (Y_{bf}). Y_{bf} is the maximum yield predicted by the boundary line given the biophysical constraint.
(iv) Boundary lines were then fitted on the graphs, assuming a nonlinear relationship between bunch weight due to a factor and the corresponding factor values. This was followed by adding the boundary line obtained from the predicted yield due to each corresponding biophysical factor in the model.

The yield gap proportions were calculated as the difference between the attainable yield (Y_{att}) and Y_{bf} (Wairegi et al., 2010). Y_{att} was the highest bunch weight measured on farmers' fields. Where yield gap data was skewed (that is, high kurtosis > 1), median was presented instead of the mean. The statistical analyses and data visualization were carried out using R-statistical software (R Core Team, 2018) and the ggplot2 package (Wickham, 2016).

RESULTS AND DISCUSSION

Plant incidence values for Xanthomonas wilt of banana (XW), banana bunchy top disease (BBTD) and Fusarium wilt were low and below 0.2 % at all altitude bands (Table 1). It has to be noted that plant incidence levels presented in Table 1 reflect levels at one point in time and provide a quick impression of pathogen impact. Cumulative plant incidence values over a year or years or crop cycle would be far higher. For example, large numbers of plants affected by XW had been removed in the years, months and weeks prior to the survey. Hence, percentage XW plant incidence as measured during the surveys does not reflect the overall severe damage done over time by this pathogen. A susceptible plant affected by XW, BBTD or Fusarium wilt does not yield an edible bunch. As such any plant affected by any of the three diseases can be discarded from yield calculations, and thus the incidence of these diseases can be equated to percentage yield loss.

Gold et al. (1999) reported nematode and weevil numbers and damage to build up gradually, with often cross-generation effects, making it difficult to link yield level with corm damage levels for a specific plant in a perennial banana mat. Wairegi et al. (2010) however, using the boundary line method, has been able to compute yield gaps due to damage caused by nematodes and weevils.

In the current study, nematode root damage effects were most profound on the yield of 'FHIA17' and moderate for the east African highland cultivars 'Indarama' and 'Igisahira' (Figure 1). 'FHIA17' is susceptible to the burrowing nematode *R. similis* (Viaene et al., 1997; Moens et al., 2005), while all highland banana types have been reported to be susceptible to nematodes (Speijer et al., 1999; Ssango et al., 2004). No nematode effects were in contrast observed on the yield of 'Km5' which is a robust dessert banana (*Musa* AAA).

Weevil damage was generally low across all the four cultivars sampled. Yields of the East African highland bananas (EAHB) declined more severely with an increasing level of weevil corm damage. The EAHB are highly susceptible to banana weevils, with more severe losses reported at the low humid altitude zones of East and Central Africa (Gold et al., 1999; Kiggundu et al.,

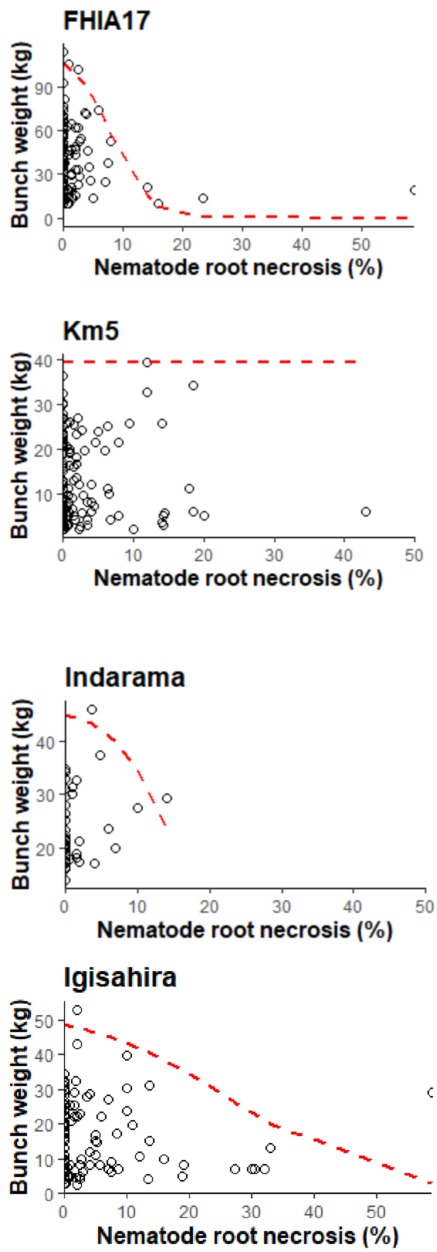

Figure 1. Relationships between banana yield and both root necrosis percentage and corm weevil damage percentage. The dotted lines represent the boundary lines, obtained through a boundary line analysis, whereas the points represent the observed bunch weights of assessed plants. Graphs are presented for four banana cultivars (FHIA17, Km5, Indarama and Igisahira) that dominate at the studied field sites.

2003). Weevils had minor to moderate effects on the performance of, respectively, ‘Km5’ and ‘FHIA17’. These two cultivars have been reported to be tolerant to banana weevils (Nowakunda et al., 2000; Kiggundu et al., 2003).

The percentage mean yield gaps due to nematode root necrosis were high in ‘Indarama’ (16.3%), ‘FHIA 17’ (12.7%) and ‘Igisahira’ (11.6%) while low in ‘Km5’ (1.8%) (Figure 2). Yield gaps due to the banana corm weevil damage were high for ‘FHIA17’ (10.4%), ‘Igisahira’ (6.7%) and low in ‘Indarama’ (3.3%) and ‘Km5’ (1.2%). Wairegi et al. (2010) also reported significant yield gaps due to nematodes and weevil larva, but only focused on east African highland banana cultivars grown in farmer’s fields. Pest constraints (nematodes and weevils) were reported to be particularly important in Central Uganda (1,100-1,300 masl), but not in South- and South-western

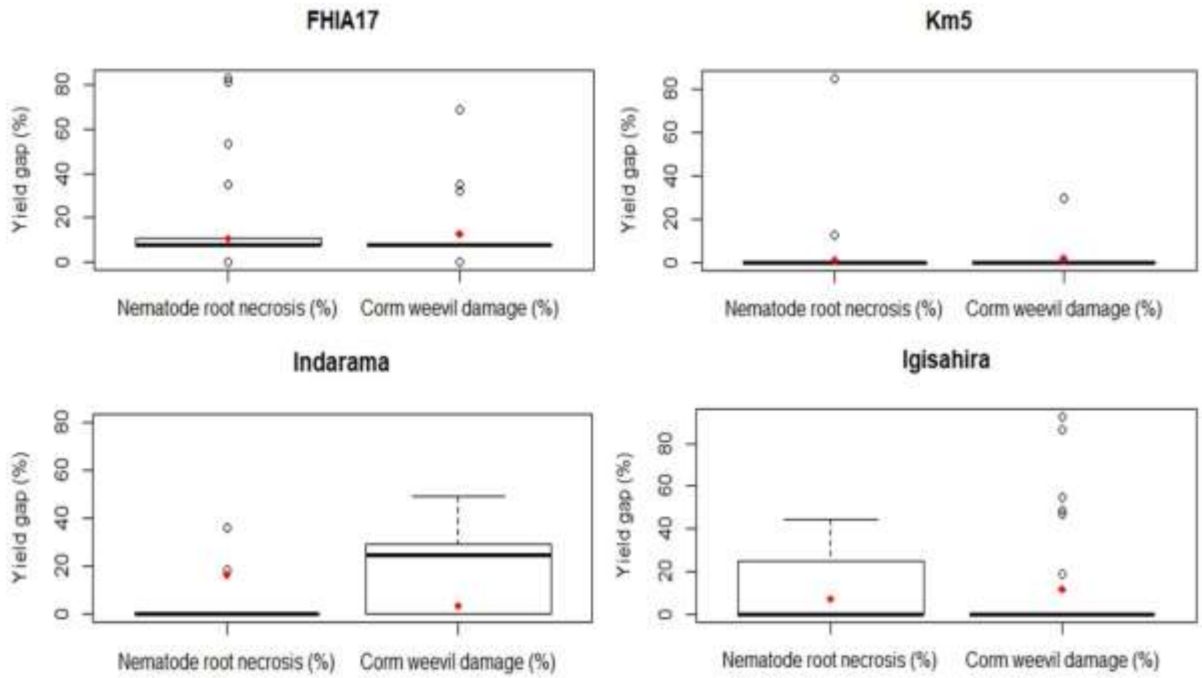

Figure 2. Percentage yield gaps due to nematode root necrosis and corm weevil damage, expressed as the percentage of the attainable bunch weights. The solid bold lines across boxes are medians. The red points represent the mean percentage bunch weight loss by each factor. The boxes represent the interquartile range (25–75th percentile), circles outside the central box represent outliers by between 1.5 and 3 times the interquartile range, while bars represent the smallest and largest observations which are not outliers. Graphs are presented for four banana cultivars (FHIA17, Km5, Indarama and Igisahira) that dominate at the studied field sites.

Uganda which has a higher altitude (1,300-1,800 masl). In Central Uganda, a yield gap median of 10.3 and 5.5% was, respectively, found for nematode and weevil damage effect (Wairegi et al., 2010).

The 'FHIA17' plants had the highest bunch weights compared to the highland AAA-EAH type bananas and 'Km5' (Figures 1 and 3). Bunch weights collected from healthy looking plants varied greatly for the 4 assessed cultivars, and at all altitudes (Figure 3). As these plants were all disease-free, it can be assumed that the yield variation is mainly due to variations in abiotic factors (e.g., soil fertility, level of input use, altitude or temperature effects) and possibly due to inter-plant competition within mats.

A large variation in bunch weights and thus yield gaps for bunches harvested from disease-free plants for all four cultivars was observed across the three altitude bands combined (Figure 4). The boundary lines show severe declines in yields at the upper altitudes, with more profound effects observed in 'FHIA17' and 'Indarama' (Figure 3). This is supported by field observations that show increasingly poor banana plant growth and yield at altitudes above 1900-2,000 masl (Rubaihayo and Gold, 1993; Karamura et al., 1998; Sivirihauma et al., 2016). Irrespective of the altitude bands, higher yield gaps were observed for the 'Igisahira', 'Km5', 'FHIA17' and least for 'Indarama' plants with gaps of 26.9, 22.8, 22.8 and 8.9%, respectively (Figure 4). Interplant competition often results in suboptimal bunch sizes/weights. The presence in the study sites of small and large mats with varying levels of interplant competition might have contributed to the observed wide range in bunch weights for the various cultivars. For 'Km5' specifically, plants tended to produce a lot of suckers/lateral shoots thus increasing competition for resources, ultimately resulting in smaller bunches.

CONCLUSION AND RECOMMENDATIONS

Banana bunch harvests and weights vary significantly at production zones in western Burundi due to a multitude of biotic constraints. The often sub-optimal, medium and small bunch sizes found on visibly healthy mats indicate that significant improvements in bunch weights could also be attained through the application of agronomic/field management practices that enhance soil fertility, soil moisture content and/or soil health. Efforts to improve productivity should be reappraised and be geared towards combining approaches to identify and overcome the most important constraints simultaneously, covering both agronomy/enhanced field/soil management and pests and diseases control. Various integrated pest

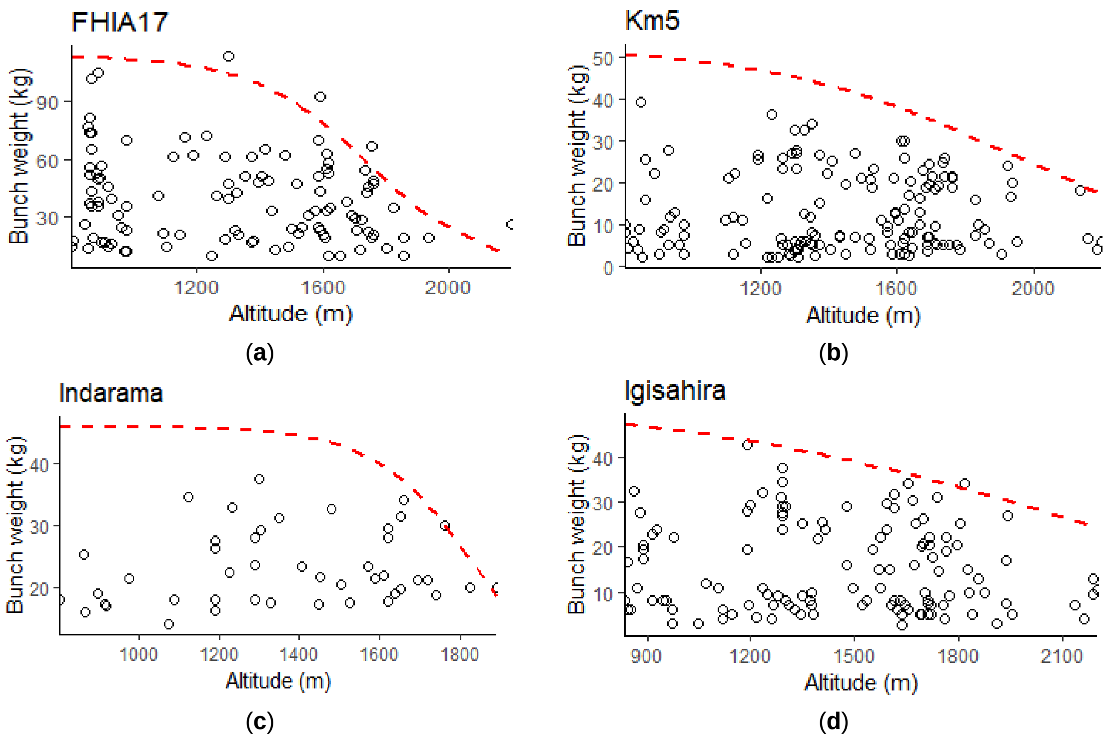

Figure 3. Relationships between banana yield and altitude. The dotted lines represent the boundary lines, obtained through a boundary line analysis, whereas the points represent the observed bunch weights of assessed apparently healthy plants. Graphs are presented for four banana cultivars (FHIA17, Km5, Indarama and Igisahira) that dominate at the studied field sites.

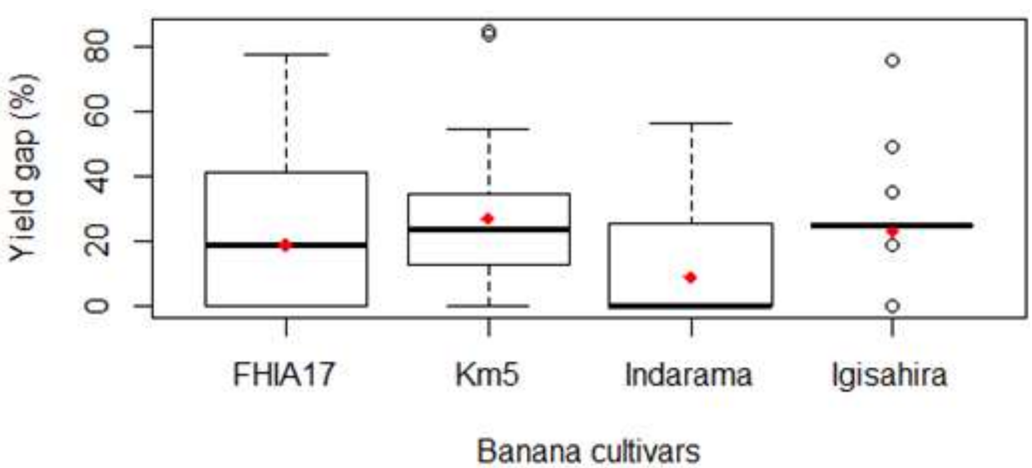

Figure 4. Percentage bunch weight loss or yield gap for 4 cultivars (FHIA17, Km5, Indarama and Igisahira) across the three altitude bands (altitude ranges from 800 to 2,200 masl) in western Burundi. Data were collected on disease-free plants.

management/disease control options are available ranging from tolerant or resistant germplasm to cultural control options. In addition, effective Integrated Soil Fertility Management/agronomic options include intercropping with N-fixing legumes, application of mulch, integration of field boundary bands of fast-growing shrubs/grasses and the integration of small ruminants. Simple and robust methods for estimating yield gaps due to pests and diseases, and abiotic constraints can inform/guide on the need to apply agronomic and/or disease control efforts. Rigorous knowledge communication of gained insights to extension agencies and farmer communities should also be high on the research for development agenda. In the current study, the assessment of the yield gaps due to diseases was challenging as farmers continuously uproot or cut down infected plants. Field monitoring, with the involvement of farmers, over time will be necessary for a more accurate assessment of yield gaps due to diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Quantitative trait loci (QTL) mapping of resistance to coffee berry disease (*Colletotrichum kahawae* Waller & Bridge) in *Coffea arabica* L. variety Rume Sudan

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Coffee Berry Disease (CBD) is a major constraint that limits *Coffea arabica* production, whose resistance is governed by three genes, T, R that are dominant and recessive k in varieties Hibrido de Timor (HDT), Rume Sudan (RS) and K7 respectively. This study identified the genomic region occupied by R-gene using F_2 genotypes from varieties RS and SL28; and Single Nucleotide Polymorphic (SNP) markers obtained through Genotyping by Sequencing. Redundant markers were removed and 699 markers obtained for linkage mapping and quantitative trait loci (QTL) analysis. The Linkage map spread over 5525.39 cM across eleven coffee chromosomes (Chr). The QTL was analyzed by both Interval Mapping (IM) and Inclusive Composite Interval Mapping (ICIM) using SNP markers and CBD resistance mean scores of the F_2 genotypes and their parents. Three QTLs, qCBD 1-1 in Chr 1, qCBD 2-1 and qCBD 2-2 in Chr 2 were significantly associated with CBD resistance, detected by both IM and ICIM at LOD ≥ 2.5 (P≤0.05). Two flanking markers that were closer to the three QTLs; 100025973|F|0-59:T>C-59:T>C at a distance of 3 centi Morgans (cM) from qCBD 1-1 and 100034991|F|0-44:C>T-44:C>T, that was flanking in both qCBD 2-1 and qCBD 2-2 at 12.5 cM, whose SNPs were significant (P≤0.05), are recommended for validation and use in marker-assisted breeding.

Key words: Coffee berry disease, linkage map, quantitative trait loci, genotyping by sequencing, single nucleotide polymorphism, SL 28, R-gene.

