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Full Length Research Paper

Cultural practices and pesticides contamination level of tomato in two gardening sites in the region of Boucle du Mouhoun, Burkina Faso

Abdoudramane SANOU[1*], Kiessoun KONATE[1], Hemayoro SAMA[2], Roger DAKUYO[1], Kabakdé KABORE[1], Mamounata DIAO[1], Crépin Ibingou DIBALA[1] and Mamoudou Hama DICKO[1]

[1]Laboratory of Biochemistry, Food Biotechnology and Nutrition, Department of Biochemistry and Microbiology, University Joseph KI-ZERBO, 03 BP 7021 Ouagadougou, Burkina Faso.
[2]Laboratory of Biochemistry and Applied Chemistry (LABIOCA), Department of Biochemistry and Microbiology, University Joseph KI-ZERBO, 03 BP 7021 Ouagadougou, Burkina Faso.

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The present study consisted of evaluation of cultural practices and pesticides contamination level of tomato in some gardening sites in the region of Boucle du Mouhoun (Burkina Faso). A survey of phytosanitary practices among 30 producers was carried out at the Di and Dedougou sites. The pesticide contamination level of tomatoes collected was determined by the QuEChERS method. The survey revealed a low level of literacy among producers (60%), an increased use of pesticides without adequate protection materials, non-compliance with prescribed doses, use of unregistered pesticides (28%) and poor management of packaging. Chromatophic analysis of the samples detected 16 active substances predominantly organophosphorus (62%). 62.5% of detected compounds (Omethoate, Parathion methyl, Diazinon, Methydathion, Pyridaphethion, Pyrimiphos methyl, Alachlor, 2.4 DDT, lindane, permethrin and Imazalil) are not authorized by the CSP. Among authorized compounds, Dimethoate, Malathion, and Cypermetrin contamination levels higher than the MRLs have been observed. These results confirmed risks of environment contamination and sanitary of farmers and consumers linked to their exposure and consumption of tomatoes. Measures should be taken to raise awareness among market gardeners of good pesticide use practices and the adoption of alternative methods to pesticides to protect environment, gardeners and consumers health.

Key words: *Lycopersicon esculentum*, pesticides, contamination, LMRs, Burkina Faso.

INTRODUCTION

Agriculture is the main occupation for 80% of the active population of Burkina Faso and contributes nearly 40% of the Gross Domestic Product (GDP) (CAPES, 2007). The cultivated areas estimated at 3.6 million hectares are

*Corresponding author. E-mail: sanouabdoudramane@gmail.com.

dominated by cereal crops, cash crops and vegetable crops. Among vegetable crops, the tomato is the second most important crop in terms of area and production. Its production is estimated to 289872 tons/year (Son, 2018). In many countries, the tomato (*Lycopersicon esculentum, Mill.*) is an important vegetable crop grown (Mutari and Debbie, 2011) and highly consumed because of its richness in nutrients especially carotenoids, vitamins and phenolic compounds. These nutriments are very beneficial for human health and intervene into prevention non-communicable diseases (Victoria et al., 2017; Oboulbiga et al., 2017).

In Burkina Faso, vegetables are mainly grown during the dry season (January-June) and the sector faces significant constraints with a high level of exposure to climatic risk, principally drought and high pests' pressure. These constraints can cause high losses in yield. Losses caused by insects and diseases in tomato production can exceed 30% (Toé, 2010), so pesticides are widely applied in agriculture to control pests and to improve yields (Kolia, 2015). However, pollution of water sources and the environment can occur due to several factors including run-off of pesticides applied to plants, the washing of clothes worn by those spraying crops in the vicinity and/or in surrounding watercourses, and cleaning sprayers (Naré et al., 2015). Unintended exposure to pesticides or ingestion of contaminated food can be extremely dangerous to humans, other living organisms and environment as they are designed to be poisonous (Sarwar, 2015).

The contamination pathways are multiple, including direct exposure (farmers for example), eating foods or liquids (water) containing pesticide residue, or inhalation (or contact) of pesticide-contaminated air. Even very low levels of exposure may have adverse health effects at early development (Damalas and Eleftherohorinos, 2011). In those situations, children are the more susceptible to pesticides than adults due to their physical makeup, behavior and physiology (Lehmann et al., 2017). Unfortunately, studies already carried out on certain market gardening sites in Burkina Faso reported the existence of poor phytosanitary practices: non-compliance with doses prescribed, non-compliance with the rules of protection and hygiene during treatments, poor management of the empty pesticide containers (Naré et al., 2015). In addition, the Burkina Faso, Ministry of Health also reported in 2019 the death of dozens of people due to contamination of pesticide residues. The region of *Boucle* of *Mouhoun* is a large tomato production area of which *Di* and *Dedougou* are major market gardening production sites, mainly for tomatoes. This production ensures local consumption and above all exports of tomatoes to various countries including Europe. Its annual tomato production is estimated at 29723 tons/year (MARHASA, 2014). Unfortunately, data on phytosanitary practices, the types of pesticides used and the risks to the health of consumers and the environment are very limited. This study proposes a comprehensive assessment cultural practices and pesticides contamination level of tomato in theses gardening sites.

MATERIALS AND METHODS

Sites description

The study was conducted in March to May 2018 in two gardening sites of the region of *Boucle du Mouhoun* which is one of the 13 regions of the administrative division of Burkina Faso. Located in the northwest of the country, the *Boucle du Mouhoun* region, which capital is Dedougou, occupies about 12% of the national territory, an area of 34,497 km^2. The region is located in the Sudano-Sahelian zone and has two seasons: a long dry season (between 6 and 8 months) and a rainy season (3 to 5 months). The region's economy is essentially based on agriculture and animal husbandry, which occupy about 90% of the population. Market gardening is one of the main counter-season activities in the region. The first collecting site is the market garden site of Di, located in the department of Di and the second site is located in the town of Dedougou (Figure 1). The main crops of the two sites are onion, pepper, green bean, potato, maize, tomato, cabbage and aubergines. Thirty (30) samples of fresh tomatoes were collected from two production sites including *Di* and *Dedougou*.

Field investigations

A survey of 15 tomato producers selected randomly in each site was carried out. The choice of these sites was motivated by the geographical position near the border, to determine whether fraudulent introduction of pesticides had occurred.

Sample collection

For each site, 1 kg of ripe tomato fruit was randomly collected from 15 plots. Tomato samples from the same plot constituted one sample. The tomato samples were labeled and then transported to the laboratory where they were kept at -20°C for the different analyses.

Pesticides extraction and chemical analysis

Pesticides residues in tomatoes were extracted using a modified AOAC 2007.01 QuEChERS (Quick Easy Cheap Rugged and Safe) extraction method. For chromatographic analysis, the samples were washed with distilled water and then crushed and 5 g of each sample was taken from the 50 ml falcon tubes. 5 g of sample were homogenized with 10 mL acetonitrile and vortexed for 1 min. The extraction of samples was realized by centrifugation (3000 tr/min for 5 min) with 1 g of sodium chloride (NaCl), 1 g of sodium citrate (Na Citrate), 0.5 g of Na citrate anhydrous and 4 g of magnesium sulfate ($MgSO_4$). The purification of the extracts was carried out by centrifugation with salts (sulphate of anhydrous sodium) and carbon black graphitized (GCB) to mobilize the coloured substances (chlorophyl and carotene) that are non-active by precipitation. The supernatant obtained from the frozen extract after centrifugation was recovered in a vial using a Pasteur pipette. The analysis of the extracts was carried out using a chromatograph in gas phase (Agilent Technologies) that has a micro-detector that captures electron (GC-µECD/GC-FPD, Hewlett Packard). A capillary

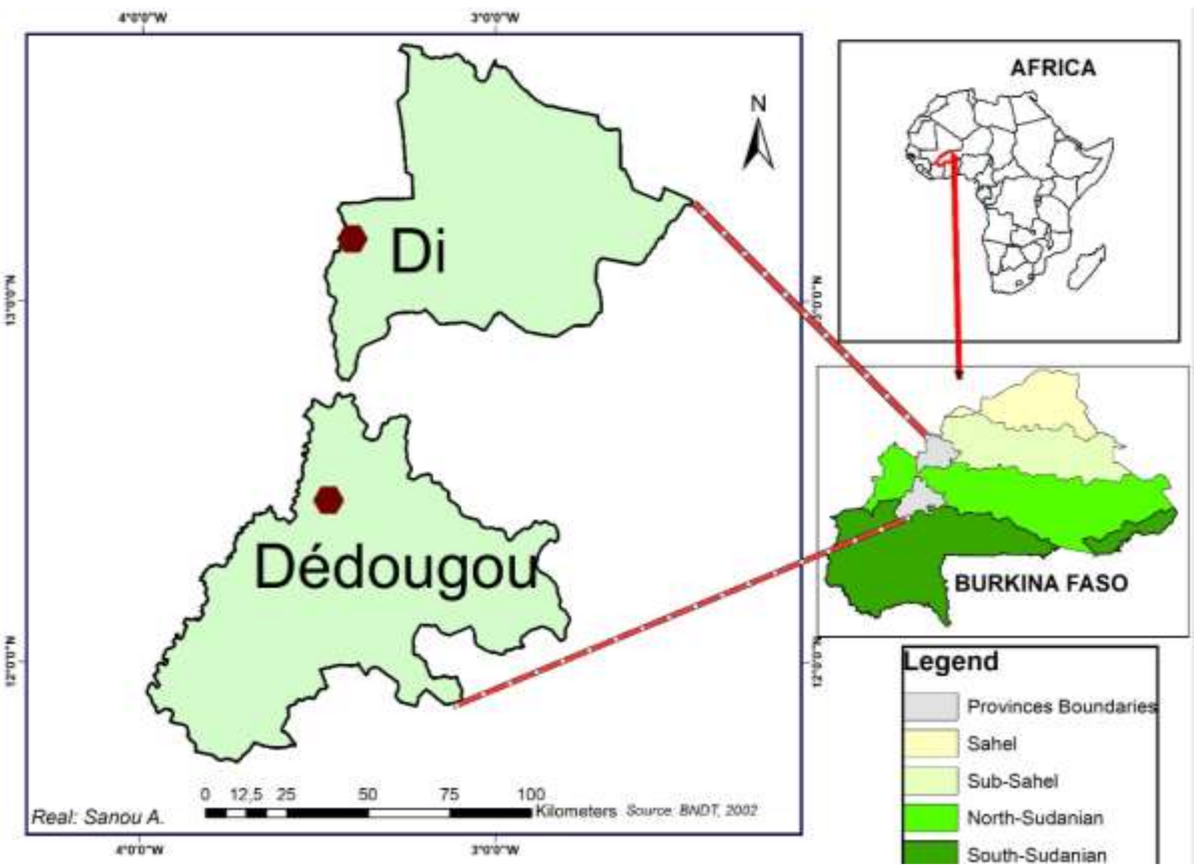

Figure 1. Location of collection sites.

chromatographic column of type dB-17 MS. It had a length of 30 cm, an internal diameter of 250 µm and a thickness of 0.25 µm. Nitrogen of high purity was used as the carrier gas. The injection was carried out using Split/Splitless injection technique with an injection volume of 2 µl. The temperatures of the apparatus were as follows: Room of injector programmed at 275°C with a pressure of 20.72 psi; Column (75°C during 0.5 mn, 75-300°C with a flow of 10°C/mn and 300°C during 7 mn); Detector (325°C).