INTRODUCTION

Coffee Berry Disease (CBD) is caused by the fungal pathogen *Colletotrichum kahawae* Waller & Bridge (Waller et al., 1993) and is a major constraint to *Coffea arabica* L. production in African countries with a possibility of occurrence in other coffee-growing countries in the world (Van Der Vossen et al., 2015). CBD epidemics destroy 50-80% of susceptible Arabica coffee varieties during prolonged wet and cool weather conditions (Hindorf and Omondi, 2011) if no control is applied. The preventive control of CBD using programmed fungicide sprays increases production cost by 30-40% and contributes to environmental pollution (Gichuru et al., 2008); and therefore, the need for resistant varieties as the best alternative approach in the management of this disease.

C. arabica L. is a simple tetraploid (2n = 4x = 44) and the only tetraploid species of coffee, formed out of two diploids species, *Coffea canephora* and *Coffea eugenioides* (Lashermes et al., 2011). This species is genetically less diverse in comparison to its parental diploid species (Baruah et al., 2003), a situation that has been associated with its susceptibility to diseases (Prakash et al., 2002) and hinders molecular breeding tools development for the breeding process (Sant'Ana et al., 2018). The first case of CBD was reported in 1922, in coffee plantations on the slope of Mt Elgon in Western Kenya (McDonald, 1926), from where it spread to other Arabica coffee producing countries in Africa (Hindorf and Omondi, 2011). Studies on the source and selection for resistance to CBD date back to 1974 (Robinson, 1976) but significant breakthrough on selection was on the discovery of the hypocotyl inoculation test on six-week-old seedlings. This revealed that the mechanism of CBD resistance in the field conditions was similar to the reaction of CBD inoculum in the hypocotyl of a six-week-old seedling (Van Der Vossen et al., 1976).

Initial studies on the inheritance of CBD resistance indicated a continuous variation in a naturally occurring heterozygous population, leading to the belief that CBD resistance is polygenic (Van Der Graaff, 1978). However, this belief was dismissed by the study of Van der Vossen and Walyaro (1980). The study revealed that there are only three genes that confer resistance to CBD in *C. arabica*. These genes are the R-gene in the variety Rume Sudan, T-gene in HDT and a recessive k-gene found in both K7 and Rume Sudan. The T and R genes are dominant while the k gene is recessive and only confers partial resistance to CBD in a homozygous state. The R-locus has multiple alleles (R_1R_1) in Rume Sudan and R_2R_2 that are less effective in Pretoria (Van der Vossen and Walyaro, 1980; Hindorf and Omondi, 2011).

The development of a new coffee variety takes more than 25 years due to the perennial nature of this crop. This period can be reduced drastically by using marker-assisted selection (MAS) and hence the need to identify markers associated with the traits of interest in coffee (Moncada et al., 2016). Genetic resistance to CBD has been characterized by various studies in Kenya. The locus for the T-gene was mapped by Agwanda et al. (1997) using Random Amplified Polymorphic DNA (RAPD) markers. The same locus was identified by Gichuru et al. (2008) using Simple Sequence Repeats (SSR) markers and Amplified Fragment Length Polymorphisms (AFLP) markers and christened it as *Ck-1*. Similarly, detection and mapping of the R- gene in Rume Sudan is desirable and believed to be realized through QTL mapping using Genotyping-by-Sequencing (GBS) based single nucleotide polymorphism (SNP) markers.

GBS is a powerful, cost-effective method for identifying single-nucleotide polymorphisms (SNPs) on a whole-genome scale. This technique encompasses reduced representation genome sequencing based on partial restriction enzyme digestion, usually with a methylation-sensitive restriction enzyme, followed by barcoded adaptor ligation and next-generation sequencing of highly multiplexed samples, usually 96, samples per lane (Elshire et al., 2011; Vining et al., 2017). The Diversity Array Technology Pty Ltd (DArT, Canberra, ACT, Australia), was developed as a cost-effective sequence-independent ultra-high-throughput marker system. The DArT develops markers through a microarray hybridization method that produces a thousand of polymorphic loci in a single assay (Alam et al., 2018). Various studies have utilized DArT- GBS based SNP markers in coffee (Moncada et al., 2016; Garavito et al., 2016; Sant'Ana et al., 2018). The process of quantitative trait loci (QTL) mapping is useful in the detection of genes that could either have positives, negatives or both effects on the trait of interest. It helps in identifying genomic regions conveying disease resistance in bi-parental populations (Sant'Ana et al., 2018). This process leads to the identification of linked molecular markers that may be used for gene pyramiding in the breeding process for durable resistance to diseases (Kthiri et al., 2019). This study aims to identify the genetic region for R-gene that confers resistance to CBD in *Coffea arabica* variety Rume Sudan through bi-parental QTL mapping using GBS-based SNP markers.

MATERIALS AND METHODS

Phenotypic and population structure analysis of the mapping genotypes

The study materials comprised 108 *Coffea arabica* genotypes including 106 F_2 genotypes and their parents, Rume Sudan and SL28 maintained at Coffee Research Institute of the Kenya

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Agricultural and Livestock Research Organization (KALRO-CRI) in Ruiru, Kenya. Ruiru is located within the upper midland (UM2) at 1^0 06'S and 36^0 45'E and an altitude of 1620 m above sea level (Jaetzold et al., 2006). Rume Sudan is a *Coffea arabica* landrace (Scalabrin et al., 2020) with the R gene in the R locus that confers resistance to CBD (Van Der Vossen and Walyaro, 1980); while SL28 is a commercial Kenyan cultivar that is high yielding and of excellent cup quality but highly susceptible to CBD. The F_2 individuals were obtained by crossing the parents and subsequent selfing of their F_1 generation. The F_2 genotypes were classified their resistance to CBD based on their F_3 progenies using hypocotyl inoculation method on a standard scale of 1 – 12 of Van der Vossen et al. (1976). The population structure analysis of the study genotypes was carried out by Principal Component Analysis (PCA) based on marker data using the PCA-clustering functionality tool within the KDCompute plugin system (https://kdcompute.igss-africa.org/kdcompute/plugins).

Genotyping of SNP markers

Genomic DNA samples were extracted from fresh leaves of each of the 108 genotypes using a standard cetyltrimethylammonium Bromide (CTAB) protocol of Doyle and Doyle (1987). The quality and quantity of the DNA samples were evaluated through a Spectrophotometer (Specgene, UK; at 260/280 nanometers) and by running it through 0.8% agarose gel electrophoresis. The DNA concentration was adjusted to 50 ηg/µl. The genomic DNA samples were sent to Diversity Arrays Technology (DArT) Pty Ltd, in Canberra-Australia (http://www.diversityarrays.com) for sequencing and identification of SNP markers. The GBS was performed as described by Elshire et al. (2011). The genomic DNA was digested with methylation-sensitive restriction enzyme. This type of enzyme was chosen due to its partial sensitivity to DNA methylation, thus avoiding repetitive element regions and frequency of DNA cutting (Elshire et al., 2011; Brito et al., 2017). After digestion, the DNA was ligated to adapters and then combined into pools of 96 samples and amplified with primers compatible with the adapter sequences (Camacho et al., 2019). After polymerase chain reaction (PCR), the pooled products were purified and quantified for sequencing on the Illumina HiSeq 2500 flow cell (Illumina platform) (Elshire et al., 2011; Akohoue et al., 2020).

The SNP calling was carried out by DArT-soft14 algorithm within the KDCompute pipeline developed by Diversity Arrays Technology (http://www.kddart.org/kdcompute.html). In the primary pipeline, the FASTQ files were first processed to filter poor quality sequences to ensure that the assignments of the sequences to specific samples carried in the barcode split region were consistent and reliable (Barilli et al., 2018; Li et al., 2018; Nemli et al., 2017). The identical sequences were collapsed into FASTQ call files that were used in the secondary pipeline for DArT P/L's proprietary SNPs calling algorithms (DArT-soft14) pipeline in the processing of the sequence data (Barilli et al., 2018). Since open access genome assembly with reliable sorting of homologous sequences for *C. Arabica* is not yet available (Scalabrin et al., 2020), the filtered sequence reads were aligned against the *C. canephora* reference genome (http://coffee-genome.org/coffeacanephora) to determine the corresponding genomic positions.

The genetic linkage map construction

The high-density linkage map for the Rume Sudan x SL28 F_2 populations and their parents was carried out using QTL IciMapping version 4.2 (http://www.isbreeding.net/). Before the map was constructed, redundant SNP markers were removed using the BIN functionality tool, implemented within the QTL IciMapping software (Meng et al., 2015). The obtained markers after binning were used in the construction of genetic linkage maps using the MAP functionality tool within the software. The map function was by stepwise regression to select the most significant markers and a likelihood ratio test to calculate the logarithm of odds (LOD) scores for each marker by a criterion of > 2.5 LOD and a maximum distance of 30 cM between two loci. Three steps were involved in building a linkage map: grouping, ordering, and rippling. The grouping was carried out with a LOD score > 2.5 that was used to declare the linkage relationship between two markers such that markers with LOD higher than the threshold were grouped. The Recombination Counting and ORDering (RECORD) algorithm was used for ordering markers while rippling was by the sum of adjacent recombination fractions (SARF) to confirm the marker order (Meng et al., 2015; Sitonik et al., 2019, Awata et al., 2020). The recombination fraction between two linked loci was used to sort the markers with the Kosambi mapping function (Kosambi, 1943) in centi Morgans (cM).

Analysis of the QTL for resistance to CBD

The SNPs markers obtained from BIN together with the phenotypic scores of CBD infection of the F_2 genotypes that had strong parental relationships and their parents were used in QTL analysis using the QTL functionality of the QTL Ici-Mapping software v4.2 (Meng et al., 2015). The conventional Interval Mapping for additive QTL (IM-ADD) and inclusive composite interval mapping for additive QTL (ICIM-ADD) methods was adopted in QTL analysis. The QTL Ici-Mapping software carries out QTL analysis by incorporating additive genetic effects (Zhang et al., 2008; Meng et al., 2015). The LOD value of ≥ 2.5 with a window scan step of 1 cM and 1000 permutation test was used as the threshold to declare the significance of the QTLs defined at P≤0.05 (Kim and Reinke., 2019; Awata et al., 2020). Stepwise regression was adopted to determine the percentages of phenotypic variance explained (PVE%) by individual QTL, additive and dominance effects at LOD peaks (Awata et al., 2020). In the QTL naming, the letter "*q*" indicates QTL followed by the abbreviation of the trait name (CBD), the chromosome and lastly the marker position.

The markers that were closely linked with the significant QTLs were statistically tested using the scores of their alleles, where the alleles with significant differences (P≤0.05) by *t*-test were confirmed as significant SNP markers (Xiong et al., 2019; Tsai et al., 2020).

RESULTS

Phenotypic and SNP marker data analysis

The GBS based Diversity Arrays sequencing (DArTseq) generated 1635 SNPs markers from the F_2 genotypes and their parents. After filtering, 1170 SNP markers were anchored to the 11 coffee chromosomes that were utilized for further analysis in the study. The phenotypic data analysis is described in a recent study by Gimase et al. (2019). The PCA clustering analysis of 108 genotypes revealed three main clusters: A, B and C (Figure 1). The "A" genotypes clustered with SL 28, "B" with Rume Sudan while "C" was not grouped with any of the two parents.

The "C" cluster comprising 20 genotypes that portrayed a weak relationship with the rest eliminated to obtain

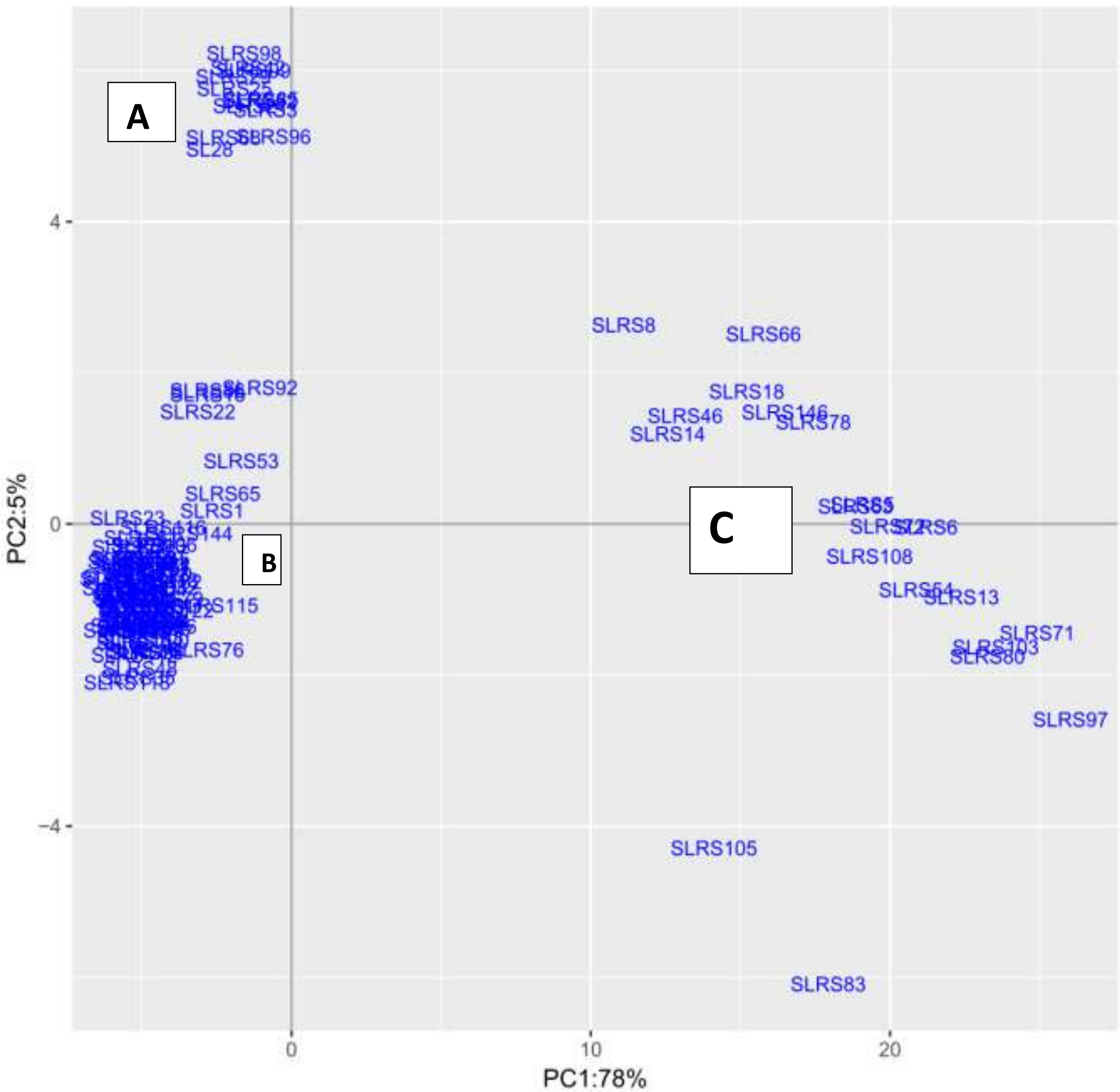


Figure 1. Population structure analysis on the relatedness of the 106 F_2 genotypes to their parents using PCA clustering.

88 genotypes that were used in QTL analysis. The first principal component (PC1) was the most important, which accounted for 78% of the total variation (Figure 1).

Genetic linkage map construction

After binning of the 1170 DArT-Seq SNP markers, 471 markers were highly correlated and therefore removed. A total of 699 high-quality SNP markers were obtained and used in the construction of the linkage map. The 699 SNP markers were spread over 5525.39 cM across eleven genetic linkage groups (LG), equivalent to coffee chromosomes with an average marker distance of 7.904 cM and a maximum interval size of 18.378 cM (Table 1). The highest genetic distance was recorded on chr 6 at 800.56 cM while the lowest was at chr 7 at 265.98 cM. The highest mean distance between two markers was

Table 1. Whole-genome genetic linkage maps for the Binned makers indicating the size and mean distances per chromosome.

Linkage group	No. of SNP markers	Size (cM)	Mean distance (cM)
Chr1	85	393.32	4.627
Chr2	44	773.31	17.575
Chr3	78	379.65	4.867
Chr4	132	781.33	5.919
Chr5	94	388.09	4.129
Chr6	80	800.56	10.007
Chr7	26	265.98	10.23
Chr8	56	383.95	6.856
Chr9	28	439.29	15.689
Chr10	29	532.99	18.379
Chr11	47	386.92	8.232
Whole Genome	699	5525.39	7.905

in chr 10 at 18.378cM while the lowest was in chr 5 at 4.128cM. The highest numbers of markers were on chr 4 (132) while the lowest number was on chr 7 (26) (Table 1).

QTL analysis

Significant QTLs for CBD resistance were detected by both the conventional Interval Mapping for additive QTL (IM-ADD) and inclusive composite interval mapping for additive QTL (ICIM-ADD) based on LOD threshold value ≥ 2.5 defined at P≤0.05. The IM-ADD detected a total of 19 QTLs at LOD ≥ 2.5 (Figure 2a) that accounted for 56.5% of the total phenotypic variation explained (PVE) by the model. Out of the 19 QTLs, only four QTLs had an admixture of negative additive and dominance effect (Table 2). The three were qCBD 1-1 in Chromosome 1 and qCBD 2-1, qCBD 2-2 in chromosome 2. Similar to IM-ADD, ICIM-ADD detected a total of 41 QTLs at LOD ≥2.5 (Figure 2b) that explained 65% of the total PVE. Out of the 41 QTLs, only five had an admixture of negative additive and dominance effects (Table 3). The individual percentage PVE by the QTLs ranged from 0.8-3.95% for IM and 0.7-2.1 for ICIM.

Three QTLs, qCBD 1-1, in Chr 1 and qCBD 2-1, qCBD 2-2 in Chr 2 were detected by both IM-ADD and ICIM-ADD with similar flanking markers/quantitative trait nucleotides(QTNs), where 100025973|F|0-59:T>C-59:T>C and 4421602|F|0-12:T>C-12:T>C were left and right flanking markers for qCBD 1-1 (Figure 3A). The SNP markers 4428977|F|0-55:C>A-55:C>A and 100034991|F|0-44:C>T-44:C>T were flanking as left and right marker in qCBD 2-1 while in qCBD 2-2, 100034991|F|0-44:C>T-44:C>T and 4426086|F|0-37:G>A-37:G>A were flanked as left and right marker respectively (Figure 3b). For the two QTLs in Chr 2, the SNP marker ID 100034991|F|0-44:C>T-44:C>T was flanking as either left or right in qCBD 2-1 and qCBD 2-2 respectively. The three QTLs accounted for 6.4% PVE and 3.4% PVE in IM and ICIM analysis respectively.

The left and right flanking marker for qCBD 1-1 (Figure 3a), was at a distance of 3 cM and 13.5 cM from the QTL in both IM and ICIM analysis (Tables 2 and 3) while in qCBD 2-1 and qCBD 2-2, the SNP marker 100034991|F|0-44:C>T-44:C>T was flanked as left and right marker respectively (Figure 3b), at a distance of 16.5 and 12.5 cM in IM and ICIM respectively while the other flanking markers were at a distance of 37.5 and 31.5 cM for both loci in IM and ICIM respectively (Tables 2 and 3). Two markers, 100025973|F|0-59:T>C-59:T>C and 100034991|F|0-44:C>T-44:C>T in chr 1 and 2 respectively, were very close to the three significant QTLs. The statistical *t*-test analysis of the two markers revealed significant differences (P≤0.05) in their allelic means (Table 4).

DISCUSSION

The phenotypic variation among the 106 F_2 genotypes with their parents and inheritance segregation ratio of the gene for resistance to CBD was reported in an earlier publication (Gimase et al., 2019). Evaluation of population structure on the relatedness of the 106 F_2 genotypes and their parents using the PCA revealed that a total of 20 F_2 genotypes were weakly related with the rest of the genotypes and were therefore excluded from QTL analysis. The analysis of population structure reduces type I error during association mapping (Camacho et al., 2019). The mapping population was confirmed as a suitable population for genetic mapping of a dominant gene-based and Chi-Square test based on 3:1 Mendelian ratio of segregation (Gimase et al., 2019).

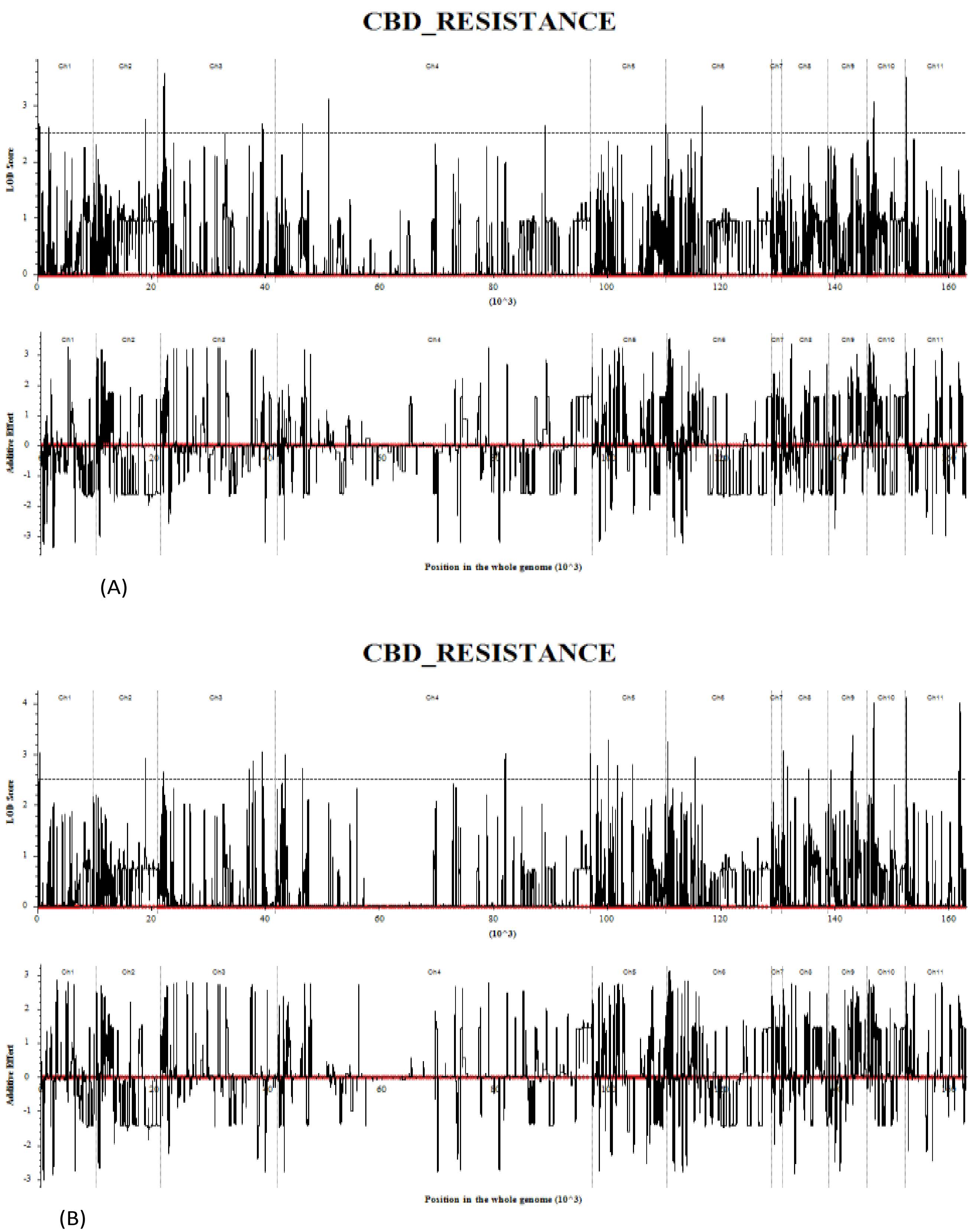


Figure 2. Whole-genome LOD profile and additive gene interaction effects mapping using IM and ICIM as (a) and (b) respectively. The SNP location is indicated as a red dot. Positive additive effects were related to the susceptible parent SL 28 and negative values were related to the resistant parent RS.

Table 2. The QTLs for CBD resistance detected by IM with negative additive and dominance gene interaction effects

QTLName	Chr	Postn (cM)	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom	Left CI	Right CI
qCBD 1-1	1	3	100025973|F|0-59:T>C-59:T>C	4421602|F|0-12:T>C-12:T>C	3.6948	1.1664	-1.173	-0.208	0	16.5
qCBD 2-1	2	9231	4428977|F|0-55:C>A-55:C>A	100034991|F|0-44:C>T-44:C>T	2.755	2.5918	-1.948	-1.396	9193.5	9247.5
qCBD 2-2	2	9280	100034991|F|0-44:C>T-44:C>T	4426086|F|0-37:G>A-37:G>A	2.755	2.6022	-1.957	-1.385	9263.5	9317.5

Table 3. The QTLs for CBD resistance detected by ICM with negative additive and dominance gene interaction effects.

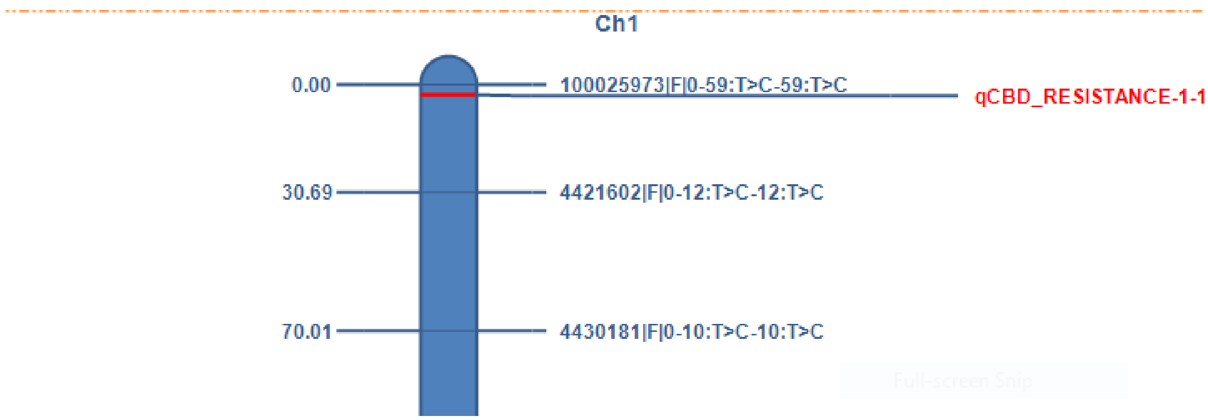
QTL name	Chr	Position	Left marker	Right marker	LOD	PVE (%)	Add	Dom	Left CI	Right CI
qCBD 1-1	1	3	100025973|F|0-59:T>C-59:T>C	4421602|F|0-12:T>C-12:T>C	3.695	0.768	-1.173	-0.208	0	16.5
qCBD 2-1	2	9274	100034991|F|0-44:C>T-44:C>T	4426086|F|0-37:G>A-37:G>A	2.915	1.313	-1.803	-1.447	9261.5	9305.5
qCBD 2-2	2	9237	4428977|F|0-55:C>A-55:C>A	100034991|F|0-44:C>T-44:C>T	2.915	1.311	-1.800	-1.453	9205.5	9249.5

Table 4. A t-test (P≤0.05) for two SNP markers that was closely associated with the three significant QTLs for resistance to CBD in Rume Sudan.

SNP marker ID	Chr	Allele 1	Means±SD	Allele 2	Means±Sd
100025973|F|0-59:T>C-59:T>C	1	T	0.83±0.02[a]	C	0.63±0.32[b]
100034991|F|0-44:C>T-44:C>T	2	C	0.83±0.07[b]	T	0.92±0.01[a]

This study generated a genetic linkage map from 699 SNP markers spreading over 5525.39 cM across eleven genetic linkage groups (LG), with an average marker distance of 7.904 cM at maximum interval size of 18.378 cM. Although saturated genetic linkage maps of *C. arabica* are useful for mapping resistance genes and identification of markers linked to those genes, they are not widely available due to low molecular polymorphism and the polyploidy nature of this species (Pestana et al., 2015). Despite this constraint, several studies attempted to generate these types of maps for Arabica coffee. Pestana et al. (2015) used fewer makers (111) to construct a relatively smaller linkage map with a total length of 976.8 cM. The LG size ranged from 18.4 cM (LG 12) to 234.6 cM (LG 1) with a distance between two adjacent markers varying from 0 cM to 29.4 cM, with the average distance of 9.9 cM; while the mean distance between markers within each linkage group varied from 5.3 cM (LG 5) to 20.1 cM (LG 10). Moncada et al. (2016) constructed a relatively advanced linkage map using 848 (both SSR and SNP) markers that represented all 22 *C. arabica* linkage groups with a total map length of 3840 cM. The average distance was 4.52 cM between markers and a maximum interval size of 35 cM, where the length of each linkage group ranged from 535.9 cM for LG 1 to 22.7 cM for LG 22 and the number of markers ranged from 129 markers on LG 1 to 8 markers on LG 22. The linkage map in this study equally forms a basis for future research studies on *C. arabica* since it consists of sequence-based SNPs that can be widely used by the research community (Moncada et al., 2016). Both conventional interval mapping (IM) and Inclusive composite interval mapping (ICIM) were used in this study for QTL analysis. The ICIM is an effective two-step statistical approach that allows separation of co-factor selection from the interval mapping process, to control the background

(a)



(b)

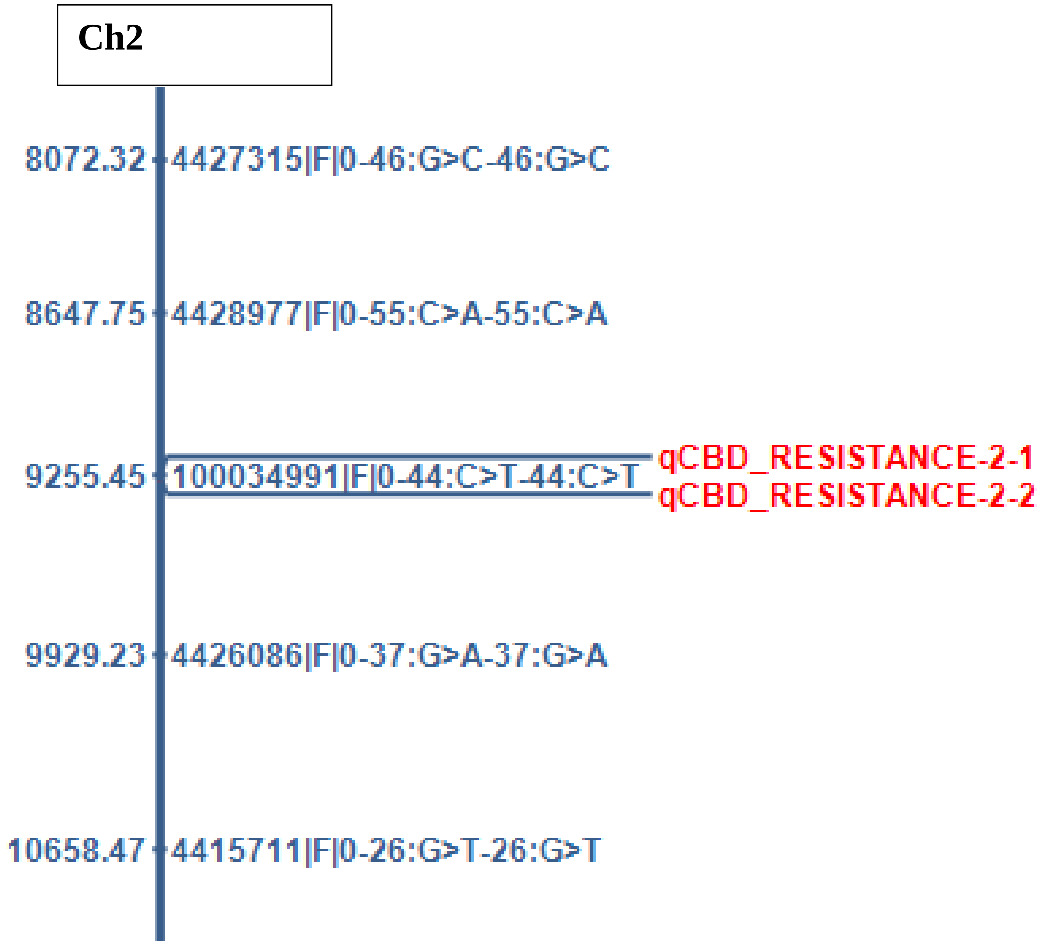


Figure 3. Linkage maps showing the positions of the three QTLs that were significantly detected by ICIM-ADD with a combination of both negative additive and dominance interaction effects in Chr 1 and 2 as (a) and (b) respectively.

effects and improve the mapping of QTL with additive effects (Horn et al., 2015; Awata et al 2020). ICIM has a proportion of lower False Discovery rate (FDR) than composite interval mapping (CIM).

Three QTLs, qCBD 1-1, qCBD 2-1 and qCBD 2-2 were detected by both IM and ICIM. The qCBD 1-1, detected by both IM-ADD and ICIM-ADD had a LOD score of 3.69 with similar flanking markers,100025973|F|0-59:T>C-59:T>C and 4421602|F|0-12:T>C-12:T>C as left and right flanking markers respectively. This locus was right at the beginning of coffee Chromosome 1, where the left flanking marker was at 0 cM; while the right marker was at 16.5 cM. The QTLs, qCBD 2-1 and qCBD 2-2 detected by ICIM in Chr 2 were similar to two QTLs, detected by IM, with same flanking markers at LOD score 2.8 and 2.9 in IM and ICIM respectively. The SNP marker, 100034991|F|0-44:C>T-44:C>T was flanked in both QTLs, as either left and right respectively. These QTLs were placed towards the end of the second linkage group. All the three QTLs had negative additive and dominance effects implying that the additive-by-additive and dominance-by-dominance interaction effects between the flanking markers for each QTL (Zhang et al., 2008) was acting against the trait that is, reducing CBD infection in the host genotypes (Horn et al., 2015; Kim and Reinke, 2019). Although the PVE% cumulatively by the QTLs was high, the individual score was low. This is attributed to the high number of the significant loci detected by the model (Curtolo et al., 2017) and the small size of the mapping population that was utilized in QTL analysis (Zhang et al., 2008).

The flanking markers for qCBD 2-1 and qCBD 2-2 revealed polymorphic occurrence (presence/ absence of allele) within the parental genotypes; therefore best suited to diagnostic marker design for MAS (Rouet et al., 2019). Abundance and polymorphic occurrence are some of the features for an ideal marker for MAS (Babu et al., 2004). Two SNP markers were closer to the three QTLs where the SNP marker 100025973|F|0-59:T>C-59:T>C was flanked as left marker for qCBD 1-1 at a distance of 3 cM; while 100034991|F|0-44:C>T-44:C>T was flanked in qCBD 2-1 and qCBD 2-2 as either left and right respectively at a distance of 12.5 cM in ICIM. The genetic variation between the alleles of this markers was significantly different (P≤0.05) based on *t*-test analysis, confirming further that the SNPs were significant. In a related study, Xiong et al. (2019) used a *t*-test to confirm significant SNP involved in resistance to low nitrogen traits in wheat mutant population; while Zhou et al. (2018) used a *t*-test to declare SNP significantly associated with plant height (P≤0.05) in maize.

Previous work by Van der Vossen and Walyaro (1980) on the inheritance of CBD resistance based on F_2 progenies of 11 *C. arabica* varieties with different levels of resistance revealed that the variety, Rume Sudan carries two genes for resistance to CBD. The dominant R- gene has multiple alleles occurring as R_1R_1 and recessive k gene. The study further reported that Rume Sudan transmits high levels of resistance to CBD that was indicated by a high negative General Combining Ability (GCA) effects. In this study, three QTLs significantly associated with CBD resistance, with a combination of both negative additive and dominance gene interactions against CBD infection. The three QTLs are synonymous to the R gene associated with resistance to CBD in *C. arabica* variety Rume Sudan as described in the previous studies.