Statistical analysis

The Tukey's test, the descriptive statistics and the creation of the various graphs were established using Excel and XLSAT software version 2016.

RESULTS

Socio-demographic characteristics

The socio-demographic characteristics of the farmers taken into account in this study are shown in Table 1. The results showed that in the two gardening sites, the met producers were mostly men (93.33%) and their age ranged from 26 to 51 years. 60% of the producers are not educated and only half belong to an agricultural group and therefore did not receive any technical supervision or advisory support from market gardening technicians.

Phytosanitary practices on market gardening sites and risks to health and the environment

To control the main pests of tomatoes, producers generally use synthetic chemical pesticides. In order to characterize the main families of used pesticides and their origin with a view to assessing the health and environmental risks associated with their use, field investigations were initiated. The results showed that all the producers surveyed at both sites used pesticides to control tomato pests' control. The used pesticides were purchased directly from the surrounding markets without any guarantee of conformity and quality or provided by the Society of Fibres and Textile (SOFITEX), for the control of cotton pathogens. The dosage, formulation and application of pesticides are generally done by growers, often their children or their employees, but in some plots the phytosanitary treatment is carried out by women. The amounts of pesticides applied by market gardeners are variable in the two study sites. Surveys revealed that the rates applied ranged from 16 to 48 L per 0.25 ha. Only

Table 1. socio-demographic characteristics of the farmers.

Characteristic	Social status of producers	Proportion (%)
Sex	Man	93.33
	Woman	6.67
Education's level	Uneducated	60
	Primary	33
	Secondary	7
Agricultural group	Associated	50
	Unassociated	50

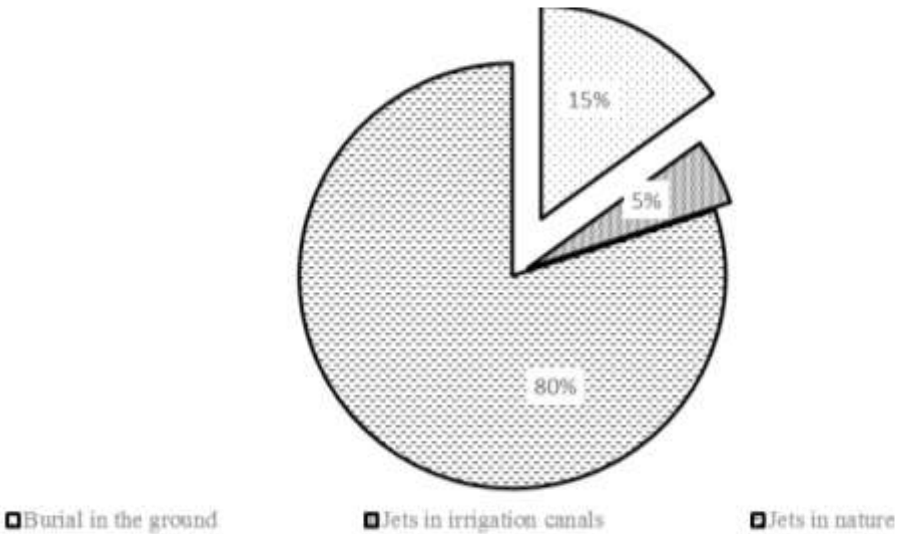

Figure 2. Management of pesticide packaging after use.

26% of producers revealed that they respected the doses prescribed by the manufacturers. The spraying frequencies recorded were 45, 25 and 30% for respectively less than a week, a week and more than a week. The study also revealed that producers observed on average week between the last spray and the harvest. In addition, the study revealed no investigated producer had complete protective equipment and only 33% use rudimentary materials of protection (masks, gloves, etc). As for the management of packaging after treatment, the recorded data (Figure 2) revealed that 80% of the market gardeners threw their packaging into the irrigation canals, 15% buried it in the ground after use and 5% threw it directly into the nature.

The Table 2 presents the main pesticides inventoried on the 2 sites of tomato production. The results showed that all the used pesticides were mostly insecticide. According to target and toxicity, these pesticides belong to categories 2 and 3. 28% of pesticides marketed in these areas are strictly prohibited by the Sahelian Committee of Pesticides (CSP).

Pesticides residues in collected tomatoes and risk of contamination

The analysis of the chromatographic profile of the collected sample permitted the detection and the quantification of 16 target pesticides: Diazinon, dimethoate, Fenitrothion, Malathion, Methyldathion, Omeothoate, Parathion methyl, pyridaphenthion, Pirimifos methyl, profenofos, alachlor, 2.4 DDT, lindane, Cypermetrin, permetrin and imazalil. Among the detected pesticides (Figure 3), the organophosphorus pesticides are the most important (62%), followed by organochlorine (19%), pyrethrinoids (13%) and carbamates (6%). The quantification of the detected pesticides revealed that only the levels of methyl parathion, 2.4 DDT, Alachor and Imazalil varied according to the samples collected sites. Also, it is noteworthy that for some samples, threshold

Table 2. The used pesticides on the 2 sites.

Commercial name	Nature of pesticides	Authorized by CSP	Category of toxicity
ACARIUS 80 EC	Insecticide	no	II
ADUMA WURA	Insecticide	no	II
ATTAKAN 344 EC	Insecticide+ herbicide	yes	II
CAIMAN ROUGE	Insecticide	yes	II
CONQUEST 88 EC	Insecticide	yes	II
COGA 80 WP	Insecticide	yes	III
CYPERCAL 50 EC	Insecticide	yes	III
EMACOT	Insecticide	yes	II
LAMBDA super 25EC	Insecticide	yes	III
PACHA 25EC	Insecticide	yes	II
RAMBO	Insecticide for mosquito/cockroach	no	II
ROUNDUP 360 SL	Insecticide	No	II
TANGO 500 EC46	Insecticide	yes	II
TITAN 25EC	Insecticide	yes	II

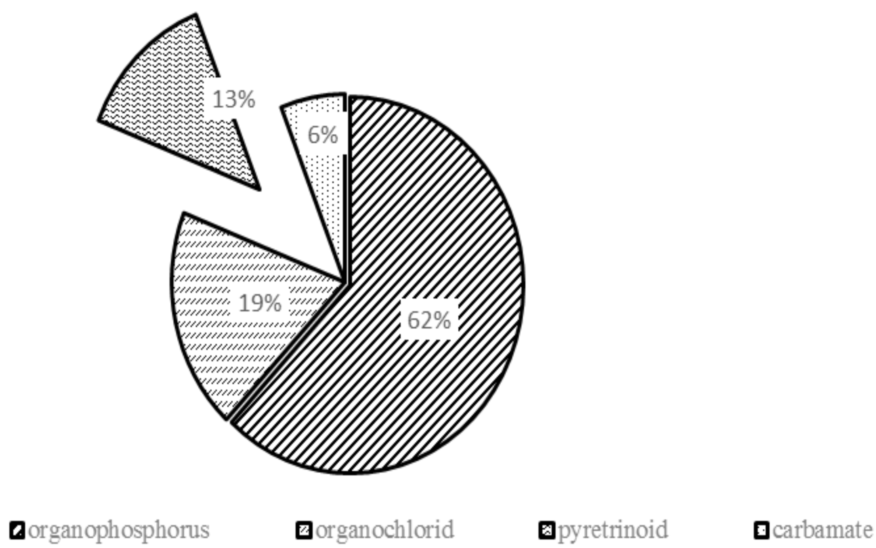

Figure 3. Main families of detected pesticides.

limits proposed by the European Directive 98/83/EC (1998) on the quality of vegetable intended for human consumption were exceeded. Indeed, the levels of 2.4 DDT in tomato samples from the site of Di and Alachore in tomato samples from both sites were above the maximum residue limits (Table 3).

Table 4 reveals that 78% of the active ingredients detected are prohibited by the CSP. Also, it is noteworthy that for some samples, threshold limits proposed by the European Directive 98/83/EC (1998) on the quality of vegetable intended for human consumption were exceeded. Indeed, only 37.5% of medium level of actives compounds detected in tomato samples was lower than the MRLs.

DISCUSSION

The study revealed a low level of education among producers. In addition, the study revealed an insufficient level of training and monitoring of growers on the use of pesticides and dosages. As a result, pesticides are for the most part applied without appropriate means of protection at inappropriate doses. Such practices do not promote the proper use of plant protection products through ignorance and by using the appropriate product according to the target. Similar results were reported by Son et al. (2017) who also reported that the level of education is a determining factor for the mode of application, the persistence, the respect of the expiration

Table 3. Comparison of different active compounds per collection site.

Compound	Site	
Active compounds	**Dedougou**	**Di**
Omethoate	2.01±0.52[a]	2.3±0.01[a]
Parathion methyl	0.05±0.01[a]	0.6±0.02[b]
Dimethoate	0.24±0.15[a]	0.4±0.3[a]
Diazinon	0.8±0.3[a]	0.6±0.1[a]
Fenitrothion	0.12±0.6[a]	0.1±0.03[a]
Malathion	0.07±0.02[a]	0.7±0.02[a]
Methydathion	0.09±0.05[a]	0.13±0.02[a]
Pyridaphenthion	0.45±0.02[a]	0.41±0.26[a]
Profenofos	0.2±0.1	0.23±0.4
Pyrimiphos methyl	0.17±0.21[a]	0.19±0.01[a]
Alachlor	0.06±0.02[a]	0.15±0.03[b]
2.4DDT	0.03±0.02[a]	0.07±0.01[b]
Lindane	0.05±0.01[a]	0.04±0.02[a]
Cypermethrin	0.14±0.03[a]	0.11±0.01[a]
Permetrin	0.1±0.02[a]	0.14±0.03[a]
Imazalil	0.129±0.01[a]	0[b]

Table 4. Authorization and contamination level of different active compounds.

Active compounds	Authorized by the CSP *	Median (ug/kg)	Maximum (ug/kg)	LMR (ug/kg)
Omethoate	No	1.32	2.7	0.01
Parathion methyl	No	0.03	0.32	0.5
Dimethoate	Yes	0.2	0.68	0.01
Diazinon	No	0.23	2.4	0.02
Fenitrothion	Yes	0.07	0.13	1
Malathion	Yes	0.14	0.79	0.02
Methydathion	No	0.08	0.18	0.1
Pyridaphenthion	No	0.18	0.68	0.05
Profenofos	Yes	0.23	0.86	1
Pyrimiphos methyl	No	0.016	0.24	0.01
Alachlor	No	0.15	1.74	0.5
2.4DDT	No	0.05	0.08	0.05
Lindane	No	0.02	0.03	0.01
Cypermethrin	Yes	0.08	1.12	0.05
Permetrin	No	0.11	3.46	0.05
Imazalil	No	0.04	1.01	0.05

*CSP: Comité Sahélien des pesticides (2017).

times as well as the precautions to be taken before, during and after the application of pesticides. According to Kanda et al. (2013) and Wognin et al. (2014), pesticide use requires a minimum of theoretical and practical knowledge to avoid health and environmental risks. Indeed, in the context of the use of pesticides, the doses and instructions for use must be respected. In our case, the majority of growers cannot read or write, so cannot do the calculations to adjust the rates to be applied. They cannot understand the labels on the crop inputs, which are usually written in English or French. This contributes to an increased risk of intoxication and environmental pollution (Son et al., 2017). According to Agnandji et al. (2018) overdosing pesticides during treatment, may cause contamination of vegetables as well as environmental compartments.