Conclusion

Coffee Berry Disease remains a limiting factor in the production of Arabica coffee in Africa with the possibility of occurring in other Arabica coffee-growing countries globally. Breeding for resistance to diseases takes up to 30 years, a period that can be reduced significantly by Marker-Assisted selection (MAS). This study identified three genetic regions, one in coffee pseudo-chromosome 1 and two in pseudo-chromosome 2 that are associated with CBD resistance in *C. arabica* variety Rume Sudan. The study further revealed that two SNP markers were closer to the three QTLs where the SNP marker 100025973|F|0-59:T>C-59:T>C was flanking as left marker for qCBD 1-1 at a distance of 3 cM; while 100034991|F|0-44:C>T-44:C>T was flanking in qCBD 2-1 and qCBD 2-2 as either left and right marker respectively at a distance of 12.5 cM. The SNPs of these markers were significant following a *t*-test analysis (P≤0.05). These SNPs are likely to co-segregate with the three loci and are identified as candidate DNA markers for the R-gene conferring resistance to CBD in Rume Sudan and recommended for validation and adoption as diagnostic markers in marker-assisted breeding.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Intrapopulation phenotypic variation in Piartal (*Chenopodium quinoa* Willd.) from the Department of Boyacá, Colombia

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***Chenopodium quinoa* Willd., is an interesting plant with a great adaptation to adverse environmental factors and exceptional nutritional qualities. It shows great genetic variation, which organization remains poorly documented. In Boyacá, there are few studies on the morphological characterization of cultivated materials, and there is no certified planting material, resulting that the farmers are planting a mixture of materials. Qualitative and quantitative descriptors and principal component and cluster analyses were used to characterize the structure of the intra-population phenotypic variation in Piartal quinoa materials grown in the Department of Boyacá. It was observed that the first two components, CP1 and CP2, explained more than 70% of the total observed phenotypic variation, and there was a significant contribution from all variables to the two components, except those related to the lower leaves, where P2, P3 and P4 presented defoliation and DP (CP2 and P6). The cluster analysis showed that the individuals of the Piartal were grouped mainly by morphological characteristics associated with plant height, panicle length, pigmented axillae, and leaf characteristics. Results showed that the variance in morpho-phenological traits was concentrated at the intra-population, due the high variation at the inter-individual level. A more efficient selection process should be carried out to find "pure" varieties.**

Key words: Andean culture, genetic diversity, improvement, morphological descriptors.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is an annual, dicotyledonous species that belongs to the Chenopodiaceae family, cultivated from sea level to 4,000 m, with wide agroecological adaptation and to different types of soils. Native to South America, it continues to be cultivated in different regions of that

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continent, especially in countries such as Colombia, Chile, Bolivia, Ecuador and Peru, being recently introduced in Europe, North America, Asia and Africa (Zurita et al., 2014). It is estimated that more than 80% of its world production is concentrated in countries such as Peru, Bolivia and Ecuador (Zurita et al., 2014). It also occurs in the coastal areas of southern Chile and the Andean valleys of southern Colombia, more precisely in the department of Nariño, Cauca, Boyacá and Cundinamarca (Agronet, 2020), where it has currently had a great boost due to its agronomic potential and different benefits derive from the production, industrialization and commercialization of its products (Chura et al., 2019). It is considered a cereal with excellent nutritional properties, among which its high protein content stands out, since it has all the amino acids, trace elements and significant amounts of vitamins C, E (tocopherols) and B (B1, B2 and B3); along with important minerals (Ca, K, Fe, Mg, Mn and P), and isoflavones that can contribute to its antioxidant properties. Quinoa is gluten-free and its high-quality fatty acids (Pereira et al., 2019). The seed coat contains saponins, previously considered an antinutrient due to its bitter taste, currently it is extracted for industrial and biomedical purposes (El Hazzam et al., 2020). It has a high adaptability, due to its domestication process and high genetic variability. It is a species that can tolerate different types of stress such as salinity, cold, high solar radiation, freezing night temperatures and phytosanitary tolerance factors (Ebrahim et al., 2018; Hinojosa et al., 2018; Ali et al., 2019). Due to its economic potential and because it represents a food security crop for Andean communities, in the last decade its production has been encouraged among farmers, agro-industrial companies and institutions (Rojas et al., 2015).

Morphological characterization, including SEM study on seed coat ultrastructure, helped in the taxonomic delineation while the size and morphology of fruit surface were found diagnostic for segregating the wild and cultivated species and influence the genetic variability (Mishra et al., 2017). The genetic bases of several quinua traits was identified several decades ago, but the first true genetic descriptions more recently provided the starting point for improvement of quinua. Several genetic tools have been developed, and today morphological and molecular markers are an effective way to enhance breeding efficiency (Ruíz et al., 2014). Quinua is one of the Andean crops with little research in the area of genetics and plant breeding, although, it has a high variability in characteristics such as plant color, flowers, nutritional contents and metabolites of interest (Bazile et al., 2014). Collecting, conservation and characterization studies are necessary for the development of strategies to improve of this species.

In Colombia, quinoa, in the last decade it has had an important recognition by government organizations at the national and international levels, promoting actions aimed to replant and shaping its supply chain (Delgado et al., 2009). It is a small-scale crop, where the indiscriminate combination of varieties, together with a low level of technology, reduces their quality and profitability (Morillo et al., 2017). Due to the lack of cultures with a single variety, technological problems arise such as heterogeneity in morphological characteristics and maturation times of individuals (Delatorre et al., 2013). Although preferably autogamous, quinoa shows notable inter- and intra-population genetic variation, easily observable in rural plots, and quantifiable by molecular markers (Del Castillo and Winkel, 2014). For morphological markers, global studies on quinoa diversity have shown variability in the phenotypic characteristics of the evaluated germplasm (Chura et al., 2019; Maliro and Njala, 2019).

In this country, there have been morphoagronomic characterization studies on quinoa materials cultivated on the Bogotá savanna and in Nariño (Veloza et al., 2016; García et al., 2018). In Boyacá, Infante et al. (2018) carried out a morphological characterization of quinoa varieties grown in that department; Morillo et al. (2020) evaluated 19 quinoa materials in the Department of Boyacá with 27 morphological descriptors. The results of these studies showed that the evaluated materials present great variability in both qualitative and quantitative characteristics, which can be used for the selection of materials.

None of these studies attempted to explain the distribution of genetic variation between the different levels of organization of the species, especially in commercial materials like Piartal. The objective of this research was to determine the intrapopulation phenotypic variation in the quinoa materials Piartal in the Department of Boyacá in order to analyze the structure of the variation of the morphological markers and, thus, be able to establish strategies for obtaining "pure" planting materials with high yield and adaptation to local conditions, that respond to the needs of producer and consumer.

MATERIALS AND METHODS

Location

This study was carried out in the Department of Boyacá in the municipalities: Sichoque, Sogamoso, Monguí, Tunja y Combita on farms where cultivation was already established, and different morphotypes were selected, with a total of 27 samples Piartal materials (Table 1).

Morphoagronomic characterization

For the morphoagronomic characterization, a completely randomized stratified simple sampling was used, which consisted of identifying the plants in the field that showed phenotypic differences in characteristics such as panicle color, presence of pigmented axillae and colored striae (morphotypes); the number of repetitions

Table 1. Geographical location of Piartal collection sites.

Municipality	Coordinates	Population (N)
Siachoque	5°30´0.6´´N 73°29´52.6´´W	P1 (3)
Sogamoso	5° 40´41" N 72° 56´ 38"	P2 (6)
Monguí	5°43′21″N 72°50′57″W	P3 (6)
Tunja	5°33′16″N 73°21′09″W	P4 (6)
Combita	5°38′02″N 73°19′23″W	P5 (6)

Table 2. Morphological descriptors used for the morphological characterization of Piartal materials.

Qualitative	Quantitative
Panicle color at physiological maturity	Panicle length (LP) (cm)
Panicle shape	Panicle diameter (DP) (cm)
Stem color	Plant height (AP) (cm)
Upper and lower leaf color	Number of teeth upper leaf (DHS)
Upper and lower leaf shape	Number of teeth lower leaf (DHI)
Upper and lower leaf edge	Main stem diameter (DT)
Presence of teeth on upper and lower leaves	Upper leaf length (LHS) and width (AHS)
Presence of pigmented axillae	Length (LHI) and width of lower leaves (AHI)
Axilla color	
Plant size (growth habit)	
Presence of stria marks	
Stria color	
Stem shape	

depended on the presence of these characteristics in the culture. The morphological characteristics proposed by Bioversity International were evaluated *in situ* at physiological maturity (Morillo et al., 2020) (Table 2).

Data analysis

A multivariate analysis was carried out with the data obtained from the morphoagronomic characterization using the statistical programs NTSYSpc® and InfoStat. A principal component analysis was used with a correlation matrix between the characteristics, performing a linear transformation of the original data, which generated a new set of independent variables. With the NTSYSpc® statistical package, a hierarchical cluster analysis was performed using the mean taxonomic distance matrix between the qualitative and quantitative characteristics and the hierarchical grouping algorithm (UPGMA), for which the squared Euclidean distance and the full link algorithm were applied (Figure 1).

RESULTS AND DISCUSSION

Regarding the evaluation of the qualitative characteristics of the Piartal quinoa material in the populations P1 (Siachoque), P2 (Sogamoso), P3 (Monguí), P4 (Tunja) and P5 (Cómbita), it was found that the most variable characteristic was panicle color, where P2, P3 and P5 showed a 1:1 segregation between green and purple, all P1 individuals exhibited a purple panicle color and P4 had a green color. The shape of the panicle for P1 was 100% glomerulated, in P5 intermediate and amarantiform and simple for the rest of the populations evaluated (P2, P3 and P4). It was observed that the edge of the leaf was serrated and with teeth for the populations P2, P4 and P5, and 100% serrated for P1 and 50% whole and 50% serrated for P3. For the characteristic presence of pigmentation in the axillae, it was found that the individuals in the population P1 had no axillae, P5 did not have pigmented axillae, P2 was 50% purple color and 50% no axillae, and P4 had 50% purple and 50% pigment free. The stem color was green for P1, P4 and P5 and yellow for P2 and P3. The color of the upper and lower leaves in P1 and P4 was green. In populations P2, P3 and P5, they showed defoliation of the lower leaves during the evaluation and a green-yellow color in the upper leaves. It was observed that most of the Piartal individuals had a rhomboidal and triangular leaf shape, presence of teeth on upper and lower leaves, angled stem, simple growth habit, and green striations, except for P5, which present red striae.

The analysis of the qualitative characteristics showed that the variable of the panicle was the most diverse since, within the populations (Table 3), there was segregation between green and purple. Similar results

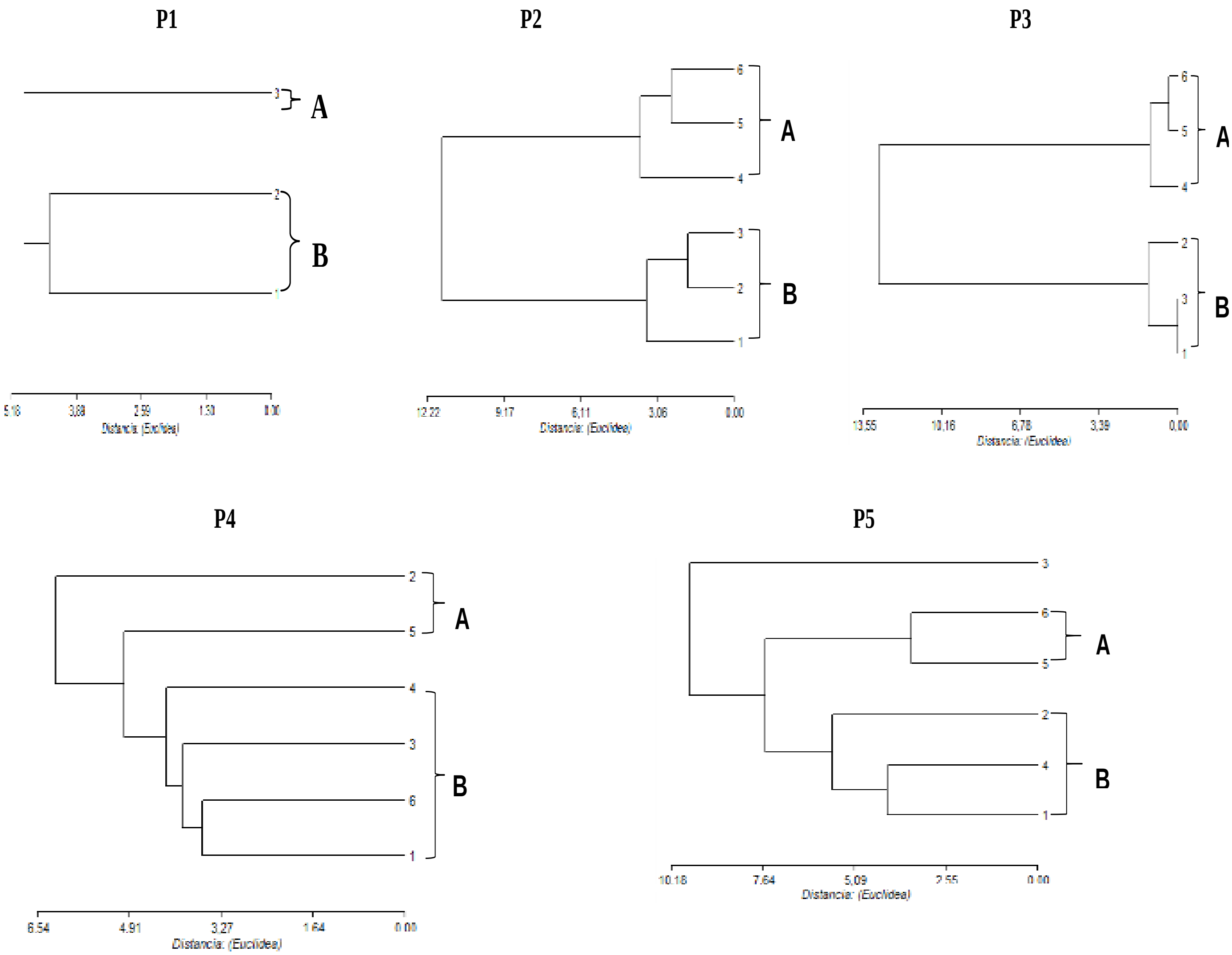

Figure 1. Dendrogram of the hierarchical classification analysis for the Piartal material.'

were obtained by Morillo et al. (2020), when evaluating 19 quinoa materials in the Department of Boyacá. Alanoca and Machaca (2017) reported that the expression of this characteristic is greatly affected by the morphological changes that quinoa shows during its maturation. In general, the color characteristic, both in stems and in other structures, also presented variation between and within the evaluated populations, contrary to the results obtained by Infante et al. (2018), who described Piartal as a material with more stable morphological characteristics. However, other germplasm evaluation studies have also shown segregation in color and in other qualitative characteristics in quinoa (Del Castillo and Winkel, 2014; Alanoca and Machaca, 2017; Afiah et al., 2018; Al-Naggar et al., 2018; Morillo et al., 2020). The color characteristic in different plant structures was variable, and this variation is subject to the phonological stage of the crop, as reported in other studies (Noulas et al., 2017; De Santis et al., 2018).

In the evaluation of the quantitative characteristics of the Piartal quinoa material in the five populations, according to the cv, the more variable characteristics were AHS (14.62-112.9%, P3, P2, respectively), DHS (23.53 - 110.4% for P1 and P3, respectively), DP (33.52%, P2), LP (24.25% -31.23%, P4 and P2, respectively), DHI (28.27%, P4), AHI (26.62%, P5), LHI (22.54%, P1) and AP (22.26%, P1) (Table 3). In the principal component analysis, it was observed that the first two components, CP1 and CP2, explained more than 70% of the total observed phenotypic variation, and there was a significant contribution from all variables to the two components, except those related to the lower leaves, where P2, P3 and P4 presented defoliation and DP (CP2, P4) (Table 3).

The AP and DT characteristics were between 16.5-177 and 1.33-435 cm, respectively, similar to that found by

Table 3. Descriptive statistics of the quantitative morphological variables and main components for the Piartal materials.

Variable	N	Average	S.D.	CV (%)	Range	CP1	CP2
P1							
AP	3	77.33	17.21	**22.26**	32	0.95	-0.31
DT	3	3.97	0.25	6.34	0.5	-1	0.05
LP	3	27.92	1.47	5.27	2.95	0.98	0.19
DP	3	24.44	4.46	18.25	8.91	1	0.03
DHS	3	15.53	3.66	**23.53**	7.3	0.4	0.92
DHI	3	23.30	4	17.17	8	0.45	0.89
LHI	3	4.47	1	**22.54**	2	-0.64	0.77
AHI	3	4	0.47	11.72	0.9	-0.97	0.25
LHS	3	7.27	0.40	5.56	0.7	0.83	0.55
AHS	3	5.37	0.76	14.23	1.5	-0.38	0.92
P2							
AP	6	166.50	20.42	12.27	55	-0.68	0.71
DT	6	4.15	0.72	17.29	1.7	-0.68	0.66
LP	6	29.27	9.14	31.23	27	-0.77	0.09
DP	6	28.98	9.72	33.52	27	-0.81	0.35
DHS	6	7.33	8	110	16	0.85	0.48
LHS	6	2	2.38	114	5	0.87	0.49
AHS	6	1.33	1.51	112.9	3	0.85	0.52
P3							
AP	6	160	21.84	13.65	60	0.89	0.21
DT	6	4.28	0.79	18.41	2.1	0.95	0.23
LP	6	28.13	3.85	13.70	9.7	0.13	0.98
DP	6	24.28	3	12.36	8.4	-0.29	0.95
DHS	6	9	9.94	110.44	20	0.96	-0.27
LHS	6	2.75	0.52	1.9	1.5	0.95	0.19
AHS	6	1.77	0.26	14.62	0.5	0.98	-0.19
P4							
AP	6	132	30.38	23	90	0.83	-0.35
DT	6	7	0.92	13	2.5	-0.66	0.47
LP	6	31.18	3.60	11.53	10	0.96	0.16
DP	6	30.55	5	16.41	13.6	-0.16	0.09
DHS	6	16	3.58	22.36	10	0.71	0.69
LHS	6	3.6	0.53	14.80	1.3	0.82	0.39
AHS	6	2.58	0.70	26.98	1.5	0.78	-0.32
P5							
AP	6	177.33	29.87	16.84	90	-0.75	0.28
DT	6	4.5	0.81	17.94	2.5	-0.77	0.34
LP	6	26.35	6.48	24.58	17.9	0.18	-0.76
DP	6	20	1.54	7.68	4.3	0.75	-0.22
DHS	6	10.33	6.38	61.71	18	0.21	0.72
DHI	6	11	6.16	56	16	0.76	0.44
LHI	6	5.55	1	18.68	2.5	0.87	0.42
AHI	6	4.78	1.27	26.62	2.8	0.90	0.31
LHS	6	5.95	1	16.73	2.2	-0.21	0.95
AHS	6	4.87	1	21.54	2.2	-0.16	0.98

Morillo et al. (2020). On the contrary, Infante et al. (2018) reported an AP of 135 to 170 cm and DT of 3 to 4 cm. These studies show that the phenotypic behavior of quinoa does not have particularly extreme values for any of the evaluated variables and that the variability of the morphological characters results from environmental factors that can be controlled by genes with pleiotropic or epistatic effects (Farooq et al., 2018). The values found in the other quantitative variables were similar to those reported in other genetic diversity studies on quinoa (Afiah et al., 2018; Ebrahim et al., 2018; Infante et al., 2018; Morillo et al., 2020).

The analysis of correlations showed that the highest value was obtained with the variable AP, which, in P1, had a perfect and negative correlation with AHI (r = -1), a negative correlation with DT (r = -0.97) and a positive, significant correlation with DP (r = 0.94). DP was negatively and significantly correlated with AHI (r = -0.96) and positively correlated with LP (r = 0.99). LP had negative correlation values with AHI (r = -0.91), and AHI was positively correlated with DT (r = 0.98) (Table 4). In P2, positive and significant correlations were found for DHS and AHS (r = 0.98), DHS and LHS (r = 0.97), AP and DT (r = 0.87), AP and DP (r = 0.80) and DP and DT (r = 0.74). P3 had the highest number of positive and significant correlations between the evaluated variables; the highest was AHS with DHS (r = 0.98). In P4, significant and positive correlation values were obtained for AP and LP (r = 0.82), LHS and DHS (0.82), DHS and LP (r = 0.79) and LHS and LP (0.78), and negative values were obtained for DT and AP (r = -0.74). In P5, the positive and more significant associations were for AHS and LHS (r = 0.99), AHI and LHI (0.99), AP and DT (r = 0.91), and DHI and DHS (r = 0.78), and there were significant negative associations for LHS and LP (r = 0.81) and AHS and LP (r = 0.81) (Table 4).

The results showed a significant correlation between AP and DT for all populations, as also been observed by Spehar and Santos (2005) when evaluating the agronomic performance of selected quinoa and by Madrid et al. (2018) in the evaluation of morphological characteristics related to the improvement of yield in quinoa. LP and DP were positively associated with AP in P1, P2 and P4, indicating that individuals with a greater height develop larger panicles, as found by Alanoca and Machaca (2017), Farooq et al. (2018) and Morillo et al. (2020).

Based on the correlations of all the variables and the evaluated populations, the phenotype of the material was highly influenced by the environment. Correlation studies are an important step in quinoa improvement programs since the information that is obtained is useful for estimating the correlated response to selection for the formulation of selection indices (Afiah et al., 2018; Al-Naggar et al., 2018, Ebrahim et al., 2018).

The cluster analysis showed that the individuals of the Piartal quinoa material were grouped mainly by morphological characteristics associated with plant height, panicle length, pigmented axillae, and leaf characteristics, as reported by Morillo et al. (2020); the clusters showed a lax distribution of the materials with an association of the characteristics presence or absence of striae, growth habit, color, shape, length and diameter of the panicle, seed/plant yield and weight of 1000 grains, results that were consistent with morphological characterization studies on quinoa (Curti et al., 2014, Infante et al., 2018, Farooq et al., 2018).

In this study, as in study by Morillo et al. (2020), no groupings were observed according to the site of origin of the materials, as observed when evaluating the intra and inter-population phenotypic variation in seven quinoa populations from the Bolivian altiplano, in which the morpho-phenological markers separated the quinoa from the most limiting sectors for agriculture (southern plateau and cold zones of the northern plateau) from quinoa cultivated in more temperate zones (Del Castillo and Winkel, 2014). These results are consistent with that reported by Farooq et al. (2018) where all quinoa accessions showed good growth in subtropical and semi-arid climatic conditions in Pakistan. In addition, the studies carried out by Noulas et al. (2017) demonstrated not only the wide adaptation of quinoa materials to the agroclimatological conditions of Greece but also the variation of quinoa phenotypic characteristics according to the environment.

The morphoagronomic characterization of the Piartal quinoa materials in the five evaluated municipalities showed high intrapopulation phenotypic variability that depended on the agroclimatological conditions of each site (Infante et al., 2018; Morillo et al., 2020), mainly as the result of the fact that quinoa is a rustic crop with broad agroecological adaptation that can tolerate different types of stress and that is a food security crop for the Andean community since with farmers who have maintained and selected seeds for generations (Alvarez et al., 2018). However, the presence of morphotypes in quinoa crops is not a desirable condition since it means that there are still no pure materials or local varieties but only materials in the process of domestication, which is a limitation for the implementation of cultivation technologies. For example, populations can have differences in the maturity stage of the plants, which can complicate uniform agronomic management. In addition, the size and color of the seeds are different between materials and within each material, which prevents the development of machinery for threshing processes as has been done for cereals with uniform grain sizes and diameters.

Conclusion

The morphoagronomic evaluation of the intraspecific variation of the Piartal quinoa material in the Department

Table 4. Pearson correlation analysis for the quantitative variables (P ≥ 0.001) in the Piartal material.

P1

	AP	DT	LP	DP	DHS	DHI	LHI	AHI	LHS	AHS
AP	1									
DT	-0.97	1								
LP	0.87	-0.97	1							
DP	0.94	-1	0.99	1						
DHS	**0.09**	-0.35	0.56	0.42	1					
DHI	0.15	-0.40	0.61	0.47	1	1				
LHI	-0.85	0.68	-0.49	-0.62	0.45	0.40	1			
AHI	-1	0.98	-0.91	-0.96	-0.16	-0.21	0.81	1		
LHS	0.62	-0.80	0.92	0.85	0.84	0.87	-0.11	-0.67	1	
AHS	-0.65	0.43	-0.20	-0.36	0.70	0.65	0.95	0.60	0.19	1

P2

	AP	DT	LP	DP	DHS	LHS	AHS
AP	1						
DT	0.87	1					
LP	0.61	0.54	1				
DP	0.80	0.74	0.40	1			
DHS	-0.21	-0.32	-0.54	-0.53	1		
LHS	-0.25	-0.26	-0.62	-0.55	0.97	1	
AHS	-0.21	-0.22	-0.57	-0.54	0.98	1	1

P3

	AP	DT	LP	DP	DHS	LHS	AHS
AP	1						
DT	0.84	1					
LP	0.33	0.33	1				
DP	**-0.06**	**-0.04**	0.88	1			
DHS	0.80	0.82	-0.12	-0.54	1		
LHS	0.79	0.96	0.32	**-0.09**	0.86	1	
AHS	0.84	0.89	**-0.06**	-0.46	0.98	0.89	1

P4

	AP	DT	LP	DP	DHS	DHI	LHS	AHS
AP	1							
DT	-0.74	1						
LP	0.82	-0.60	1					
DP	0.21	0.33	**-0.04**	1				
DHS	0.32	-0.24	0.79	-0.17	1			
LHS	0.41	-0.18	0.78	-0.26	0.82	0.21	1	
AHS	0.70	-0.37	0.64	-0.06	0.26	-0.47	0.70	1

P5

	AP	DT	LP	DP	DHS	DHI	LHI	AHI	LHS	AHS
AP	1									
DT	**0.01**	1								
LP	-0.36	0.44	1							
DP	**0.04**	**-0.03**	0.17	1						
DHS	0.46	-0.39	-0.87	0.12	1					
DHI	**-0.02**	**-0.07**	**-0.09**	0.58	**-0.01**	1				
LHI	**0.05**	0.50	-0.23	-0.53	-0.11	-0.13	1			

Table 4. Contd.

AHI	-0.17	0.49	0.10	-0.59	-0.37	-0.43	0.88	1		
LHS	0.10	0.52	-0.37	-0.51	0.33	-0.37	0.60	0.44	1	
AHS	0.20	0.34	-0.35	-0.73	0.29	-0.66	0.54	0.49	0.91	1

Values in bold are not significant

of Boyacá showed a wide segregation of the phenotypic characteristics especially related to the color of different plant structures. This variation that occurs at the inter-individual level in farmers' fields, in the materials that they plant and select cycle after cycle is not desirable, since it reduces the quality and profitability of the crop, and also suggests selection and purification processes efficient that lead to obtaining "pure" materials with better yields and adapted to local conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Grass species for range rehabilitation: Perceptions of a pastoral community in Narok North sub-county, Kenya

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Land degradation is a global problem leading to a diminished production capacity of the rangelands. The consequence is reduced potential to provide ecosystem services and increased vulnerability to the inhabitants. Biological soil water conservation measures can reverse the trend. Different communities prefer different grass species for rehabilitation as communities vary in location, needs, priorities, preferences and the type of livestock reared. This study, therefore, sought to identify the suitable grass species for soil erosion and rehabilitation from the community in Keekonyoie ward In Narok county, Kenya. Data collection was through individual interviews, focus groups, key informant interviews and field observations. Results showed that level, indicators, causes and impacts are known to the community. *Cynodon plectostachyus* (76%), *Chloris gayana* (73%), *Pennisetum clandestinum* (69%), *Cymbopogon citratus* (46%) and *Themeda triandra* (42%) were most preferred for rehabilitation and soil erosion control. The primary reason for the grasses choice was a yearlong provision of livestock feed. Needs and livelihood priorities significantly influence decision-making among the Maa-speaking community in Keekonyoie ward. We recommend consideration of community needs, priorities and preferences in the selection of grass species for rehabilitation to increase the adoption measures that can reverse land degradation

Key words: Indigenous knowledge, community perceptions, range grass species, rehabilitation, land degradation, Narok.

INTRODUCTION

Soil erosion is the most widespread form of land degradation in the world (Lal, 2001, 2003, 2014; Nkonya et al., 2016; Pimentel and Burgess, 2013) and in Kenya (Mganga et al., 2010; Mulinge et al., 2016). Wind and water erosion are the major forms of soil erosion resulting in degraded soils (Lal, 2014). Degraded soils are characterised by limited ability to sink atmospheric carbon, decline in plant nutrient reservoir as well as gene pool (Kimble et al., 2016). Degraded soils directly reduce vegetation cover resulting in bare land and hence risking a range of ecosystem services and livelihoods in the arid and semi-arid rangelands. Human, natural factors and

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conditions and the physical features and conditions of the land aggravate the erosion process in the rangelands. The resultant is eroded hillsides, denuded plains, massive erosion shelves and deep sheer sided gullies (Sindiga, 1984; Odini et al*.,* 2015).

Many measures have been used to control land degradation in different parts of the world. This includes the use of soil water conservation (SWC) methods like terraces, cut-off drains, semi-circular bands, ditches, water pans and stone bunds. These measures are, however, often expensive to implement and are labour intensive, making them only available and relevant to large-scale commercial entities (Riginos et al., 2012), or donor-funded rehabilitation support projects on community land. Despite the physical SWC measures being expensive, they are successful in runoff and soil erosion control (Wolka, 2014; Ruto, 2015; Saiz et al*.,* 2016). A biological/ vegetative tool is another SWC measure that controls soil erosion and rehabilitates degraded land. It is a technique whose use has increased in the recent past due to its availability, affordability, ease of establishment and management, low labour requirement, and its ability to provide livestock feed in the arid and semi-arid rangelands (Gachene and Mureithi, 2004; Riginos et al., 2012). Trees and or grasses are used. Trees require a longer time to establish, resulting in a longer period of time before firmly executing the role of soil erosion control. Grasses have a shorter establishment time and spread and cover the ground within a short time. Quicker and faster range rehabilitation can be achieved by grasses because they are easy to establish and grow rapidly and colonise a large area due to their prolific growth nature. Moreover, vegetative tools have been found to increase the soil organic carbon at a maximum rate of 1.06Mg/year (Garcia-Diaz et al., 2018) However, selection of best grass species for rehabilitation of degraded rangelands is key to successful restoration. The grasses vary in ecological adaptability, growth characteristics, as well as preferences by the community. As indicated by Mekonnen et al*.* (2016) the choice of grass species for rehabilitation should consider the availability of the target species, as well as their adaptability to the local environment. Also, they should be drought tolerant, establish within a short time, have a good seedling ability, high seedling survival and provide viable seeds (Mnene, 2005; Opiyo, 2007). The grasses should also be able to stabilise soil conservation physical structures and improve hydrological properties of the soil (Nyangito et al., 2009; Garcia-Diaz et al., 2018) while producing adequate biomass for livestock feed.

Successful range rehabilitation and erosion control using grass species has been done in many countries (Troung et al., 2004; Visser et al., 2007; Mganga et al., 2010; Terefe, 2011; Wanyama et al., 2012; Mganga et al., 2015; Ogwa and Ogu, 2014; Amare et al., 2014; Manyeki et al., 2015; Mekonnen et al., 2016). Whereas some productivity and rehabilitation suitability studies of grass species for arid and semi-arid environments are already done in Kenya (Mganga et al., 2010; Opiyo et al., 2011), little attention has been given to community views on suitable grass species that best fits their needs. Besides, variations on communities' views exist with respect to location, species performance, grass uses and preferences. According to Kangalawe (2012) and Ricart et al. (2019) local perceptions, attitudes and knowledge have far greater implications to their environment including resource management and control of land degradation. Local communities know what plants are available in the wet and dry seasons, species that are more persistent and drought tolerant, and this information complements the modern scientific knowledge in selecting species for rehabilitation (Wasonga et al., 2003; Wekesa et al., 2015). Understanding the community perceptions, needs and priority grass species that address existing environmental and livelihood challenges enhances the selection of appropriate grass species for adoption by communities for Sustainable Land Management (SLM). This study was therefore conducted to 1) determine the community's perceptions on soil erosion and degradation 2) identify grass species suitable for rehabilitation of degraded rangelands as perceived by the community.

MATERIALS AND METHODS

Study area

The study was conducted in Suswa ward, Narok County located in the Southwest of Kenya (Figure 1). The county lies between longitudes 34° 45\` E and $36^0$ 00\` E and between latitudes 0^0 45\`S and $2^0$ 00\` S. The temperatures are varied and ranges from 10^0C in the highlands to 26.5°C in the lowlands (Jaetzold et al., 2010; National Environmental Management Authority, 2009). The rainfall pattern is bimodal with long rains from mid- March to June and the short from September to November. The rainfall is uneven with high altitude areas receiving 1200 to1800 mm per annum while the lower altitude regions receive 500m or less per annum (Ojwang et al., 2010). The topography ranges from 1000 to 2500 m in plateaus in the southern parts to the mountainous parts that reach up to 3098 m above sea level (Serneels and Lambin, 2001; Jaetzold et al., 2010). Different soil types are found in the county and include andosols, luvisols, Phaeozems, vertisols and acrisols (Sombroek et al., 1982; Jaetzold et al., 2010). In Suswa, the soils are mainly humic andosols, dark brown, friable and smeary; sandy clay to clay with acidic humic topsoil and the area is characterized by sharp gradient highly liable to soil erosion (Ruto, 2015). The vegetation is predominantly grassland intercepted by trees. *Tarchonanthus camphoratus* and *Acacia drepanolobium* are the dominant tree species. Perrenial grasses include *Cymbopogon citratus, Harpache schimperi, Themeda triandra, Sporobolus fimbriatus* and *Aristida adoensis* among others. Forbs include *Euphorbia inequilatera, Satureia biflora* and *Borreria stricta* among others (Ombega, 2018).

Narok county is home to multiple land uses. In the highlands, the dominant land use is large scale crop farming of wheat while in the mid-elevation is more of small-scale farming while in the lower and

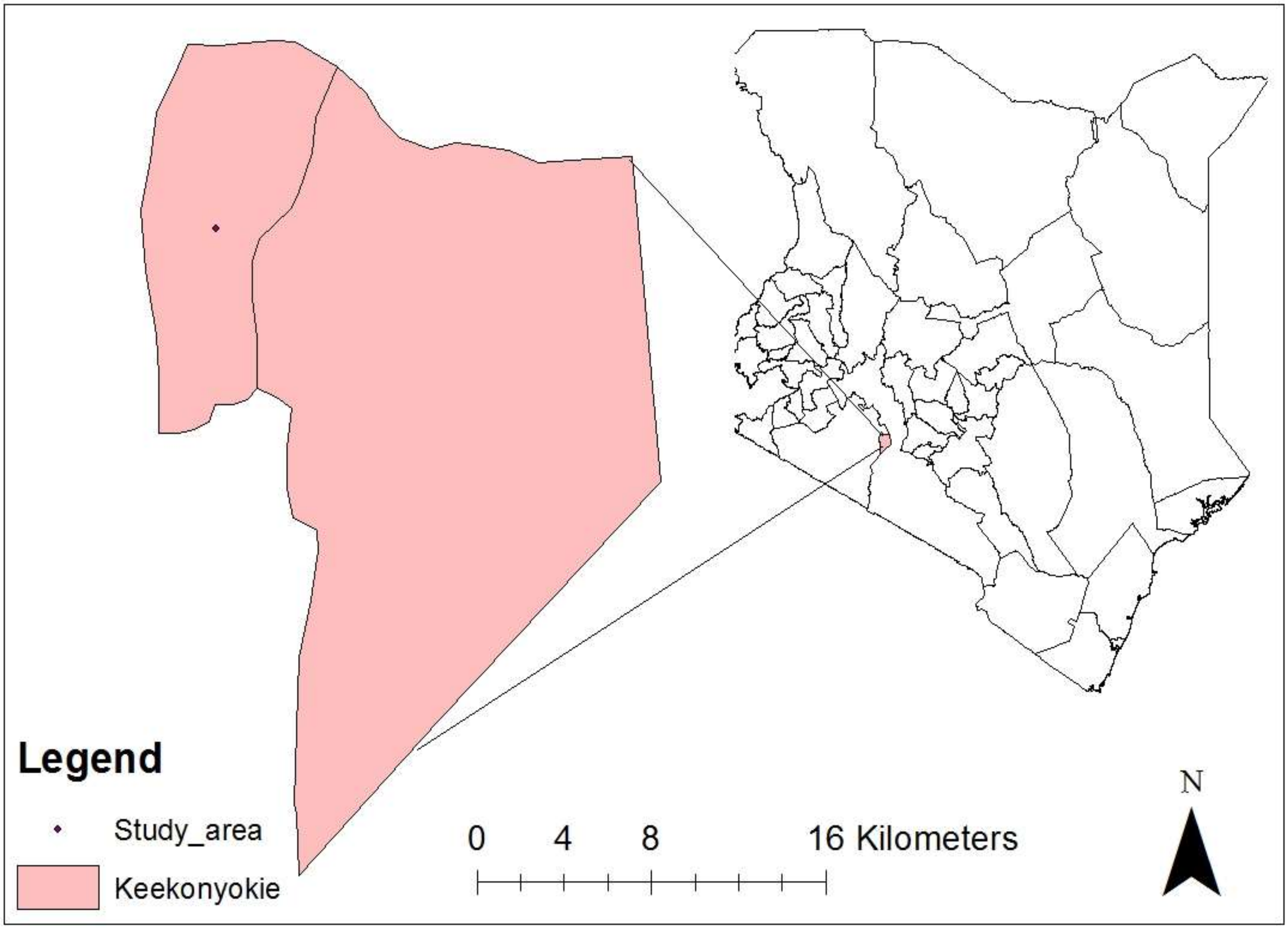

Figure 1. A map showing Keekonyoie ward in Narok County in relation to the map of Kenya Source: Hannah Kamau 2018.

drier areas is livestock production. Indigenous breeds of sheep, goats and cattle are main kinds of animals reared; however, the communities have recently started cross-breeding with exotic breeds (Maina, 2013). Other land uses include beekeeping[1] and rearing of poultry (Odini et al., 2015; Ruto, 2015). In Keekonyie ward the community is predominately agro-pastoral regardless of communal land tenure. Charcoal production is a major environmental concern in the area (Odini et al., 2015).