The results also showed treatments were carried out without adequate protective equipment. In addition, the packages are thrown into the irrigation canals. Our results corroborate those of Tyagi et al. (2015) in India, Belhadi et al. (2016) in Algeria, Doumbia and Kwadjo (2009) in Côte d'Ivoire and Son et al. (2017) in Burkina Faso who reported that few producers used adequate protective equipment when treating crops with pesticides. Lack of protective equipment and poor management of pesticide wastes are at the root of acute poisoning cases in growers (Lehmann et al., 2017; Son et al., 2017). Lehmann et al. (2017) reported the pollution of certain rivers in Burkina Faso by pesticide residues in market gardening areas. Also, as some water reservoirs may be used for fish farming, there is a risk of contamination of humans through the transfer of pesticide residues as observed in Benin through tilapia under similar conditions (Agbohessi et al., 2012). Potential health effects are of particular concern because of the presence of children and women on pesticide application plots. Various studies have reported an increased risk of disease or malformation in humans, generally related to pesticides (Son et al., 2017). Among women, the possible consequences of exposure during pesticide use are spontaneous abortion and premature and malformed newborns (Multigner, 2005).

The marketing of pesticides is regulated by international and national laws. The source of acquisition of these products is an important parameter in the control of this legislation. The study shows that the local market and SOFITEX are the two sources of procurement of pesticides. Similar results have been reported by Ouédraogo et al. (2016) who reveal that in western Burkina Faso, 85.5% of the phytosanitary products are acquired on the market.

Acquisition of pesticides in market increases the health risks and risk fraud of unauthorized products. This is more worrying because unauthorised products and some pesticides for cotton crops are used in tomato cultivation and can induce health risks. These practices have been observed on several market gardening sites (Son et al., 2017) in Burkina Faso; Agnandji et al. (2018) in Togo and Muliele et al. (2017) in Congo.

Quantitative analysis revealed different active ingredients is not significantly different depending on the production areas with the exception of alachlor, Imazalil and DDT. The analysis revealed the presence of 16 active compounds with a dominance of organophosphorus. These results confirm these of Tarnagda et al. (2017) that has detected 13 actives compounds on *Loumbila* areas. Our results are contrary to those of Son et al. (2017) who show a dominance of organochlorines in western and north region of Burkina Faso. These results could be explained by the diversity of pesticides used depending on the crop areas. The presence of organochlorine active ingredients on the production sites demonstrates the existence of fraud in the local pesticide trade. These organochlorines are strictly prohibited by the Sahelian committee of pesticides because of their toxicity. Among these prohibited products, we note the 2,4 DDT detected in our samples. The particularity of these two organochlorines (alachlor, DDT) highly concentrated in *Di* compared to *Dedougou* could be justified by the proximity of this area of the Malian border. It thus constitutes an area susceptible to pesticide fraud. A comparison of the concentrations of active substances in relation to the maximum residue limits showed worrying levels for 62.5% of the active substances.

Similar results have been obtained by Kolia (2015) for other active ingredients. Bioaccumulation of these active compounds constitutes serious carcinogenic risks.

Conclusion

In order to characterize the cultivation practices of tomato producers from market gardening sites in *Di* and *Dedougou* with a view to assessing their health risks to consumers, the present study was initiated. Investigations carried out on the two sites showed non-compliance with the conditions of use of pesticides, the use of unauthorised pesticides by some producers and poor management of pesticide packaging. These poor agricultural practices are linked to the low level of education and monitoring of producers and present numerous health and environmental risks. In addition, the quantification of pesticide residues in tomato samples from the two tomato sites detected 16 active compounds with a predominance of organophosphorus. These results confirm the health risks associated with the consumption of these tomatoes and raise the urgent need to train market gardeners on the judicious use of synthetic pesticides, manure and chemical fertilizers to guarantee the health of consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of a motorized cassava peeler with four lining materials

Ishmael Nartey Amanor[1*] and Emmanuel Yaovi Hunnuor Bobobee[2]

[1]Department of Mechanical Engineering, Cape Coast Technical University, Cape Coast, Ghana.
[2]Department of Agricultural and Biosystems Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

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Cassava processing requires peeling, which is mainly done manually and not effective for large scale processing, hence, the need to mechanise the peeling process. A motorized cassava peeler with four drum and disc linings (concrete, metal, rubber and wood) was designed. The prototype was tested at three disc speeds; (250, 350 and 500 rpm) to determine peeling capacity, peel removal efficiency, and flesh loss. The capacity for the concrete, metal, rubber and wooden discs increased with increased speed from 180-360, 360-1440, 120-540 and 80-144 kg/h, for speeds 250-500 rpm, respectively. Metal and concrete discs recorded the highest peel removal efficiencies of 75.97 and 78.33%, respectively at 350 rpm. The average flesh loss for concrete, metal and rubber discs increased with increasing speeds from 19.66-26.57, 20.94-34.83% and 7.98-31.06%, respectively for 250-500 rpm speeds. The wooden disc on the other hand had no regular pattern with highest value of flesh loss of 12.95% at 500 rpm. Regarding optimum performance, rubber and concrete discs were better, comparing peel removal efficiency and percent flesh loss for all speeds. The study shows the rotating disc linings had more effect on peeling than the drum linings.

Key words: Cassava, lining material, disc material, evaluation, peeling quality index.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a starchy root crop, with its edible parts being the roots and the leaves (Ferraro et al., 2016). The root part may vary significantly in length from 15-100 cm as well as in terms of weight from 0.5-2.0 kg. The roots are staple food, which is the main source of carbohydrate and energy for the approximately 800 million people living in the tropical and sub-tropical areas of the world (FAO, 2013; Morgan and Choct, 2016). Cassava is a primary food security crop in Africa due to its resistance to drought and plant disease, flexible planting and harvesting cycles. It is cultivated in small farms and often in fields which are left aside as fallow or marginal areas in Africa (Angelucci, 2013).

In the attempt to bridge the food production gap in Ghana, serious attention is being paid to the development and promotion of some traditional starchy staples (Amponsah et al., 2017). As a consequence, cassava is one of the priority staple crops as defined in FASDEP II

*Corresponding author. E-mail: inamanor@gmail.com, ishmael.amanor@cctu.edu.gh. Tel: +233541164732.

together with maize, rice, yam and cowpea (Angelucci, 2013). Ghana is the 5th world producer of cassava, with about 18 million tonnes of cassava of which an estimate of about 68% is available for human consumption (FAO, 2016). In recent times, the crop has found new and profitable uses in industry and contributes 22% of Ghana's Agriculture Gross Domestic Product (AGDP) (OECD-FAO, 2016). Ninety-nine percent of fresh cassava roots available in Ghana goes directly into human food whilst one percent serves other industries like animal feed, textile industry, etc. (Vanhuyse, 2012).

Fresh roots need to be processed within 48 h from harvest (Adebayo et al., 2014). Their deterioration can be delayed through special post-harvest treatments such as waxing or storage in plastic bags after fungicidal treatments, which are all expensive (Meridian Institute, 2009). Available data shows that up to about 34% of the cassava produced in Ghana is lost along the food chain (Office of Grant and Research, 2015). The processing of cassava into more storable forms offers an opportunity to overcome the perishability of the fresh produce (ibid). According to the Food Research Institute (FRI) of the Council for Scientific and Industrial Research (CSIR), the short shelf life of cassava requires efficient marketing, fresh consumption or processing, which manual processing is slow and tedious. Several post-harvest cassava processing (grating, chipping, etc.) had been mechanised successfully, however, there is less success in mechanisation of peeling (Olukunle and Jimoh, 2012). Egbeocha et al. (2016), reported on a lot of mechanical peelers available in Africa, and stated that because of low efficiency and losses, cassava peeling is still a major manual activity. This situation has made it essential to provide an efficient equipment to reduce losses associated with mechanical peeling.

The objective of this study was to evaluate two motorized cassava peelers with four different lining materials (concrete, metal, rubber and wood) using two local cassava varieties (*Asi-Abayiwa* and *Dabon*) at three different disc speeds.

MATERIALS AND METHODS

The two prototype motorized cassava peelers having a batch loading weight of 6 kg were constructed at the Department of Agricultural and Biosystems Engineering, KNUST workshop. The primary material for the construction was mild steel, which was secured from the open market. One was constructed with a fixed concrete lining material and the second prototype fitted with removable metal, wooden, and rubber lining materials. The peelers were tested and evaluated at the department's workshop. The cassava used for the evaluation was purchased freshly after harvest from the *Ejisu* market.

Description of peeler

The electrically operated cassava peeling equipment in Figure 1 was developed.

The peeling equipment consist of a cylindrical drum with an outer diameter of 500 mm, thickness of 3 mm and a depth of 600 mm which is covered at one end by base cover of 5 mm thickness. The drum assembly is supported by three 350 mm long stands, which were made with mild steel pipes with a diameter of 76.2 mm. The base of the stand is welded to a 100 mm squared flat plate for stability on the ground. A hopper of 512 mm diameter sits atop the cylinder. Inside the cylindrical drum is a rotating disc having a diameter of 420 mm, with an abrasive disc attached. The rotating disc is connected to a solid shaft of diameter 40 mm, and 250 mm length. The shaft together with the rotating disc is driven by a single phase, 5 hp DC motor with a rotational speed of 1450 rpm, held by a motor seat bolted to the sides of the cylindrical drum. A concrete lining 15 mm thick was cast round the inner part of one prototype to serve as the peeling element and the other prototype left to accommodate the other drum linings which are changeable. The sizing of the abrasive drum and disc linings was such that there was a clearance of 22 mm to allow water and peels from the cylindrical drum to escape. An opening was also made at the base of the drum to allow the peels to exit to outside. A gateway of dimensions 200 mm × 300 mm on the drum permits the peeled cassava to be discharged from the drum.

Cassava varieties

Two of the popular local cassava varieties in the market; *Asi-Abayiwa* and *Dabon* was used for the experimental testing and evaluation of the peeler. The cassava roots that were used to evaluate the peeler were obtained from cassava plants aged between 12 and 20 months after planting (MAP). The physical and mechanical properties of the varieties that affects mechanical peeling were determined.