Research design

Keekonyoie ward was purposively selected because of the gullies and the past rehabilitation interventions under 'mainstreaming sustainable land management (SLM) in agropastoral systems of Kenya's project. The target population included households living close to the gulleys in four (Olepolos, Enkiloriti, Eluai and Olesharo) villages within the ward, state and nonstate experts on livestock production, pasture management and soil conservation, early adopters of soil water conservation (SWC) measures in the study area.

Data collection and analysis

Individual interviews, focus groups, key informant interviews (KIIs) and field observation were the methods used for data collection. Primary data were collected from May to August 2016. A total of 33 household heads selected randomly from the purposively selected households (living one kilometer radius from the gulley) were interviewed through the interpreter. The open and close-ended questions are related to perceptions of grass uses, and abilities to control soil erosion. Data collected from the individual interviews were supplemented with focus groups, KIIs and field observation. Five focus groups each with 8-12 participants (Gill et al., 2008) were held in the villages at different locations as chosen by the village elder. Discussion with the same group was held twice on two consecutive days where the first day was the focus group and the second field observation to identify the grass species mentioned. Questions discussed during the focus groups included but not

Table 1. Demographic and socio-economic characteristics of respondents (n=33).

Variable	Respondents (n=33)	Percent
Household characteristics		
Male-headed households	28	85
Female-headed households	5	15
Age of the household head		
20-35	5	15
36-49	11	33
50-70	8	24
>70	9	27
Education of household head		
Formal	11	33
Informal	22	67
Average household size	7± 2	
Average TLU	20.1± 11.786	
Land and pasture production characteristics		
Severe level of land degradation	33	100
Causes of land degradation		
Climatic	16	48
Anthropogenic	17	52
Proportion of respondents that planted grasses (dummy)	**9**	**27**
Rehabilitation challenges using grasses		
Insufficient rainfall	33	100
Seedling mortality	26	79
Recurrent dry spells	33	100
Defoliation by animals	20	61
Destruction by flash floods	28	85

Source: Survey Data, 2016.

limited to the past rehabilitation interventions within the area, grass species perceived suitable for rehabilitation, grass species found during the wet and dry seasons, and reasons for mentioned preferred grass species for soil erosion control. The focus groups were conducted in local language (Maa) through the interpreter. A total of eight KIIs comprising experts in pasture management, livestock production and SWC with the government, state and non-state actors operating in the study area and early adopters of biological SWC measures were interviewed. A comprehensive literature review was done to contextualise the study and provide secondary data on community perspectives on grass species and rehabilitation.

The data collected were coded and analyzed using Microsoft Excel 2010 to generate descriptive statistics. Field notes were collated and consolidated into different topics to validate and complement individual interviews.

RESULTS

Demographic and socio-economic characteristics of respondents

Most (85%) of the households sampled are male headed and the average family size is 7 persons. Age of the respondents ranged from 20 to 80 years. Average tropical livestock unit (TLU) kept by the households is 20.1 (Table 1).

Agro-pastoralists' perceptions and knowledge on land degradation

Respondents agreed (100%) that the area is severely degraded (Table 1). Evidence provided was the presence of gullies, loss of vegetation and high soil deposits in the lowlands. The communities indicators of land degradation are presence and depth of the gulley, presence of undesirable plants growing and absence and decline of desirable plants. Above 50% of the interviewed attributed land degradation to anthropogenic causes. The community perceived increase in human population, overgrazing, cultivation on slopes and bush clearing for charcoal burning, fence and shelter (*manyatta*) building were the man-related causes of degradation. Communities perceived prolonged dry spells that often lead to drought, low and poorly distributed rainfall were what constituted climatical causes of land degradation.

Table 2. Common grass species found in the study area and their uses.

Grass species		Preference by livestock species	Other grass uses
Scientific name	**Local names (Maa language)**		
*Cynodon Plectostachyus***	*Emurua*	All livestock (cattle, sheep, goat, donkey)	
Digitaria macroblephara	*Erikaru*	Cattle and sheep	
Chloris gayana	*Olekiramatian*	All livestock (cattle, sheep, goat, donkey)	Fodder production
Cymbopogon citratus **	*Olung'u*	Cattle and sheep (dry season only)	Thatching, rehabilitation
*Aristida adoensis***	*Onkosos*		
Sporobolus fimbriatus	*Olperesi*	Sheep, goats (feed on inflorescence)	
Hyparrhenia lintonii	*Ologorroing'ok*	All livestock (cattle, sheep, goat, donkey) (cattle consume more)	
Pennisetum clandestinum	*Olobobo*	Donkeys and sheep	Fodder production
Themeda triandra	*Olperesi Orasha/Orkijitaonyokie*	Preferred by goats	Thatching, plastering
Setaria verticillata	*Olorepirepi*	All livestock (cattle, sheep, goat, donkey)but more preferred by sheep	
*Tragus barteronianus***	*Onkosos*		
Cyperus spp	*Oseyia*	All livestock (cattle, sheep, goat, donkey)but more preferred by goats	
Pennisetum mezianum	*Not specified*		
Cenchrus ciliaris	*Oshankash*	All livestock (cattle, sheep, goat, donkey) but more preferred by cattle	
Brachiaria brizantha	*Ormagutian*	All livestock (cattle, sheep, goat, donkey)	
*Harpachne schimperi***	*Onkosos*		
Not specified	*Mutanduro*	Cattle and sheep	
Not specified	*Ngonyoro*	Sheep and goats	
Not specified	*Oltiol (found in the forest)*	Cattle	
Not specified	*Olparakae*	Cattle and sheep	

** Represents dominant grasses as identified by the community in the study site.
Source: Focus Group Discussions (n=5); Survey Data, 2016.

The consequences of land degradation mentioned by the agro-pastoralists are crop failure and low yield, land fragmentation, death of animals from falling off the cliff of the gulley, separation from relatives by the barriers. The advantages perceived by the respondents as a result of land degradation are formation of ballast especially in the gullies used for construction, provision of sand that they sell and the gullies form dry feed reserves as animals cannot graze there on normal occasions.

Common grass species found in the study area and their uses

During the focus groups, 20 grass species were identified (Table 2). Nine of the grasses were identified as dry season livestock forages namely: *Cymbopogon citratus, Cynodon plectostachyus, Sporobolus fimbriatus, Chloris gayana, Eragrostis superba, Pennisetum mezianum, Cenchrus ciliaris, Hyparhennia lintonii* and *Aristida adoensis*. Out of the nine species, *Cenchrus ciliaris, Cynodon plectostachyus* and *Chloris gayana* were reported to be highly preferred by the livestock. *Cymbopogon citratus* was only fed when the

Table 3. Grass species perceived suitable for rehabilitation and soil erosion control and their reasons.

	Respondent		Reasons for selection						
Scientific name	**Frequency**	**Percent**	**Livestock feed**	**Rapid growth**	**High biomass**	**Perennial**	**Drought tolerance**	**Continuous grass cover**	**Stabilize SWC**
Cynodon plectostachyus	25	76	x	x	x	x	x	x	
Chloris gayana	24	73	x		x	x	x		x
Pennisetum clandestinum	23	69	x			x	x	x	
Cymbopogon citratus	15	45		x		x	x		x
Themeda triandra	14	42	x			x	x		x

n=33; SWC= Soil water conservation.
Source: Focus group Discussions (n=5); Survey Data, 2016.

animals did not have any other feed and it gave milk a distinctive citral kind of taste. During the wet season, livestock utilised annuals including *Setaria verticillata*, "mutanduro" (in Maa language) and *Sporobolus fimbriatus*. Agro-pastoralists preferred mutanduro to the other annual grasses citing its distinctive taste in the milk. Fast growing grasses following rains mentioned by the respondents were *C. citratus, Cynodon plectostachyus* and *Sporobolus fimbriatus.* The respondents perceived *Themeda triandra* to have declined in abundance.

The major grass use in the study area is livestock feed. Other uses mentioned by the community were thatching, plastering and rehabilitation. The community considers *C. citratus* more durable to *T. triandra* for use in thatching. *T. triandra* was used in traditional huts plastering where the respondents cut it in small pieces and mix it with mud.

Above a quarter per cent of the respondents had planted grasses and out of which 11% had planted *Cymbopogon citratus* for rehabilitation purposes. Most (89%) planted Chloris gayana and *P. clandestinum* to provide for the livestock. *P. clandestinum* was planted around the homesteads and water pans due to the species high water demand. The respondents reported that *Chloris gayana* provided high biomass yield where the establishment was successful. Challenges of planting grasses were reported to be insufficient rainfall, seedling mortality, recurrent dry spells, animals grazing on young grasses leading to uprooting and destruction by flash floods (Table 1).

Perceived suitable grass species for rehabilitation and their reasons

From the 20 identified grass species, the respondents perceived five grass species to be best suited for soil erosion control and rangeland rehabilitation (Table 3). The chosen grasses were *C. plectostachyus, C. gayana, P. clandestinum, C. citratus* and *T. triandra*. Livestock feed provision was the primary reason for the choices of the grasses. Land rehabilitation was considered secondary to the use of livestock feed. Stabilizing the already SWC structures concerned the respondents.

DISCUSSION

The Maa speaking community inhabiting the study area is aware of their surrounding environment and possesses a great pool of knowledge about their environment, which is no different from other communities in Tanzania and Ethiopia, respectively (Kangalawe 2012; Walie, 2015). The knowledge possessed by the community forms their decision-making tool on key issues of degradation, pastoralism and alternative livelihoods. The indicators used by the communities to describe the extent of degradation were close to what the modern scientists use. The Maa speaking community of Narok County considers the presence of gullies, gulley depth and presence of desirable and undesirable species for their livestock when making their rehabilitation decisions. Based on the indicators, the pastoral communities know where to take their animals during wet or dry seasons to control erosion. Jandreau and Berkes (2016) observed a similar phenomenon at the Maasai Mara where the community uses forage characteristics like grass height, keystone species and grass colour in making rehabilitation decisions. In Dejen in Ethiopia, presence of gullies and rills was the major indicator followed by decline in agricultural productivity and soil colour change (Tegegne, 2014). The indicators perceived by community closely match with those of scientific findings. It was equally evident that anthropogenic induced land degradation was common in the study area arising from agricultural activities like unsustainable cultivation methods on hillsides and indiscriminate bush clearing (*personal observation*). Degree of slope, unsustainable farming methods, deforestation, intense rainfall and lack of physical SWC structures have been reported severally as the main results of land degradation (Tegegne, 2014; Kusimi and Yiran, 2011; Saguye, 2017). Diversification of livelihoods has increased with many starting poultry farming and bush clearing for charcoal. Mganga et al. (2015) observed a similar scenario of increased charcoal production from indigenous trees while working with the Akamba agropastoral community in South Eastern Kenya. The case is no different in Ethiopia where Gashu and Muchie (2018) reported livelihood changes alternatives to be sale of firewood and charcoal. It is evident that alternative livelihood strategies that are being adopted are no good in alleviating land degradation, therefore more community awareness programmes should be channelled towards educating communities of sustainable alternative livelihood options. The livelihood change can be attributed to declining land and net primary productivity because of degradation. Additionally, the increase in human population within the area and fragmentation of land influence the lifestyle of the community by increasing rearing of small ruminants because of their tolerance to undesirable species and their ability to utilise the rough terrain created by degradation (Odini et al., 2015).

It is evident that different grass species are perceived differently with respect to location, community perceptions, and priorities. The pastoral community in the study area uses grasses as livestock feed, for thatching and plastering, and rehabilitation. The grass species considered suitable for rehabilitation are the ones that provide adequate livestock feed. This agrees with Sacande and Berrahmouni (2016) who noted that prioritization of species not only depended on aspirations and conservation status but importantly their way of life. As opposed to modern scientists who link the plant characteristics like root length, root biomass and diameter, cover and plant density as good for rehabilitation, the community acknowledges the amount of biomass produced by the grass species for livestock as another most desirable consideration for rehabilitation. These findings agree with those of Mganga et al. (2015) in Kenya; Sacande and Berrahmouni (2016) in Ethiopia; Visser et al. (2011) in Tunisia; Sjögersten et al. (2013) in China which found that the livestock feed was a criterion used in selecting the grass species for rehabilitation.

*C. plectostachyus'*s higher preference compared to *C. gayana, P. clandestinum, C. citratus* and *T. triandra* demonstrates their need for yearlong livestock feed. In a study that crosscut Burkina Faso, Mali and Niger, 105 of 193 grass species selected by communities was for their value in livestock feed (Sacande and Berrahmouni, 2016). *C. plectostachyus* is also available in many areas and establishes rapidly from splits or seeds (Harlan et al., 1969). Additionally, the grass species is also preferred by all kinds and classes of livestock in the area. Geissen et al. (2007) found that grass species was important in slowing the speed of runoff thereby controlling erosion while working in Mexico. *C. gayana* preference for rangeland rehabilitation was attributed to its high biomass and palatability to all livestock in the study area. Koech et al. (2016) found that the species produces high biomass even under limited water conditions. *P. clandestinum* was preferred by the community for its growth form and its ability to spread and cover the land. The grass has however been observed to effectively grow in high altitude and rainfall areas (Fukumoto and Lee, 2003; Mears, 1970), explaining why it was only planted near homesteads and water sources. The lower preference accorded to *C. citratus* can be attributed to the citral content that lowers its palatability (Thomas et al., 2012). *T. triandra* was least preferred species because of its rapid decline in abundance after establishment. This can be attributed to the grazing and trampling because it is highly sensitive to poor management (Snyman et al., 2013).

Wasonga et al. (2003) and Mutu (2017) observed that calamities and lack of resources make the pastoralists flexible in decision-making and utilisation of resources. The chosen grasses by the Maasai community as best for rehabilitation demonstrate the flexibility in decision-making depending on their needs and way of life. Indigenous knowledge among the pastoral community is increasingly evolving to suit the needs of the community and cushion them from future calamities.

Conclusion

The community in the study area is aware of the land degradation status and clearly understands the indicators, causes and effects of land degradation. Loss of vegetation and declined abundance of keystone species is one of the indicators and impacts of land degradation respectively. The needs of the community are well articulated and the major concern is livestock feed regardless of the status of the land known. Decision-making of the choice of grass species for soil erosion control and rehabilitation of the land is based on its ability to provide livestock feed. Soil conservation and range rehabilitation are secondary reasons for provision of livestock feed in choosing suitable grass species for rehabilitation of degraded lands. There is need for a tradeoff, therefore, between the local community needs, priorities and beliefs and land rehabilitation and restoration objectives. In addition, need arises to quantify the ability of the grasses to control soil erosion and restore degraded land while providing adequate livestock feed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Phenotyping sorghum [*Sorghum bicolor* (L.) Moench] for drought tolerance with special emphasis to root angle

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The study was conducted to characterize early maturing sorghum genotypes under moisture stress and non-stress environments based on root angle. Phenotyping of 23 early maturing sorghum genotypes was carried out under post-flowering moisture stress and non-stressed environment using randomized complete block design (RCBD) in two replications at Werer Agricultural Research Center in 2018 off season. The genotypes were selected based on root angle data that varied from 13.0 to 26.75°. The analysis of variance revealed significant variation among genotypes for most of the traits. Post-flowering drought reduced grain yield by 21% and all the traits showed a reduction in value except flag leaf area. Grain yield showed positive correlations with seedling vigor, grain filling rate, thousand grain weight and panicle weight while negative correlations with number of fertile tiller and panicle exertion for both environments. Root angle revealed positive correlation with grain yield, grain filling rate and thousand grain weight while there was negative phenotypic correlation with panicle exertion in the stressed environment. Therefore, selection for high correlated traits could aid breeding program to develop genotypes with superior yield under both environments flag leaf area, chlorophyll content, harvest index and root angle traits could be used as morphological marker for drought tolerance screening in sorghum since there was positive correlation with yield observed for stressed environment only. The result revealed the importance of intermediate to slightly wider root angle for drought tolerance of early maturing sorghum genotypes by enhancing lateral water absorption of the roots under silty clay soil.

Key words: Correlation, early maturing sorghum, morphological marker, post-flowering drought, root architecture, root traits.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops in the world as well in

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Ethiopia. In Ethiopia sorghum is major staple food and the second preferred grain for making flat bread (Enjera) which is the most common traditional food in the country. East Africa is considered to be the center of origin and diversity for sorghum and Ethiopia is the third largest producer from Africa next to Nigeriya and Sudan (Rakshit et al., 2014; FAOSTAT, 2017). Cultivation of sorghum takes the third larger area under wide agro-ecology of Ethiopia and highly preferred in dry lowlands where drought predominates (Demeke and Marcantonio, 2013; FAOSTAT, 2017). Even if sorghum has the ability to cope with many stresses including heat and moisture, its production highly affected by drought occurred during reproductive stage in arid and semi-arid regions of the world (Ejeta and Knoll, 2007).

Terminal drought is a common phenomenon in Ethiopia especially in southern, southeastern, eastern and northeastern part of the country where sorghum is dominant and the main livelihood of the population (Geremew et al., 2004; Brhane et al., 2006). A severe drought during post anthesis leads to loss of chlorophyll and grain pre-maturation which bring about 55% or more yield loss in sorghum (Assefa et al., 2010).

Drought resistance and drought escape is the two main drought survival strategies (Ludlow and Muchow, 1990). Drought resistance is a complex trait that shows high level of interaction with environment (Cooper et al., 2006). Stay green phenotype is the most common selection criteria for post-flowering drought in plants. However, identification and analysis of a new set of plant traits with sound and positive correlation with yield and drought tolerance is compulsory to feed the ever increasing human population in a Blue revolution (Borrell et al., 2000; Rauf and Sadaqat, 2008; Pennisi, 2008).

Under water limited condition researchers identified root traits that increase the extraction of resources from the soil. Researchers have been working to identify specific root traits targeted for plant improvement under drought and nutrient limitation conditions (Comas et al., 2013; Lynch et al., 2014). Thus, recently much more focus was given to root system architecture and root angle is one of the important root traits in drought tolerance breeding (Mace et al., 2012; Rostamza et al., 2013; Ali et al., 2015).

Mace et al. (2012) indicated an association between the Quantitative Trait Loci (QTL) identified for nodal root angle at the leaf six stage and both yield and the stay green drought response in sorghum. Also, nodal root angle in the seedling stage is associated with subsequent root system architecture and can potentially affect water extraction patterns of mature plants suggesting the trait importance to improve drought tolerance in sorghum. Moreover, root biomass and distribution of matured plant could be predicted through analyzing root angle growth at early stage (Kato et al., 2006; Sanguineti et al., 2007; Manschadi et al., 2008). Incorporation of nodal root angle in a breeding program requires at least a moderately high-throughput platform. However, where there is unavailability of this platform above ground phenotyping and indicating possible association of root angle traits with morphological markers could be an indicator as a screening technique for sorghum improvement in moisture stress area. Since there is no single plant character to identify plants with improved performance under moisture stress condition, phenotyping to rank the contribution of trait towards the desirable plant response in a given environment is crucial. Moreover, earliness and drought tolerance are farmers' preferred trait and a key factor to enhance adoption of improved sorghum varieties in Eastern lowlands of Ethiopia (Mekbib, 2008).

In order to exploit the most from root angle trait in maximizing water uptake under drought condition, it is imperative to be complemented with appropriate shoot characteristics associated with high yield. Ethiopian early maturing sorghum genotypes have never been studied for their response to drought adaptation in relation to root angle and possible association with shoot phenotype. Hence the objectives of this research are to characterize early maturing sorghum genotypes under moisture stress and non-stress environments and to evaluate the effect of root angle for drought tolerance.

MATERIALS AND METHODS

Experimental location

Two experiments (stressed and non-stressed) were conducted at the field of Werer Agricultural Research center in the 2018 off season. The center is located in Eastern Ethiopia Afar region (9°16'8" N, 40° 9'41"E and with altitude of 750 m.a.s.l).

Experimental materials

The experiment was conducted using 23 early maturing sorghum genotypes (Table 1). The genotypes were selected based on their root angle data generated at Jimma University College of Agriculture and Veterinary Medicine greenhouse (Menamo et al., 2017).

Experimental design and management

The experiment was carried out using randomaized complete block design under two moisture regimes (non-stress and stress conditions) and each of individual experiment was replicated twice. Irrigation was applied every eleven days according to the area recommended for sorghum crop; thus the two moisture regimes were achieved by ceasing irrigation at early booting stage before flowering for stressed block to induce post-flowering drought; while non-stressed block received sufficient irrigation until maturity. Fertilizer application and all other agronomic practices were done following the recommendation for the area.

Data collection

Data collected on plot basis

Days to 50% flowering (DF), Days to 75% physiological maturity

Table 1. Early maturing sorghum genotypes with root angle.

Genotype	Root angle (°)	Maintainer	Genotype	Root angle (°)	Maintainer
76T1#23	22.61	M.ARC/EIAR	Emahoye	25.17	P.ARC/EIAR
Birhan	21.66	S.ARC/ARARI	Misikir	15.91	S.ARC/ARARI
B-35	15.81	M.ARC/EIAR	Meko-I	15.56	M.ARC/EIAR
ETSL100674	13.75	M.ARC/EIAR	SC103-14E	16.53	M.ARC/EIAR
Macia	16.24	M.ARC/EIAR	Teshale	13.03	M.ARC/EIAR
A2267-2	17.88	M.ARC/EIAR	Abshir	26	M.ARC/EIAR
Dekeba	26.75	M.ARC/EIAR	ICSV 93046	18.28	M.ARC/EIAR
E36-1	19.04	M.ARC/EIAR	ICSV745	18.22	M.ARC/EIAR
ESH-1	20.09	M.ARC/EIAR	Melkam	17.96	M.ARC/EIAR
ESH-3	17.34	M.ARC/EIAR	Khwangphang	15	M.ARC/EIAR
Girana-1	22.08	S.ARC/ARARI	ICSV700	22.5	M.ARC/EIAR
ICSR14	20.75	M.ARC/EIAR			

M: Melkasa, S: Sirinka, P: Pawe, ARC: Agricultural Research Center, EIAR: Ethiopian Institute of Agricultural Research, ARARI: Amhara Agricultural Research Institute.

(DM), Seedling vigor (SVG), Stay-green (SG), Over all plant aspect (PAS), Drought Score (DRS): under stressed environment, Leaf senescence (LSC), Disease Score (Dis), Grain filling period (GFP), Grain filling rate (GFR): kg ha^{-1} day^{-1}, above ground biomass (AGBM): kg ha^{-1}, Harvest index (HI) in percentage, Thousand grain weight (TGW): in grams at moisture content were adjusted to 12% and Grain yield (YLD) kg ha^{-1}.

Data collected on plant basis

Five randomly selected plants were pre-tagged to collect all the plant basics data in the plot: plant height (PH) in cm, Panicle length (PL) in cm, Panicle weight (PW) in g, Panicle exertion (PEX) in cm, Flag leaf area (FLA) (Stickler et al., 1961), chlorophyll content (SPAD reading) using Chlorophyll Meter SPAD-502 and number of tillers (NT).

Root structures image was taken on both sides of each chamber using Tablet (T-113) by connecting with two digital cameras (CANON SX610 HS) through Wi-Fi. The tablet and camera were connected by camera connect android app. This app helps to take image using Table from camera and controlled the imaging set up and synchronized the imaging of both sides of each root chamber. The images were used to determine the root angle (RA), relative to the vertical plane (Figure 1). RA was taken from the first flush of nodal roots at a distance of 2 cm from the base of the plant (Singh et al., 2011) using *Opengelphoto* software which was designed by the University of Queensland. The observed root angle for each plant was the mean of four observations (left and right of each plant for both sides of the chamber). All the data were collected based on sorghum descriptors (IBPGR/ICRISAT, 1993) and the method adopted by National Sorghum Improvement Program of Ethiopia by using 'Fieldscorer 4 Android' software.

Soil samples like soil texture, soil pH, organic carbon, bulk density, water retention at field capacity (FC) and permanent wilting point (PWP) were taken. Moreover soil moisture contents were taken three times at booting stage, grain filling stage and at maturity stage. Six soil samples diagonally from 30 cm depths were collected from each replication to estimate soil moisture content by using gravimetric method as described by Klute (1986). Meteorological data such as minimum and maximum temperature, rainfall, sunshine hours and relative humidity were recorded. Data analysis were carried out using SAS statistical version 9.2 (SAS, 2009) and Minitab version 17.1.0.0 (2013) packages.

RESULTS AND DISCUSSION

Analysis of variance and mean performance of genotypes

Phenological and growth traits

Significant variation among genotypes was observed for flowering and maturity date under both moisture environments (Table 2). Days to flowering ranged from 58 to 68 and 59 to 66 days after emergence (DAE) in non-stressed and stressed environments, respectively with mean value of 62 days for each (data not shown). Days to physiological maturity ranged from 96 to105 DAE in non-stressed and from 95 to 101 DAE in a stress environment. Maturity date was earlier for stressed environment as a result of interaction effect which upshot early maturing genotypes to escape the moisture stress by utilizing the maximum stored water under post-flowering drought (Kadam et al., 2002). Similar findings were reported by El Naim et al. (2012) and Yaqoob et al. (2015) in sorghum.

Growth traits viz seedling vigor, plant height, panicle exertion, plant agronomic aspect, flag leaf area, number of productive tiller and disease reaction revealed highly significant variation among genotypes under both environments (Table 2).

The mean values for plant height were 188.2 cm for non-stressed and 175.5 cm for stressed environments. Therefore, here an average of 7% reduction (%R) was observed due to post-flowering drought (Table 3). Similar observation was made by Khaton et al. (2016) and Menezes et al. (2014) on sorghum. According to Ali et al. (1999) decrease in plant height might be due to the

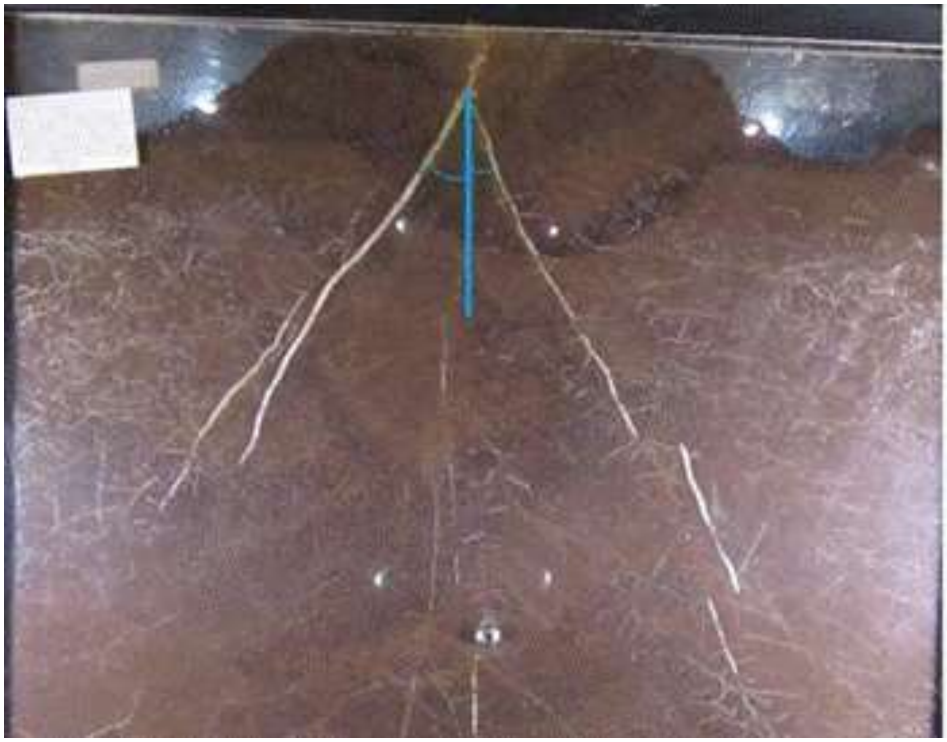

Figure 1. Root angle measuring methods at 5th leaf stage relative to the vertical line.
Source: Menamo et al. 2017.

Table 2. ANOVA and mean squares of traits for sorghum genotypes under non-stressed and stressed environments.

Traits	Mean square					
	Non-stressed			Stressed		
	Geno	Error	CV (%)	Geno	Error	CV (%)
Days to flowering	23.15**	0.81	1.45	9.51**	1.83	2.18
Days to maturity	15.01**	2.23	1.51	7.35**	1.72	1.34
Grain filling period	2.80	1.88	3.73	2.47	2.11	4.07
Seedling vigor	0.44*	0.22	22.59	0.52**	0.11	15.51
Plant height	7199.79**	100.81	5.34	6417.49**	74.47	4.92
Plant aspect	0.74**	0.20	15.27	0.70**	0.18	13.10
Flag leaf area	11021.6**	3727.84	27.42	13686.4**	2315.19	19.14
Panicle exertion	113.77**	1.34	16.56	74.09**	0.81	19.45
Number of tillers	0.52**	0.01	29.54	0.29**	5.93	2.34
Disease	0.06	0.06	11.13	0.54*	0.26	19.61
Grain yield	3234791.84**	298384.07	11.09	2315971.5**	222288.5	12.12
Grain filling rate	2485.69**	307.75	13.06	1841.3**	192.84	12.71
Tausand grain weight	57.82**	2.63	4.43	74.56**	9.81	10.01
Above ground biomass	136216211**	11476375	17.72	72877882**	6213106	15.98
Harvest index	144.70**	28.33	18.48	150.62**	15.74	14.39
Panicle length	36.79**	1.76	4.79	37.37**	3.15	6.69
Panicle weight	1626.34**	257.90	13.94	1282.8**	420.35	19.96
Stay green	0.86*	0.33	21.79	0.87**	0.20	14.62
Lefe scenecence	1.35**	0.46	21.47	2.68**	0.85	21.18
chlorophyll content	33.94	25.41	8.59	43.65*	19.27	9.00
Drought score	-	-	-	0.76*	0.29	17.34
Root angle	29.4**	0.1	1.67	-	-	-

Table 3. Means of yield and other important agronomic traits of sorghum genotypes under non-stressed (NS) and stressed (DS) environments.

Genotype	RA	GYLD		PW		TGW		SG		SPAD		PH	
	-	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
76T1#23	22.61^{d}	5432.1^{b-g}	4409.8^{b-e}	112.31^{c-g}	96.82^{b-e}	37.58^{ef}	34.00^{a-g}	3.0^{a-c}	2.5^{c-e}	59.36^{ab}	52.83^{b-d}	153.5^{hg}	144.0^{e-g}
Birhan	21.66^{e}	4507.2^{f-h}	4021.8^{b-g}	103.73^{d-g}	98.29^{b-e}	40.35^{a-e}	36.61^{a-d}	2.0^{dc}	3.0^{b-d}	62.38^{ab}	51.08^{b-d}	126.5^{ij}	112.0^{hi}
B-35	15.81^{l}	3602.1^{hi}	3192.4^{f-j}	75.39^{hg}	74.00^{d-f}	34.07^{fg}	35.95^{a-e}	1.3^{d}	1.5^{e}	66.20^{a}	65.23^{a}	108.5^{jk}	97.5^{ij}
ETSL100674	13.75^{n}	3159.2^{i}	2689.0^{h-j}	124.87^{a-f}	114.15^{a-e}	23.39^{h}	15.83^{i}	2.0^{dc}	2.0^{ed}	59.89^{ab}	45.17^{cd}	280.0^{a}	255.0^{a}
Macia	16.24^{kl}	4705.8^{e-h}	3743.6^{c-h}	95.16^{e-g}	95.55^{b-e}	31.15^{g}	30.06^{c-h}	2.5^{b-d}	3.0^{b-d}	58.30^{ab}	51.82^{b-d}	130.0^{ij}	120.5^{h}
A2267-2	17.88^{ji}	5325.2^{b-g}	2613.9^{ij}	120.47^{b-f}	114.44^{a-e}	40.98^{a-e}	29.67^{d-h}	4.0^{a}	4.3^{a}	54.06^{ab}	50.40^{b-d}	270.5^{a}	249.0^{a}
Dekeba	26.75^{a}	5903.1^{a-e}	4833.9^{a-c}	143.11^{a-c}	111.07^{a-e}	33.20^{g}	28.41^{e-h}	2.5^{b-d}	2.5^{c-e}	55.82^{ab}	50.38^{b-d}	134.0^{hi}	129.0^{f-h}
E36-1	19.04^{h}	4449.4^{gh}	3626.8^{d-i}	86.78^{fg}	82.90^{c-f}	31.32^{g}	26.03^{h}	1.5^{a-c}	2.5^{c-e}	61.52^{ab}	58.41^{ab}	135.0^{hi}	125.0^{gh}
ESH-1	20.09^{g}	5809.2^{a-f}	4803.1^{a-c}	121.30^{b-f}	94.42^{b-e}	38.30^{ed}	31.81^{a-h}	2.5^{b-d}	3.0^{b-d}	59.03^{ab}	52.84^{b-d}	177.0^{ef}	169.0^{d}
ESH-3	17.34^{j}	4550.0^{f-h}	3471.5^{e-i}	86.47^{fg}	65.31^{ef}	34.58^{fg}	27.50^{f-h}	2.3^{b-d}	3.0^{b-d}	61.65^{ab}	47.89^{b-d}	167.5^{e-g}	155.0^{de}
Girana-1	22.08^{ed}	6070.8^{a-d}	4730.8^{a-d}	146.20^{a-c}	113.70^{a-e}	42.49^{ab}	38.32^{ab}	3.5^{ab}	3.8^{ab}	55.11^{ab}	42.94^{cd}	234.0^{dc}	227.5^{b}
ICSR14	20.75^{f}	4805.5^{d-h}	4041.6^{b-g}	110.69^{c-g}	100.82^{a-e}	41.87^{a-d}	32.84^{a-h}	3.5^{ab}	4.0^{ab}	55.68^{ab}	50.79^{b-d}	138.5^{hi}	131.5^{f-h}
Emahoye	25.17^{c}	4983.0^{c-g}	4248.5^{b-f}	109.52^{c-g}	101.51^{a-e}	42.23^{a-c}	39.06^{a}	2.5^{b-d}	3.0^{b-d}	51.85^{b}	49.63^{b-d}	237.0^{dc}	208.5^{c}
Misikir	15.91^{kl}	5578.0^{b-g}	4392.7^{b-e}	129.90^{a-e}	127.29^{a-c}	38.50^{c-e}	33.28^{a-h}	3.0^{a-c}	3.5^{a-c}	55.87^{ab}	53.61^{bc}	224.5^{d}	208.5^{c}
Meko-I	15.56^{lm}	6415.2^{ab}	4494.9^{b-e}	121.84^{c-f}	117.78^{a-d}	43.94^{a}	37.60^{a-c}	2.5^{b-d}	3.3^{a-c}	62.93^{ab}	49.31^{b-d}	184.5^{e}	174.0^{d}
SC103-14E	16.53^{k}	2728.4^{i}	2240.8^{j}	75.31^{gh}	66.72^{ef}	31.17^{g}	28.70^{e-h}	2.5^{b-d}	3.0^{b-d}	60.74^{ab}	45.26^{cd}	100.5^{k}	90.0^{j}
Teshale	13.03^{o}	6355.2^{ab}	4690.0^{a-d}	140.73^{a-d}	109.60^{a-e}	37.35^{ef}	31.14^{b-h}	3.0^{ab}	3.8^{ab}	59.11^{ab}	48.80^{b-d}	247.5^{bc}	236.5^{ab}
Abshir	26.00^{b}	4878.4^{c-h}	4514.3^{b-e}	128.54^{a-e}	127.14^{a-c}	39.68^{b-e}	36.92^{a-d}	3.0^{a-c}	3.5^{a-c}	65.11^{a}	52.70^{b-d}	136.5^{hi}	124.5^{gh}
ICSV 93046	18.28^{i}	4486.9^{f-h}	3004.6^{g-j}	121.56^{b-f}	99.64^{a-e}	37.82^{ef}	26.44^{hg}	3.0^{ab}	4.0^{ab}	54.65^{ab}	42.75^{d}	282.5^{a}	253.5^{a}
ICSV745	18.22^{i}	6981.7^{a}	5136.7^{ab}	163.03^{a}	140.63^{ab}	40.14^{a-e}	36.80^{a-d}	3.0^{ab}	3.0^{b-d}	62.52^{ab}	58.07^{ab}	181.0^{e}	171.5^{d}
Melkam	17.96^{ij}	6164.3^{a-c}	5677.3^{a}	137.43^{a-d}	123.13^{a-d}	41.15^{a-e}	34.77^{a-f}	2.0^{dc}	2.5^{c-e}	61.65^{ab}	57.95^{ab}	155.5^{f-h}	146.5^{ef}
Khwangphang	15.00^{m}	1585.8^{j}	911.0^{k}	44.05^{h}	38.82^{f}	25.96^{h}	16.85^{i}	3.0^{ab}	3.0^{b-d}	50.62^{b}	42.31^{d}	260.0^{ab}	253.5^{a}
ICSV700	22.50^{d}	4822.0^{d-h}	3971.9^{c-g}	155.89^{ab}	148.97^{a}	34.20^{fg}	31.09^{b-h}	2.5^{a-c}	3.5^{a-c}	55.58^{ab}	48.28^{b-d}	263.0^{ab}	253.5^{a}
Mean	**19.05**	**4926.02**	**3889.60**	**115.40**	**102.73**	**36.58**	**31.29**	**2.6**	**3.1**	**58.68**	**50.80**	**192.4**	**175.5**
%R	**-**	**18.96**		**10.8**		**14.47**		**17.36**		**13.42**		**6.75**	

Where %R=relative percentage reduction and trait abbreviation as described in materials and methods.

reduction in cell division, cell elongation and cell enlargement caused by the stress factor. Mean minimum value for panicle exertion was 0 for both environments and mean maximum was 37.5 and 27.0 cm for non-stressed and stressed environments, respectively. Mean panicle exertion was 7.0 and 4.62 cm for non-stressed and stressed environments implying 34%R due to post flowering drought (data not shown). The result concurs with the finding of Malala (2010), Sakhi et al. (2014) and Abraha et al. (2015).