Physical properties determination

Moisture content

The moisture contents on wet basis of the peel and cassava root flesh of the two different varieties were determined by oven drying method. The mass of ten cassava peel and root flesh samples were prepared. The initial masses before drying and the final dried masses of the samples were weighed and recorded, using an electronic balance. The mass of water in the samples is the difference in the initial and final masses of the samples. The moisture contents were calculated using Equation 1.

$$Moisture\ content\ (\theta_{wb}) = \frac{\text{Mass of water in sample (W)}}{\text{Initial mass of sample } (M_t)} \quad (1)$$

Cassava peel ratio

Peel ratio is the mass of peel available for a given mass of cassava root expressed in percentage. Using an electronic balance, 5 kg of cassava root was measured and recorded before peeling and the mass of cassava root flesh obtained after peeling was measured and recorded (using *fufu* method of peeling, Figure 2). The experiment was done ten times and Equation 2 below was used to calculate the peel ratio of the varieties.

$$Peel\ ratio, p = \frac{M_1 - M_2}{M_1} \quad (2)$$

Where, M_1 is mass of cassava roots before peeling and M_2 is the mass of cassava roots flesh after peeling.

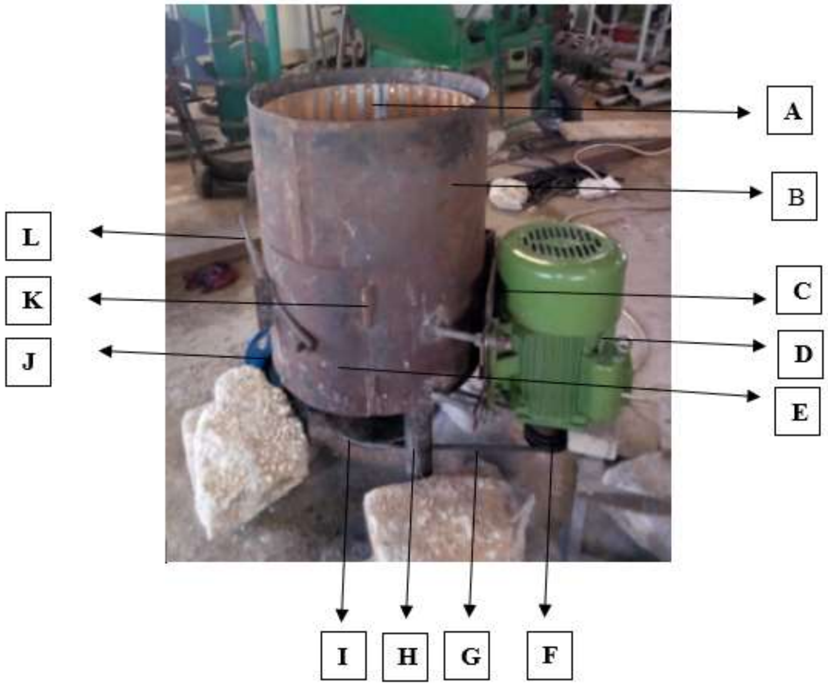

Figure 1. Motorized cassava peeler prototype. A, Lining assembly; B, Cylindrical drum; C, Motor seat assembly; D, Electrical motor; E,Gate; F, Driver pulley; G, V-belt; H, Stand; I, Driven pulley; J, Peel spout; K, Gate hinge; L, Gate handle/lock.

Figure 2. *Fufu* method of peeling.

Root diameter

In determining the average root diameter for the two varieties, ten cassava root samples were randomly selected from each variety. A digital Vernier caliper was used to measure the proximal end diameter (d_1), mid-section diameter (d_2) and distal end diameter (d_3) of all the roots (Figure 3). Equation 3 was used in determining the average root diameter of the varieties.

$$Average\ root\ diameter = \frac{d1+d2+d3}{3}\ \text{(Adetan } et\ al.\text{, 2003)} \quad (3)$$

Cassava root peel thickness

The peel thickness of the roots selected for the root diameter measurement were determined and recorded by peeling each root, using the *fufu* method of peeling. The proximal end diameter (D_1), mid-section diameter (D_2) and distal end diameter (D_3) of the peeled roots were measured, using a digital Vernier caliper. The peel thickness of the varieties was calculated using Equations 4 and 5:

$$\text{Peel thickness of the various root sections} = d_1\text{-D1},\ d_2\text{-D2 and } d_3\text{-D3} \quad (4)$$

$$Average\ peel\ thickness = \frac{\sum_{i=1}^{n} d1i\text{-}D1i + d2i\text{-}D2i + d3i\text{-}D3i}{3n} \quad (5)$$

Where, n is the sample size (10).

Bulk density

The bulk density of the two cassava varieties was determined by filling a 0.025 m^3 container with fresh cassava roots and the mass

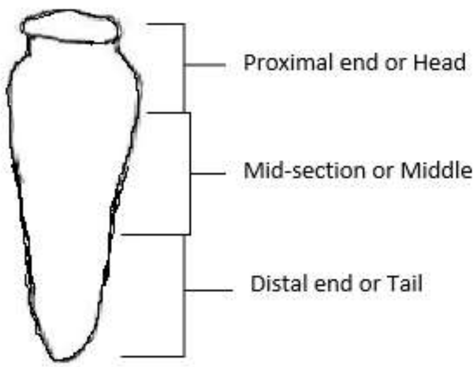

Figure 3. Cassava root morphology.

determined with the aid of an electronic balance. The bulk density was calculated as the ratio of the mass of the roots to the volume of the container. The process was replicated 10 times for both varieties.

Mechanical properties determination

The mechanical properties; shear stress, shear force, strain and Aut Young modulus of the varieties were determined using an Instron machine (model 4482). Ten samples of each variety, with a mid-section diameter of 70 mm were randomly selected for the mechanical property test. The test sample was prepared and fixed in the machine's working tool. The machine applies continual force through the shear force tool until the computer, which displays the output indicates plastic deformation (i.e. until the root peel fails). The mechanical properties of the various test samples of the varieties were recorded at full load conditions of the machine and the data were downloaded. This test is carried out to determine the force needed to cut open the peel of a cassava root.

Angle of repose and coefficient of static friction of cassava roots

The angle of repose of ten randomly selected unpeeled cassava root samples of each variety were determined along the length of cassava root samples on the four lining materials (concrete, galvanized steel, rubber and wood) as described by Nwachukwu and Simonyan (2015). Cassava root was placed on the various lining materials and was raised slowly until the frictional force between the root and the lining surface was overcome by the force of gravity and the root slides down the slope. The angle, at which the cassava roots starts to slide, was marked with a pencil on a paper and measured with a graduated protractor. The tangent of the angle of inclination (repose) is the coefficient of static friction of the cassava root on the lining material (Equation 6).

Coefficient of static friction, $\mu = \tan\alpha$ (6)

Where, μ is the coefficient of static friction and α is the angle of repose

Manual peeling

For performance evaluation, a survey of manual peeling was conducted at some cottage cassava industries in Kumasi and data were recorded. The survey focused on the efficiency in terms of time spent during manual peeling. Samples of cassava (5 kg each) were given to five different peelers and each of them was timed to record the time it took to peel the 5 kg. The procedure was replicated five times for each peeler and the average was calculated.

Experimental design

The split-split plot design was used to plan the experiment. The four different independent variables consisting of drum lining materials, disc lining materials, speed and cassava variety were grouped as follows: 4 Block = Drum lining material (concrete, metal, rubber, wood); 4 Main effect= Disc lining material (concrete, metal, rubber, wood); 2 Split plot factor = Variety (Asi-Abayiwa, Dabon): 3 Split-split factor= Speed (250 rpm, 350 rpm, 500 rpm).

Combinations = 4 x 4x 3 x 2 = 96, at 3 replications; that is treatment = 96 x 3 = 288.
The dependent variables were; capacity (kg/h), peel removal efficiency (%), percent flesh loss (%) and peeling quality index.

Experimental procedure for evaluation

The evaluation experiment on the peeler was done with four different linings; concrete, galvanized steel (metal), rubber and wood surfaces. Freshly harvested roots were randomly chosen from two popular varieties of cassava (*Asi-Abayiwa* and *Dabon*) procured from the open market in Ejisu. Experiments were run on the roots within 4 days after harvesting, as cassava starts deteriorating after 3 or 4 days after harvesting (Kolawole et al., 2011).

Preparation of samples for evaluation

Cassava root was sorted into various sizes; $\leq$50 mm, $>$50-70 mm, $>$70-90 mm and $>$90 mm, based on their average diameter as described by Adetan et al. (2003). The sorted roots were prepared for the test by cutting them into 100-200 mm long near cylindrical roots as shown in Figure 4.

Time recording

The duration (time) *t,* for an effective peeling for each experiment was monitored and recorded by observing the cassava in the peeler for satisfactory peeling. This was done severally during the preliminary testing to determine the actual time for effective peeling for the various disc lining materials at different speeds. The derived effective times for peeling for the various discs lining materials and speed combinations was used during the effective capacity testing experiment and the general evaluation.

Peeler evaluation

After all the preliminary testing, data collection and analysis, the peeler was finally run with four independent variables (drum lining material, disc lining material, speed and cassava variety) to determine four responses (capacity (kg/h), peel removal efficiency (%), percent flesh loss (%) and peeling quality index). Experiment was run on the 4 drum lining materials (that is concrete, metal, rubber and wood), 4 disc lining materials (that is concrete, metal, rubber and wood), 3 speeds (i.e. 250 rpm, 350 rpm and 500 rpm),

Figure 4. Prepared cassava roots sample ready for testing.

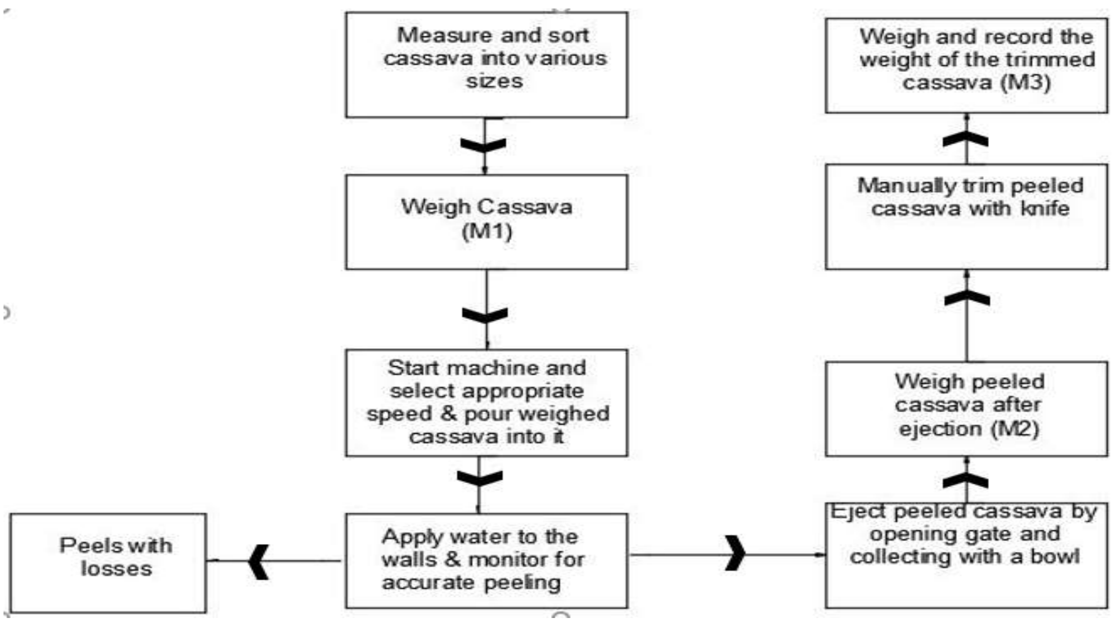

Figure 5. Flow chart of experimental procedure

and with 2 different varieties (*Dabon* and *Asi-Abayiwa*) at 3 replications. Initial mass before peeling (that is 6 kg, selected after testing), mass after mechanised peeling and mass after manual hand trimming of the mechanised peeled cassava were determined and recorded throughout the experiment. Figure 5 shows the basic procedures followed during testing.