The highest mean flag leaf area was observed from genotypes ICSR14 (351.57 cm^2) and Misikir (390.80 cm^2) while genotype Kwangphang was found to be the least (70.14 and 90.30 cm^2) under non-stressed and stressed environments, respectively. The mean value for flag leaf area showed 14% increase under stressed environment in comparison with non-stressed environment. Most of the genotypes showed significant increase in flag leaf area along the stress induction except ETSL100674, A2267-2 and SC103-14E which exhibited susceptible phenotype and poor yield performance under stress. In contrast, the highest increase was observed for the genotype Melkam which is relatively high yielder in both environments. Therefore, higher value for flag leaf area is associated with higher yield under moisture stress and could serve as an indicator for drought tolerance (Ali et al., 2009; Ali et al., 2010). Comparatively, Surwenshi et al. (2007) indicated that tolerant sorghum genotypes had greater leaf area and longer active leaf area duration under post-flowering drought.

Of the twenty-three genotypes tested, half of them exhibited tillering capacity under non-stressed condition whereas under stressed condition, only three genotypes Kwangphang, SC103-14E and A2267-2 tillered and performed poor as well. Tillering was reduced by average of 72% due to the stress and thus low tillering ability could serve as drought adaptive mechanism (Richards et al., 2002; Abraha et al., 2015).

Yield and yield components

The variation for grain yield and yield components were highly significant among twenty three sorghum genotypes on both moisture environments (Table 2). The highest grain yield was recorded for genotypes ICSV745 (6981.7 kg/ha), Meko-1 (6415.2 kg/ha) and Teshale (6355.2 kg/ha) with the mean value of 4926.0 kg/ha for non-stressed environment. Mean yield under stressed environment was 3889.6 kg/ha and genotypes Melkam, ICSV745 and Dekeba revealed 5677.3, 5136.7 and 4833.9 kg/ha, respectively to be good yielder and drought tolerant. The least yield performance under both environments was observed for genotypes Kwaangphang and SC103-14E. The mean grain yield of genotypes was reduced by 21% as stress induced and it is regarded as stress intensity of 0.21. High yield reduction or drought susceptibility was observed for genotypes A2267-2, Kwangphang and ICSV93046. On the other hand, genotypes Abshir, Melkam, Birhan and B-35 which is tolerant showed low yield reduction and less affected by drought. Post-flowering drought highly affected the yield of sorghum and similar findings were reported by Menezes et al. (2014), Khaton et al. (2016), Hamza et al. (2016) and Sory et al. (2017). Yield loss under drought condition could be driven by stomatal conductance and concomitant lowering of photosynthesis rate, smaller active leaf area and higher rates of leaf senescence coupled with altered assimilate partitioning between plant parts and reduction in both grain numbers per panicle and thousand seed weight (DaMatta et al., 2003; Naserian et al., 2007; Prasad et al., 2008).

The mean value was 28.2 and 26.5 cm for panicle length and 115.4 and 102.7 cm for panicle weight under non-stressed and stressed environments, respectively.

The stress induced resulted in an average reduction of 4.6 and 10.8% panicle length and panicle weight, respectively. This result coincided with the findings of Sakhi et al. (2014), Sara, (2015), Khaton et al. (2016) and Hamza et al. (2016) on sorghum moisture stress experiments. Thousand seed weight ranged from 23.4 to 43.9 g with mean value of 36.6 g under non-stress environment while the performance in stressed environment were from 15.8 to 39.1 g with mean of 31.3 g. Thousand seed weight of Meko-1, Emahoy and Girana-1 genotypes affected less by the stress factor. Terminal drought affects sorghum grain weight thereby grain yield and it could be triggered by the lessening in rate and productivity of photosynthesis and altered assimilate partitioning (Khaton et al., 2016; Menezes et al., 2014; DaMatta et al., 2003). Also, Assefa et al. (2010) and Prasad et al. (2008) explained the reduction in thousand seed weight as the main cause for lower grain yield in sorghum under drought condition.

Grain filling rate is expressed as kg ha^{-1} day^{-1} and shows the average weight gain per hectare once genotype achieves within a day throughout the grain-filling period. Under non-stressed environment genotypes Kwangphang and ICSV745 revealed 41.7 and 191.4 kg ha^{-1} day^{-1} to be the lowest and highest, respectively. Genotypes Kwangphang and Melkam were the lowest and the highest by having 25.7 and 153.4 kg ha^{-1} day^{-1}, respectively for the stressed environment. Mean performance was reduced by 19% due to drought stress which disrupts the soil moisture status in turn affected the sink-source balance between plant parts resulting in lower grain filling rate thereby grain yield (Okamura et al., 2018). Moreover, drought is one of the limiting factors of yield by affecting the rate of grain filling and decreased yield per panicle of plants (Rahman and Yoshida, 1985). Aboveground biomass shows the accumulation of photosynthetic product while harvest index indicates the partitioning of assimilates to economical yield or in our case grain yield (Sinclair, 1998). The mean aboveground biomass weight was 19115.9 kg ha^{-1} for non-stressed and 15599.0 kg ha^{-1} for stressed environment. Drought had greater impact on biomass production of sorghum genotypes and this finding is in agreement with Hamza et al. (2016) and Abraha et al. (2015). Also, drought reduced the harvest index of the majority of genotypes and this finding is in conformity with Majid et al. (2010) and Malala. (2010). On the contrary, Deblonde and Ledent (2000) suggested that moderate drought conditions did not influence harvest index.

Traits for drought tolerance evaluation

Significant ($P \leq 0.05$) and highly significant ($P \leq 0.01$) variation were observed among genotypes for stay green trait under non-stressed and stressed environments, respectively. Genotypes B-35, E-36, and Melkam showed higher stay greenness and lower leaf senescence at

Table 4. Genotypic (above diagonal) and phenotypic (below diagonal) correlations of grain yield with other traits under non-stressed environment.

Traits	SVG	YLD	GFR	TGW	AGBM	PEX	NT	PW
SVG		-0.444*	-0.421*	-0.132	-0.244	0.458*	0.38	-0.263
YLD	-0.312*		0.993**	0.729**	0.345	-0.598**	-0.427*	0.789**
GFR	-0.298*	0.988**		0.734**	0.399	-0.611**	-0.41	0.798**
TGW	-0.142	0.698**	0.699**		0.126	-0.417*	-0.327	0.463*
AGBM	-0.221	0.352*	0.404**	0.131		-0.428*	0.077	0.68**
PEX	0.386**	-0.564**	-0.567**	-0.405**	-0.399**		0.492*	-0.649**
NT	0.284	-0.405**	-0.384**	-0.306*	0.063	0.478**		-0.286
PW	-0.219	0.741**	0.753**	0.415**	0.658**	-0.594**	-0.261	

*, ** Significant at 5 and 1% level of probabilities, respectively and traits abbreviation as described in material and methods.

maturity under both environments. On the other hand, genotype A2267-2 found to be senescent type under both environments. The lowest scores (stay greenness) were 1.25 and 1.5 for B-35 genotype under non-stressed and stressed moisture regimes, respectively. The introduced drought brings about 17.4 and 35.6% performance reduction for stay green and leaf senescence traits, respectively.

Genotypes varied significantly for chlorophyll content and drought score for stressed environment only (Table 2). The lowest chlorophyll content (SPAD reading) was recorded by Kwangphang and the highest reading was recorded from B-35 and E-36 (stay green parents) and ICSV745, and Melkam genotypes which revealed drought tolerance according to the current study. The chlorophyll content (SPAD reading) for stressed environment ranged from 42.3 to 65.2 with mean value of 50.8. Also, mean performance was reduced by 13.4% as drought induced. Several authors reported performance reduction of sorghum genotypes for chlorophyll content (SPAD reading), stay greenness, leaf senescence and drought score due to post-flowering drought (Kassahun et al., 2010; Sara, 2015; Abraha et al., 2015; Sory et al., 2017).

According to Smart (1994), moisture stress in plants results in closing of stomata, inhibition of photosynthesis, cell division, wall and protein synthesis; however, chloroplast is the first organelle to break down under drought condition. Lichtenthaler et al. (1998) further describes the damage on chloroplast is less likely to happen in tolerant sorghum genotypes than the susceptible ones due to magnesium in their cells. Stay green genotypes maintain chlorophyll concentration, contribute to longevity of leaves, high relative water content (Razakou et al., 2013), maintenance of greenness and absorption of more nitrogen and delay in chloroplast protein degradation under drought condition (Kamran et al., 2014). However, since drought tolerance is a complex trait controlled by many genes and is dependent on the timing and severity of the stress (Ludlow and Muchow, 1990), leaf chlorophyll content alone does not assure sufficient yield under post-flowering drought condition. Therefore, introgression of these traits to adaptable and high yielding genotypes could have paramount importance for drought tolerance breeding. Accordingly, genotypes B-35, E-36, ICSV745 and Melkam could be utilized as a parent (Table 3). The variability of genotypes for traits related to leaf chlorophyll content was found to be higher for the stressed environment which exhibited an association between chlorophyll content and available soil moisture. Therefore, the testing environment has imperative importance in ease of selection for drought tolerance breeding.

Genotypic and phenotypic correlation of grain yield with other traits in stressed and non-stressed environments

The magnitude of correlation was higher for genotypic correlation than phenotypic correlation in non-stressed environment which describes the heritable association of the characters (Johnson et al., 1955). Grain yield had significant and strong positive genotypic correlation coefficients with grain filling rate, thousand grain weight and panicle weight (Table 4). The positive and significant correlation indicates that simultaneous selection of these traits under non-stressed moisture condition will bring significant yield advantage on sorghum. Correlation analysis showed that grain yield had negative significant correlation with seedling vigor but, as the data scoring was in descending order (1=vigorous; and 5=less vigorous), the association remains positive. Chalachew et al. (2017) reported that grain yield was positively associated with thousand seed weight, biomass yield, and panicle weight in sorghum. Other reports also showed the correlation of yield with thousand seed weight and panicle weight in sorghum (Tesso et al., 2011; Amelework, 2012).

Under stressed environment, phenotypic correlation was found to be higher in magnitude which depicts higher degree of unheritable environmental effect as a result of induced post flowering drought. Grain yield had a positive significant genotypic correlation coefficient with chlorophyll content (SPAD reading), flag leaf area, grain

Table 5. Genotypic (above diagonal) and phenotypic (below diagonal) correlations of grain yield with other traits under stressed environment.

Traits	SVG	YLD	SPAD	FLA	GFR	TGW	HI	PEX	NT	PW	RA
SVG		-0.39	0.01	-0.34	-0.40	0.04	-0.18	0.46*	0.51*	-0.07	-0.04
YLD	-0.36*		0.34*	0.56**	0.99**	0.70**	0.46*	-0.53**	-0.66**	0.61**	0.38*
SPAD	0.05	0.42*		0.45*	0.39	0.43*	0.52*	-0.10	-0.37	0.16	0.03
FLA	-0.27	0.59**	0.26		0.55**	0.35	0.35	-0.49*	-0.50*	0.43*	0.07
GFR	-0.37*	0.99**	0.33*	0.52**		0.68**	0.49*	-0.58**	-0.69**	0.68**	0.40*
TGW	0.001	0.71**	0.37*	0.34*	0.67**		0.52*	-0.33	-0.53**	0.44*	0.43*
HI	-0.19	0.51**	0.39**	0.32*	0.44**	0.47**		-0.08	-0.26	-0.13	0.26
PEX	0.39**	-0.56**	-0.09	-0.44**	-0.55**	-0.31*	-0.07		0.77**	-0.65**	-0.29
NT	0.46**	-0.69**	-0.32*	-0.46**	-0.65**	-0.50**	-0.25	0.76**		-0.58**	-0.26
PW	-0.05	0.67**	0.16	0.31*	0.60**	0.39**	-0.17	-0.58**	-0.50**		0.28
RA	-0.03	0.40**	0.03	0.06	0.38**	0.40**	0.23	-0.29*	-0.26	0.25	

*, ** Significant at 5 and 1% level of probabilities, respectively and traits abbreviation as described in material and methods.

filling rate, thousand grain weight, harvest index, panicle weight and root angle (Table 5).

As these traits have a positive significant correlation with yield, breeding for drought tolerance in sorghum should consider higher value of these traits in developing varieties for moisture stress areas. In agreement with these results previous finding by other workers indicated significant positive correlation of grain yield with chlorophyll content (SPAD reading), thousand grain weight and harvest index by Kumar et al. (2013); thousand grain weight, panicle weight and harvest index by Chalachew et al. (2017) in sorghum. Similarly, Kamran et al. (2014) reported chlorophyll content to have a positive correlation with grain yield. Moreover, Khaliq et al. (2008) in bread wheat and Ali et al. (2009) in sorghum also observed the positive association of flag leaf area and grain yield in moisture stress experiment. This suggests utilization of traits through selection which showed positive correlation with yield could be important if adopted as breeding strategy to increase yield in moisture stress area. More importantly, harvest index, chlorophyll content, root angle and flag leaf area showed significant positive correlation with yield under stressed environment only. Therefore, these traits could be used as morphological marker for screening of drought tolerant sorghum genotypes.

On the other hand, panicle exertion and number of fertile tiller revealed significant negative genotypic and phenotypic correlation with grain yield under both environments. Even if the two traits had a desirable character in sorghum they do have yield penalty by consuming greater assimilates which could be allocated to grain yield. Hence, considering lower value for these traits could bring significant yield advantage on sorghum. Comparable result was reported by Richards et al. (2002) for fertile tiller.

Effect of root angle for drought tolerance

Highly significant variation was observed among sorghum seedlings in root angle (Table 2). Therefore, genotypic variability for root angle trait will give us an opportunity for selection of sorghum tolerant to drought. From the 23 genotypes tested the widest mean root angle was observed for Dekeba and Abshir which revealed 26.75 and 26.0°, respectively and the narrowest was observed from Teshale: 13.0° and ETSL100674: 13.75° with mean and standard deviation of 19.05 and 3.84°, respectively (Table 3).

Positive significant correlation coefficient of root angle with yield and some yield component were observed under stressed environment and no significant association of any trait under non-stressed environment (Table 5). Similar results were reported by Pandey et al. (2015) and Ali et al. (2015). Under stressed condition, significant genotypic and phenotypic correlation were observed between root angle with yield, grain filling rate and thousand grain weight (Table 5). The associations observed were weak (<0.43) for all these traits, therefore, intermediate to slightly wider root angle had a synergic effect on drought tolerance of sorghum under silty clay soil condition. In conformity with this result, Fenta et al. (2014) observed soybean genotype with intermediate root angle was the most drought-tolerant cultivar under irrigated as well drought environments.

Comparably, Mace et al. (2012) indicated a possible association between nodal root angle and sorghum yield in the study of QTL. According to Singh et al. (2012) wide nodal root angle of sorghum could potentially enhance access to water through more horizontal root system and higher root biomass in the upper soil surface that would be advantageous to extract water from inter-row spaces; this contributes to better grain yield. In contrast, Pandey et al. (2015) observed consistence negative correlation of root growth angle with grain yield in managed drought, irrigation and rainfed wheat experiment under sandy loam soil; while no correlation was observed under silt loam soil condition. These could be as a result of soil compaction layer which limits vertical growth of the roots. Moreover, Malamy (2005) observed the variation in the expression of root traits under different soil and rainfall condition.

Early maturing sorghum genotypes had lesser root weight in comparison to late ones because more assimilate is partitioned to shoot growth to escape the stress by completing life cycle (Matthews et al., 1990). Therefore, as the root growth for early maturing sorghum genotypes is limited, its advantage to have it on the upper surfaces means moderate to wider root angle for effective top soil foraging. These suggested that under stressed environment moderate to slightly wider root angle was associated with high yield by increasing the efficiency of the root system in capturing lateral available soil resources. However, the importance of soil textural class should be kept in mind in exploiting root angle trait for drought adaptation.

Conclusion

The variation observed among sorghum genotypes in most of the traits gives opportunity for further improvement by selection and hybridization. Post-flowering drought reduces grain yield and the values for the most of the traits. The goal of the study is to characterize and establish possible selection criteria helpful for screening of sorghum for drought prune environments. Flag leaf area, SPAD, harvest index and root angle traits could be used as morphological marker for sorghum breeding program for moisture stress. The result pointed out the importance of root angle under drought condition and also, the contribution of intermediate to slightly wider root angle for enhanced grain yield under silty clay soil. Genotypes B-35, E36, Melkam and ICSV745 showed better performance in stay green and SPAD chlorophyll reading which is good indicators of drought tolerance traits and could be utilized as a parent. As per the result, genotype ICSV745 utilized maximum soil moisture to give better yield under optimum as well drought environments. In addition, for drought prone environments varieties Melkam and Dekeba are good yielders as well drought tolerant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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