Determination of performance and operational parameters

The performance of the peeler was measured using the following parameters: peeling quality index, peel removal efficiency (%), percent flesh loss (%) and capacity (kg/h) of the peeler. The following equations and parameters were very essential in determining the performance of the peeler: mass of unpeeled cassava (m_{bp}); average mass of manually peeled cassava peels (m_{mc}); mass of cassava after machine peeling (m_{mp}); mass of cassava after manual hand trimming (m_{ht}):

Peel retained after mechanised peeling, kg (A) = (m_{mp} - m_{ht}) (7)

Gross loss, kg (G) = (M_{bp} – M_{mp}) (8)

Net loss, kg (N) = (M_{bp} -M_{ht}) (9)

Percent net loss (N%) = $\frac{N}{M_{bp}} \times 100$ (10)

Peel to flesh ratio (p) = $\frac{M_{bp} - M_{mc}}{M_{bp}} \times 100$ (11)

Actual mass of cassava flesh (B) = $\frac{(100-p)}{100} \times M_{bp}$ (12)

Actual mass of peels, C (kg)= $M_{bp} - B$ (13)

Mass of peel that is removed by the peeler, C_R (kg) = C – A (14)

Mass cassava flesh that is recovered after peeling, R (kg) $= B - (G - C_R)$ (15)

Peel removal efficiency (μ_p) = $\frac{C_R}{C} \times 100$ (16)

An overall peeling quality index, (Q) = R$\times$ CR (17)

Machine capacity (T.C) = $\frac{\text{mass of unpeeled cassava (kg)}}{\text{time (h)}}$ (18)

Percent flesh loss (Pf) = $\frac{(G-C_R)}{B} \times 100$ (19)

For all the performance parameters, the results of that of the mechanised peeler were compared with the ideal (theoretical) method of manual peeling.

Data analysis

The data collected during the testing and evaluation of the peeler was tested for normality using the Anderson-Darling normality test tool in the basic statistics tool park of Minitab version 17. The normalised data was analysed at 95% confidence level, using multiple linear regression with fit model and optimised response in Minitab version 17. Microsoft Excel 2016 was used to produce all the figures and tables used in presenting the results.

RESULTS AND DISCUSSION

Physical and mechanical properties

The physical and mechanical properties of the cassava varieties used, that have influence on mechanised peeling are listed in Tables 1 and 2.

From Table 1, the moisture content, root diameter, bulk density, peel to flesh ratio and peel thickness correspond to what were reported by Adetan et al. (2003), Kolawole et al. (2007) and Charrondiere et al. (2012), with the exception of peel thickness for *Asi-Abayiwa* that falls outside the range.

From Table 2, it appears metal lining material is the most abrasive surface among all the lining materials with a coefficient of static friction value of 0.625 and 0.623 for *Asi-Abayiwa* and *Dabon* varieties, respectively. Concrete lining surface, rubber lining surface and wood lining surface follow in a descending order in terms of level of abrasiveness. The shear force value of the cassava varieties is an indication of how difficult it is to break the peel of the roots and also how faster the roots abrade during peeling. From Table 2, *Asi-Abayiwa* has a tougher peel than *Dabon* and hence *Dabon* will abrade faster than *Asi-Abayiwa*.

Manual peeling

In Table 3, the average peel removal efficiency, peeling capacity, percent flesh loss and peeling quality index of the two manual methods of peeling used (that is *fufu* method and *dough* method) are shown.

From the data of manual peeling per person (Table 3) for all two varieties the capacity of the *dough* method is higher than the *fufu* method. This has influenced the losses that occur during peeling with the *dough* method and the overall peeling quality index is also affected.

Peeling duration and peeling quality index

The effective time of peeling at the various speeds (that is 250, 350 and 500 rpm) was tested by fixing time for peeling and increasing it, until effective peeling in terms of satisfactory peeling quality was achieved. Several tests were run at each speed and the mean time (that is the one in parenthesis) for efficient peeling was determined for all the speeds as shown in Table 4.

The effective peeling duration (Table 4) was the determining factor in calculating the capacity in kg/h for the various disc lining materials. The peeling duration of the various lining materials at the different speeds confirms the abrasiveness of metal, which has the low peeling duration at all speeds, followed by concrete lining, rubber lining and wood lining having the high at all speeds. This confirms the coefficient of static friction values discussed in Table 2.

Performance of the mechanised peeler

The significance level of the various independent variables for the various dependent variables is outlined in Table 5.

The following terms are in the final model Equations in Table 6 for capacity, peel removal efficiency, percent flesh loss and peeling quality index: X_1: Drum lining, X_2: Disc lining, X_3: Speed and X_4: Cassava variety.

The independent variables that were not significant for the respective dependent variables in Table 5 were not part of the terms in the equation in Table 6 with the exception of the interactive terms.

Figure 6 indicates the capacity in kg/h for the various disc lining materials at three different speeds.

For all the four different disc lining materials, the capacity increases with increasing speed (Figure 6). The capacity range of the peeler (80– 1440 kg/h) is higher than a capacity of 10.4 kg/h reported by Agbetoye et al. (2006). Figure 6 shows higher capacity at higher speed but for all speeds, metal (galvanized steel) disc lining has the maximum capacity. The plot for capacity also showed that rubber and concrete disc lining materials are almost similar in performance, with wood disc lining material

Table 1. Physical properties of cassava varieties.

Property	Mean value		Values from literature	Source
	***Asi-Abayiwa* (V1)**	***Dabon* (V2)**		
Moisture content of flesh (%)	66.4	58.75	50 - 70	Kolawole et al. (2007)
Moisture content of peel (%)	65.9	55.1	50 - 70	Kolawole et al. (2007)
Proximal diameter (mm)	61.02	64.03	Not available	
Mid-section diameter (mm)	60.63	62.17	Not available	
Distal diameter (mm)	50.75	50.20	Not available	
Average diameter (mm)	57.47	58.8	18.8- 88.5	Adetan et al. (2003)
Proximal peel thickness (mm)	5.28	2.60	Not available	
Mid-section peel thickness (mm)	5.31	3.92	Not available	
Distal peel thickness (mm)	5.27	2.63	Not available	
Average peel thickness (mm)	5.29	3.05	1.20 – 4.15	Adetan et al. (2003)
Peel to flesh ratio (%)	17	16.5	10.6 - 21.5	Adetan et al. (2003)
Bulk density (kg/m^3)	635	625	630	Charrondiere et al. (2012)
Sample size (n)	10	10		

Table 2. Mechanical properties of cassava varieties.

Properties	Mean value	
	***Asi-Abayiwa* (V1)**	***Dabon* (V2)**
Force at maximum load (kN)	0.2899	0.2345
Stress at maximum load (MPa)	0.0753	0.0609
Strain at maximum load (mm/mm)	0.0402	0.0286
Modulus (AutYoung) (MPa)	2.62	2.129
Coefficient of static friction, μ (concrete lining)	0.469	0.466
Coefficient of static friction, μ (galvanized steel lining)	0.625	0.623
Coefficient of static friction, μ (rubber lining)	0.558	0.554
Coefficient of static friction, μ (wood lining)	0.412	0.408
Sample size (n)	10	10

An average diameter of 70 mm was considered for all varieties during the test.

Table 3. Manual peeling data of *Asi-Abayiwa* and *Dabon* varieties.

Parameter	***Fufu* method**		***Dough* method**	
	Asi-Abayiwa	***Dabon***	***Asi-Abayiwa***	***Dabon***
Percent flesh loss (%)	0	0	11.65	9.55
Capacity (kg/h)	32.07	43.64	40.59	50.96
Peel removal efficiency (%)	100	100	100	100
Peeling quality index	5.08	4.97	4.49	4.49

having the least capacity.

Figure 7 indicates the peel removal efficiency for the various disc lining materials at three different speeds.

Figure 7 shows that the peel removal efficiencies for rubber and wood disc lining material increases with increasing speed from 250 to 500 rpm with value range of 55.081 - 83.847 and 17.709 -71.567%, respectively. The peel removal efficiencies for concrete and metal disc lining material are higher at 350 rpm, with values of 78.329 and 75.969% and lower at 250 rpm, with values of 64.7 and 67.801%, respectively. The highest peel removal efficiency of 83.85% for the rubber lining is

Table 4. Effective peeling time of disc lining materials at various speeds.

Speed (rpm)	Peeling quality index (time in seconds)			
	Concrete	Metal	Rubber	Wood
250	3.41 (120)	3.82 (60)	4.11 (180)	2.52 (270)
350	3.57 (90)	3.88 (45)	4.44 (120)	2.12 (240)
500	3.17 (60)	3.70 (15)	3.82 (40)	3.79 (150)

Table 5. Significant level of independent variables.

Predictor variables	Significance level			
	Capacity	Peel removal efficiency	Percent flesh loss	Peeling quality index
Drum lining (L)	ns	ns	ns	Ns
Disc lining (D)	***	***	***	***
Speed (S)	***	***	***	***
Variety (V)	ns	ns	***	***

*** = p<0.001, ** = p<0.01, * = p<0.05 and ns = not significant.

Table 6. Model equations and root mean square values.

Equation		R^2
Capacity	$y_{ca} = -426 + 850.4X_2 - 278X_3 - 168.8X_2^2 + 155.5X_3^2 - 50.4X_2X_3$	0.6117
Peel removal efficiency	$y_{pre} = 64.60 + 15.33X_2 - 9.48X_3 - 8.458X_2^2 + 8.504X_2X_3$	0.6531
Percent flesh loss	$y_{pfl} = 7.27 + 17.70X_2 - 15.08X_3 + 1.80X_4 - 3.976X_2^2 + 4.330X_3^2 - 0.738X_1X_2 - 1.196X_3X_3 - 1.979X_2X_4 + 3.672X_1X_4$	0.7083
Peeling quality index	$y_{pqi} = 2.609 + 0.189X_2 - 0.295X_3 + 0.166X_4 - 0.0944X_1^2 - 0.2483X_2^2 + 0.3831X_2X_3 - 0.2406X_3X_4$	0.4774

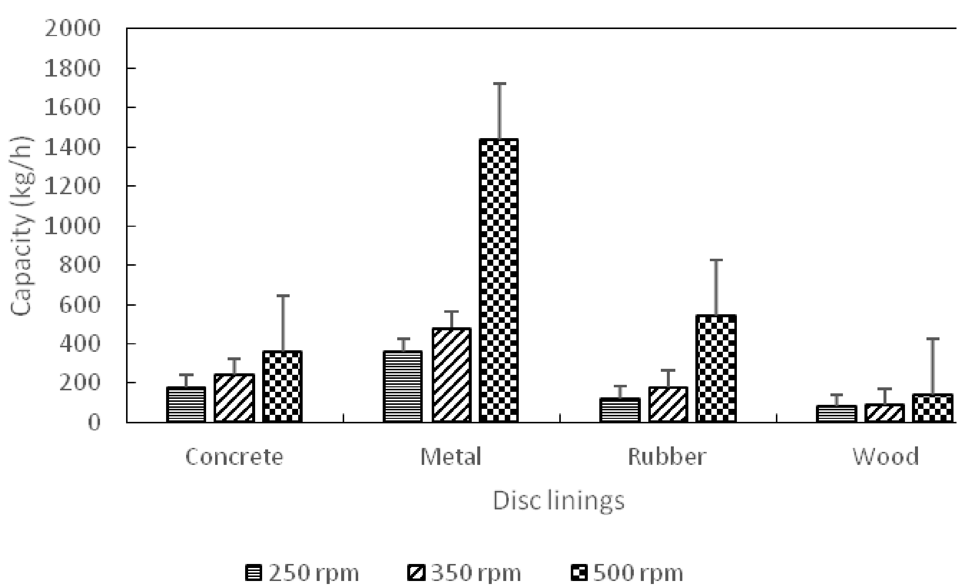

Figure 6. Graph of capacity against the various disc materials.

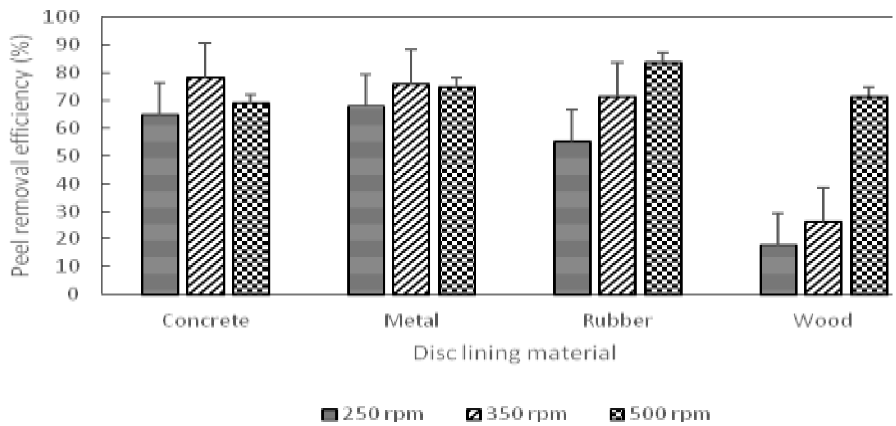

Figure 7. Graph of peel removal efficiency against the various disc materials.

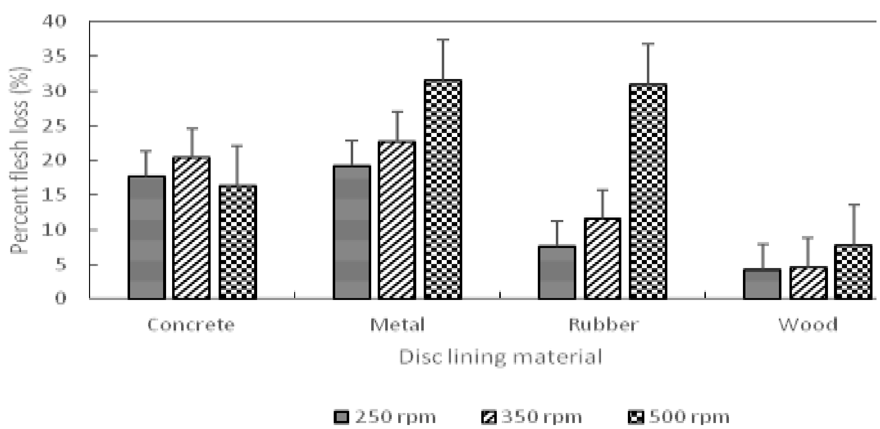

Figure 8. Graph of percent flesh loss against disc materials at different speeds for *Asi-Abayiwa.*

higher than that of Agbetoye et al. (2006), Olukunle et al. (2010), Olunkunle and Jimoh (2012) and Ajibola and Babarinde (2016) having values of 75, 80, 73.05- 82.50% and 81%, respectively.

Figures 8 and 9 indicate the percent flesh loss for the various disc lining materials at three different speeds (250, 350 and 500 rpm). Figure 8 is the percent flesh loss of *Asi-Abayiwa* cassava variety and that of *Dabon* is in Figure 9.

From Figure 8, the percent flesh loss of *Asi-Abayiwa* for metal, rubber and wood disc lining material increases with increasing speed from 250– 500 rpm with value ranges of 19.23- 31.58%, 7.66 - 30.97% and 4.31 - 7.82%, respectively. The percent flesh loss for concrete disc lining material is higher at 350 rpm, with a value of 20.38% and the least value of 16.37% recorded at 500 rpm.

Figure 9 presents the percent flesh loss of *Dabon*, which indicates an increase in percent flesh loss with increasing speed from 250– 500 rpm for metal and rubber disc lining material with value ranges of 22.64- 38.08% and 8.30 - 31.14%, respectively. The percent flesh loss for concrete and wood disc lining material is higher at 500 rpm, with a value of 36.78 and 18.08% and their least values of 4.20 and 1.90% recorded at 350 rpm, respectively.

The percent flesh loss of both varieties increases with increasing speeds for metal and rubber disc lining materials (Figures 8 and 9). Concrete and wood disc lining material have an irregular pattern of percent flesh loss for both varieties. This confirms cassava variety significant impact on the outcome of percent flesh loss. Generally, the percent flesh loss for the peeler is lower than the 42% that was reported by Olukunle and Jimoh (2012), even for the most abrasive lining materials (metal and concrete).

In terms of capacity (kg/h), the peeler performs better than the two manual methods, with peeler capacity value range of 80 – 1440 kg/h as compared to that of the *fufu* method and *dough* method, with value range of 32.07

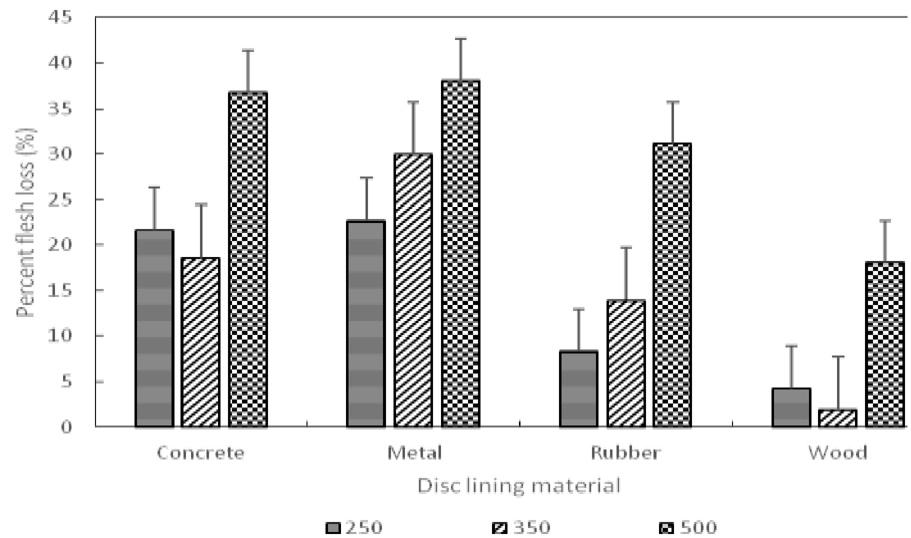

Figure 9. Graph of percent flesh loss against disc materials at different speeds for *Dabon.*

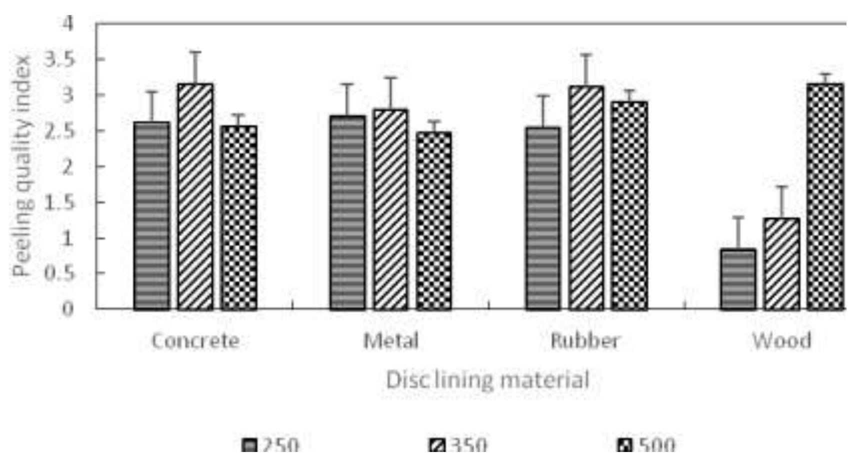

Figure 10. Graph of mean peeling quality index of all varieties against disc materials at different speeds.

– 43.64 and 40.59 – 50.96 kg/h, respectively. The difference in capacity between the three best disc lining materials and speeds combination (that is concrete at 350 rpm, wood at 500 rpm and rubber at 350 rpm) and the manual methods (*fufu* and *dough* method) are approximately 4, 2 and 3 times greater than the manual methods.

The peel removal efficiency of the peeler, discussed above, has a higher value of 83.85%, which was recorded at 500 rpm by the rubber disc lining material. This is 16.75% less than the peel removal efficiency of the two manual methods, which is 100%. The three best disc lining material and speed combination generated by the regression model (that is concrete at 350 rpm, wood at 500 rpm and rubber at 350 rpm), recorded a peel removal efficiency difference of 21.67, 28.43 and 28.73%, respectively from the manual methods.

Since peeling quality index is a function of peel removal efficiency and percent flesh loss, and peeling quality index is also dependent on variety, with every cassava variety having different ideal peeling quality index (*fufu* method of peeling in Table 3). This situation makes it difficult to select which disc lining material and speed combination that gives higher performance, hence the need to consider the average peeling quality index for the two varieties (Figure 10).

It can be deduced from Figure 10 that the peeling quality index for concrete and rubber linings is highest at

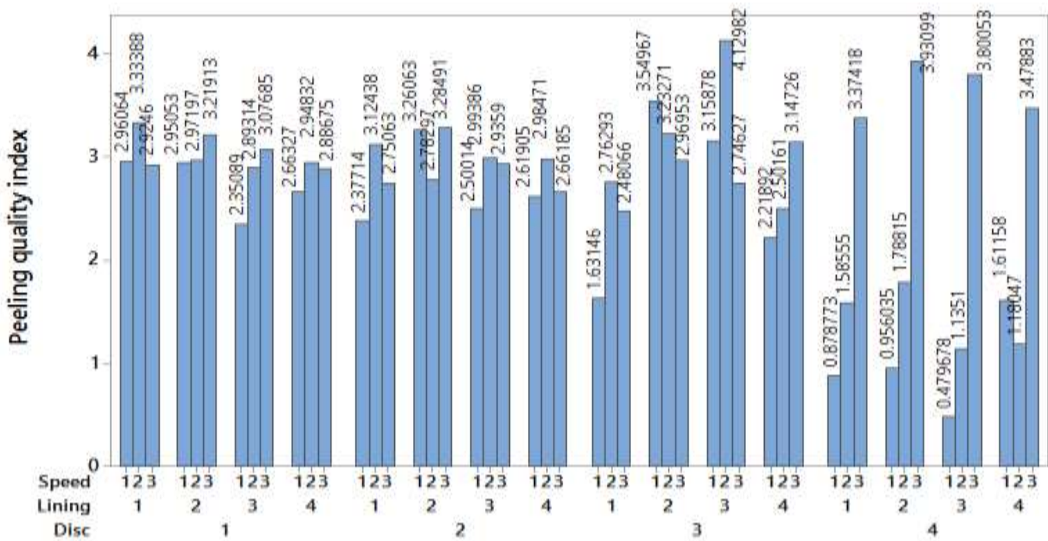

Figure 11. Peeling quality index of *Asi-Abayiwa.*

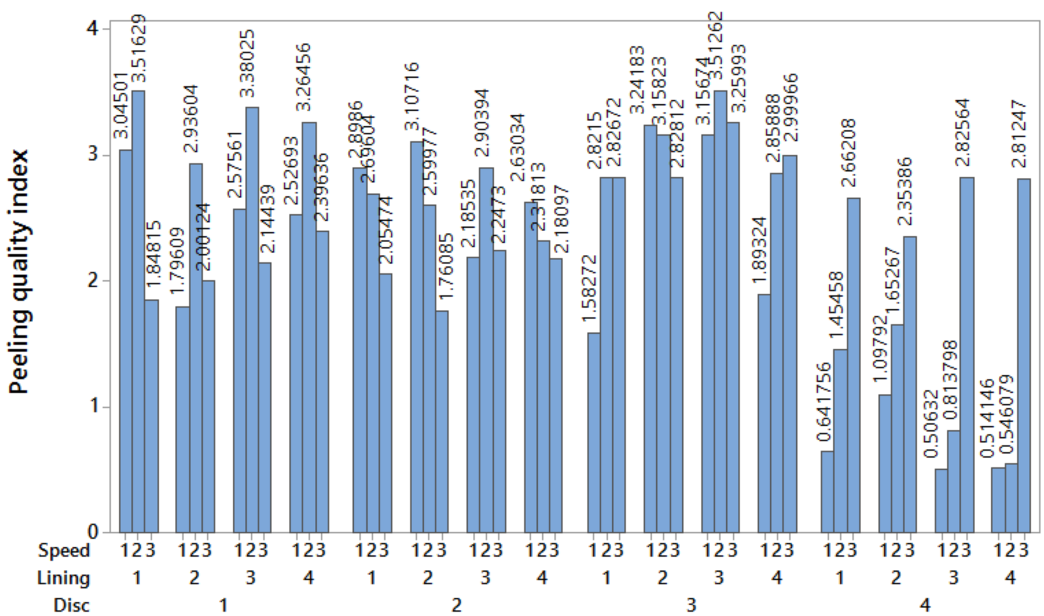

Figure 12. Peeling quality index of *Dabon.* Disc 1 and lining 1 is concrete, Disc 2 and lining 2 is metal, Disc 3 and lining 3 is rubber, Disc 4 and lining 4 is wood, Speed 1 is 250 rpm, speed 2 is 350 rpm, speed 3 is 500 rpm.

350 rpm with values of 3.16 and 3.12, respectively. Wood disc lining material recorded its highest peeling quality index at 500 rpm with a value of 3.15. These three different disc lining materials and speed combinations had the better performance among all combinations, but since peeling quality index is a function of peel removal efficiency and percent flesh loss, it is important to consider Figures 7 to 9 in deciding which combination is the best.

From the performance parameters above, the best disc lining materials and their corresponding speeds neglecting the impact of cassava varieties and drum lining materials were concrete disc lining at 350 rpm, wood disc lining material at 500 rpm and rubber disc lining material at 350 rpm in descending order. But when the impact of drum lining and variety are considered, the results are shown in Figures 11 and 12.

From Figures 11 and 12, the best combination for the mean of *Asi-Abayiwa* variety is rubber drum lining and rubber disc lining material at 350 rpm, with peeling quality index of 4.13. The best combination for the mean of *Dabon* variety is concrete drum lining and concrete disc lining at 350 rpm for a peeling quality index of 3.52. Followed closely is rubber disc and drum lining material at the same speed with peeling quality of 3.51. Although the regression model predicted the best combinations without considering drum lining material and with some response variables too, the cassava variety was not considered. The model still gave rubber disc lining material at 350 rpm as one of the top three best combinations. Hence the rubber disc lining material is the best followed by concrete disc lining material in terms of peeling quality, with a keen consideration of peel removal efficiency and percent flesh loss.

Conclusion

Two developed prototype peelers having a vertical stationary drum and a horizontal rotating disc were evaluated with two local cassava varieties; *Asi-Abayiwa* and *Dabon*. The effective capacity of the peeler for all disc lining materials and speed combinations ranged from 80 – 1440 kg/h with metal lining having the highest value. Peel removal efficiency of the peeler for all disc lining materials and speed combinations ranged from 17.7 to 83.85%. Concrete disc lining at 350 rpm, and rubber disc lining at 500 rpm gave the highest peeling efficiencies of 78.33 and 83.85%, respectively. The peeler percent flesh loss for all disc lining materials and speed combinations ranged from 4.31 – 31.58% and 1.89 - 38.08% for *Asi-Abayiwa* and *Dabon* cassava varieties, respectively with wood disc lining at 500 rpm and rubber disc lining at 350 rpm being the best combinations having values of 9.67 and 12.72%, respectively. In terms of peeling quality index of the peeler, concrete disc lining material at 350 rpm, wood disc lining material at 500 rpm and rubber disc lining material at 350 rpm recorded highest performance, with average values for the two varieties of cassava at 3.16, 3.15 and 3.12, respectively. But when all factors are considered, rubber disc and rubber drum lining material at 350 rpm performed better with an average value of 3.82.

Recommendations

1) From the outcome of the studies, it was realised that the disc lining material has greatest impact on peeling. The rubber disc material produced the best performance; further studies should be done to determine the effective diameter of the abrasive disc that will give efficient performance.
2) Further studies should be done to determine the effective spacing between the drum lining materials (for wood and rubber) and also the number, spacing and types of perforations of the metal lining material that will give a better performance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Comparing productivity of rice under system of rice intensification and conventional flooding: A switching regression approach

Francis K. Kaloi[1]*, Hezron N. Isaboke[1], Charles N. Onyari[2] and Lucy K. Njeru[3]

[1]Department of Agricultural Economics and Extension, University of Embu, P. O. Box 6-60100, Embu. Kenya.
[2]Department of Land and Water Management, University of Embu, P. O. Box 6-60100, Embu, Kenya.
[3]Department of Agricultural Economics, Faculty of Agriculture, University of Nairobi, P.O Box 29053-00625, Nairobi, Kenya.

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This paper examined the factors influencing rice productivity in Mwea Irrigation Scheme using the System of rice intensification (SRI) and conventional flooding (CF). Stratified sampling was used to obtain 364 smallholder rice farmers for interviewing. Data collection was done with the aid of a semi-structured questionnaire and analyzed using the Endogenous Switching regression Model (ESRM). The results of ESRM revealed that factors such as household size, access to extension services, involvement in off-farm work, distance from the canal, farm size, labour use, access to credit services and years spent in rice farming were found to be significant in explaining variations in rice productivity. Furthermore, the gross margin analysis showed that the returns of SRI outweigh the returns of CF, thus making SRI more profitable than CF. The study therefore recommends that Kenya government should enhance engagement with development partners to pay attention to all significant factors which are important in making decisions in the two practices of rice production.

Key words: System of rice intensification (SRI), rice, Mwea irrigation scheme, productivity.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops for more than 50% of the world population (Atera et al., 2018). Globally, about 160 million hectares are estimated to be under rice production with an annual production of approximately 500 million metric tons (Kirby et al., 2017). The demand for rice has increased steadily over the years, thus playing a major role in many countries in terms of strategic food security planning policies.In recent years, rice crop yield has slowed considerably therefore failing to keep up with the population growth thus leading to shortages and higher prices that have adversely affected smallholder rice

*Corresponding author. E-mail: kadipo.francis@embuni.ac.ke.

farmers (Denkyirah, 2015; Lee and Kobayashi, 2017)

Paddy rice is one of the commodities whose demand is rapidly increasing in Sub-Saharan Africa as a result of increased urbanization, growing importance of the crop and the challenges of attaining food security (Amos, 2014). Therefore the consumption of rice is expected to increase tremendously (Kirby et al., 2017). The current paddy rice production in Kenya is estimated at 150,000 metric tons. The output meets only about 20% of the total demand (Omondi and Shikuku, 2013). The gap between production and consumption is filled by importation to meet the domestic demands (Ndirangu, 2015). For Kenya to attain self-sufficiency in rice production, the domestic production must increase at the rate of 9.3% per year (Amos, 2014). To achieve self-sufficiency in rice production, innovative practices that reduce water use need to be put in place to enhance sustainable rice farming.

Various methods have been used to reduce water usage in rice production (Denkyirah, 2015). One of the most tried methods was the Green Revolution in Asia, which involved a series of research and technology transfer initiatives (Kassam et al., 2011). This innovation involved the development of high yielding varieties of cereal grains and modernization of farmland management techniques (Rahman, 2017). The Green Revolution was very effective and successful in Asia whereby many farmers were able to adopt the technology (Thakur et al., 2015). However, the innovation was not able to help many African countries farmers due to limited infrastructure and financial constraints (Ndiiri et al., 2013). The other innovation is the System of Rice Intensification (SRI). From the farmers' perspective, SRI is the use of existing assets differently yet increasing the outputs and reducing water use while maintaining the quality of the grain (Katambara et al., 2013). It can be inferred from Stoop (2003) that SRI is a concept on the manipulation of agronomic practices to attain higher rice yields with the use of minimal resources such as agrochemicals, seeds, and water (without continuous flooding in SRI as compared to traditional methods). The SRI is gaining popularity in all rice-growing areas of the world and that farmers can grow more rice with less water (Karki, 2010).

The key components of SRI include: water management, practiced by keeping the soil drained and saturated rather during the vegetative growth period. Furthermore, it includes flooding and drying of the fields for alternating periods of 3-6 days each (Namara et al., 2003). The second component is the planting method which involves spacing configurations and age of seedlings. In SRI, seedlings are transplanted 8-15 days after germination (Thura, 2010). Some studies suggest a line spacing of 30 cm × 30 cm. The spacing could be based on the local edaphic conditions but it has to facilitate weeding (Uphoff and Thiyagarajan, 2005). The third component is weed control which is best done ten days after transplanting and then weeding every ten days until canopy closure (Uphoff and Thiyagarajan, 2005). The fourth component entails soil fertility management. Most farmers use compost or organic manure but the amount applied varies in terms of its availability and also because there is no fixed recommended rate to follow (Ndiiri et al., 2013). The traditional method of rice growing involves continuous flooding during the vegetative growth with draining of the water during the grain ripening stage, which is a common practice in all rice-growing schemes in Kenya (Omwenga et al., 2014). The Conventional Flooding (CF) method is thus associated with higher water demand and occasioned by high losses through percolation, seepage, and evaporation (Paredes et al., 2017).

There exist various socioeconomic factors that influence the level of rice productivity in the two systems of rice farming in Sub-Saharan Africa, Kenya included. Many empirical studies have investigated the issue of crop productivity and profitability (Denkyirah, 2015). However, alternative production practices such as SRI and CF have not yet been fully investigated especially on productivity and profitability of rice. Previous studies on SRI in Mwea Irrigation Scheme include (Ndiiri et al., 2013). Authors such as Ndirangu (2015) focused on the constraints and the returns associated with SRI. Additionally, their studies focused on the perceptions of SRI. On profitability, authors such as Denkyirah (2015) focused on cash flow projections for SRI for a period of five years using the Benefit Cost Ratio (BCR) and Net Present Value (NPV) approaches. However the study did not focus on the use of other approaches in determining profitability. In Ghana, the application of gross margin approaches was done for paddy production (Bwala and John, 2018). However the study did not make a comparison of rice practices instead it focused costs and returns thus creating a research gap.

There is a need to understand what to increase or decrease in productivity of rice among smallholder farmers. Therefore the main objective of this study was to compare the productivity of rice under SRI and CF. Against this background, this study came in hardy to provide research-based information on determinants of rice productivity under SRI and Convectional flooding in Mwea irrigation scheme, Kenya. From these studies, little has been done or investigated on productivity of rice using the SRI and CF methods of crop establishment in Mwea Irrigation Scheme. ESRM have been applied to examine the impacts of technology adoption on farm outcomes when self-selection is an issue. The model accounts for selection bias making it the most appropriate in analyzing the productivity of rice under SRI and CF. This, therefore, provides a strong case of argument of using SRI to generate information on rice productivity with a view of driving policy recommendations and filling the information gap in Kenya.

MATERIALS AND METHODS

Description of study area

This research was carried out in Mwea Irrigation Scheme (MIS) in Central Kenya. MIS was selected since the first field experiments on SRI in Kenya, were conducted at Mwea Irrigation Agricultural Development (MIAD) Centre. The scheme occupies the lower altitude zones of the region with expansive low marshy areas. The altitude ranges from 1,000 - 2,200 m above the sea level, with temperatures ranging between 15 and 30°C. The rain seasons in the region are usually two, the long rains occur between March and May, while the short rains occur in October/December. The main agricultural activity is mono-cropping of rice. The crop is grown in paddies that are irrigated for six months. The main sources of water to the scheme are River Nyamindi and River Thiba which are tributaries of river Tana. There are currently over 52 villages with approximately 7320 households within the scheme (GoK, 2008).

Sampling procedure

The respondents were selected using two stage stratified random sampling. This was done with the aid of the major blocks/villages which include Karaba, Tebere, Wamumu, and Thiba. In total, 364 smallholder farmers were selected (that is 91 from each study location and each 45 from SRI and CF per block). The four study blocks were purposively selected based on their prevalence of SRI farmers. Additionally, the sampling frame was obtained from the National Irrigation Board.

Theoretical framework

Smallholders Farmer's perception is to maximize on their perceived utility. The study was based on the subjective expected utility framework. The individual expected utility of innovation can be approximated in Equations 1 and 2.

$$SEU(\pi) = \sum_i p_i^t \, U(\pi i) \quad (1)$$

$$U(\pi) = \frac{\pi^{1-RRA}}{1-RRA} \quad (2)$$

Where pi is the probability of state of nature i for the profit (π_i); RRA is the relative risk aversion coefficient and SEU is the subjective expected utility. It is expected that farmers choose the alternative with the highest utility (Equation 3). Based on the random utility theory, the global utility of a system is composed by the utility of each characteristic of the cropping system. Although profit could be one of the characteristics, farmers also maximize their utility based on other factors such as agronomic and technical.

$$U_k > U_j \quad (3)$$

$$\text{Where} = U(t_1, t_2, \ldots, t_r) + \varepsilon \quad (4)$$

$t_1, t_2 \ldots t_r$ Corresponds to the r characteristics of innovation while the error term (ε) depicts the individual determinants.

Testing for heteroscedasticity- White Man-test

White test is used to test the presence of heteroscedasticity. The null hypothesis indicates that the error variances are all equal (homoscedasticity), whereas the alternative hypothesis indicates that the error variances are multicative function of one or two variables (heteroscedasticity).

Analysis of gross margin

In order to compare profitability of SRI and conventional flooding, the gross margins were computed. The variable cost was summed to derive the total cost of production per hectare basis. Variable cost used includes hired labour, fertilizer, pesticides, and machinery operating costs. The gross margins are the difference between the gross returns and the total variable costs.

Descriptive analysis

The data was analyzed using descriptive and inferential statistics. For the socio-economic characteristics, t-test and Chi-test was used to determine the variables that were significant. The t-tests was applied for continuous variables and Chi-tests for dummy variables used in comparisons of the SRI and CF farmers.

Analysis of factors influencing rice productivity among smallholder farmers

The main approach employed in the analysis of rice productivity for the two practices is mainly the econometric approaches that use the Endogenous Switching Regression Model (ESRM). Smallholder farmers take into account the net benefits when deciding on the technologies to adopt. Thus, the technologies employed by farmers should take into account the outcomes such as yields and profits. The selection biasness may occur if they fail to take into account of the outcomes. The biasness occurs because of the smallholder farmers who would obtain less than average returns from the new technology. The selection biasness occurs when the unobservable factors influence the technology choice equation and the outcome (Abdulai, 2014). The factors may include the technical abilities of the farmers in understanding the new technologies such as SRI; while evaluating the impacts of new agricultural technologies it becomes difficult to attribute the differences on yields and returns to adoption and non-adoption. Thus in ESRM approach, the smallholder farmers are classified as both adopters and non-adopters. Therefore the model becomes the most appropriate to use since it captures the responses of the two groups.

The ESRM was used to compare the rice productivity of the farm households who participated in SRI and CF. Switching regression consists of two stages. The first stage is based on a dichotomous choice criterion function. The farmer evaluates whether or not to adopt SRI practices based on resource endowment. The adoption, I_{SRI}^* is compared to the expected utility of using CF practices I_{CF}^*.

The farmers will adopt SRI if, $I_{SRI}^* > I_{CF}^*$ and will not adopt if $I_{SRI}^* \le I_{CF}^*$. This model was appropriate for this study since it allows for analysis of the outputs in the two rice farming practices. The first stage equation can be written in a simplified form as:

$$I^* = S' \alpha + \varepsilon_v \quad (5)$$

$$\text{I = 1 if } I^*_{SRI} > I^*_{CF}. \quad (6)$$

$$\text{I = 0 if } I^*_{SRI} \leq I^*_{CF} \quad (7)$$

Where vector S includes farm and household characteristics, α is a vector of parameters to be estimated, and ε_v is a random error term with mean zero and variance σ^2 .

In the second stage, two regime equations can be specified by explaining the results of the estimated criterion function. The relationship between variable X and Y can be represented as $Y = f(X)$ and specified for each regime as:

$$Y_{SRI} = X' \beta_{SRI} + \varepsilon_s \text{ If I = 1,} \quad (8)$$

$$Y_{CF} = X' \beta_{CF} + \varepsilon_C \ if \ I = 0 \quad (9)$$

β_{SRI} and β_{CF} are parameters to be estimated. The variables in X' and S' are allowed to overlap, identification requires some variables in S' that does not appear in X' Therefore the function is estimated based on the exogenous variables specified in equation. The counterfactual outcomes (observed and unobserved) for the adopters and non- adopters can be estimated using the endogenous switching regression model (Lokshin, 1977). SRI plots with adoption (observed):

$$E(Y_{SRI}\ l\ I = 1) = X' \beta_{SRI} + \sigma_{Sv}\, \lambda_S \quad (10)$$

SRI plots with no adoption (Counterfactual):

$$kE\ (Y_{CF}\ l\ I = 1\ ) = X' \beta_{CF} + \sigma_{cv}\, \lambda_S \quad (11)$$

CF plots without adoption of SRI practices (observed):

$$E\ (Y_{CF} l\ I = 0\ ) = X' \beta_{CF} + \sigma_{cv}\, \lambda_c \quad (12)$$

CF plots with SRI (Counterfactual):

$$E(Y_{SRI}\ l\ I = 0\ ) = X' \beta_{SRI} + \sigma_{sv}\, \lambda_c \quad (13)$$

Therefore Equation 14 will determine the average treatment effects (ATT) and control for observed and unobserved heterogeneity (Noltze, 2012).

$$\text{ATT} = E(Y_{SRI}\ l\ I = 1) - E\ (Y_{CF}\ l\ I = 1\ ) \quad (14)$$

RESULTS AND DISCUSSION

Heteroscedasticity test

The white test was used to test the presence of heteroscedasticity. The null hypothesis indicates that the error variances are all equal (homoscedasticity), whereas the alternative hypothesis indicates that the error variances are multiplicative functions of one or two variables (heteroscedasticity). The results showed that the probability value of the chi-square statistics is less than 0.05. Therefore the null hypothesis of the constant variance can be rejected at a 5% level of significance. This implied the presence of heteroscedasticity in the residuals. The heteroscedasticity test had a significant chi-value indicating that the heteroscedasticity problem was present; however, correction of this problem was achieved by generating the robust standard errors after the regression.

Characteristics of the respondent according to SRI and CF productivity

The socio-economic characteristics of the smallholder rice farmers in the two practices were analyzed and the findings are presented in Table 1. The results indicated that the mean age of the respondent under SRI was 42 years while for CF was 41 years. The results were significant implying that young farmers are actively involved in farm operations. The results further showed that the male headed households dominate rice farming in MIS. This is understandable since rice farming is labour intensive and the tedious activities are required in the farm operations. Regarding education status, 79.17% of SRI farmers had obtained secondary education while 43.67% of CF farmers had primary education. This analysis further showed that more educated households are likely to record increased output since they have the ability to process information regarding the most productive practices in rice farming.

The mean household size for the SRI farmers was 4.12 and for the CF farmers were 5.12. These results revealed that farmers with relatively large-sized households have advantageous to rice farming since it enables farmers to use family labor for rice production. On average farmers in CF had large farm size (2.1 Ha) as compared to those practicing SRI (1.5 Ha), indicating that farm size has a bearing on rice output produced in these practices. Although farmers under CF had an advantage of land size, their income was relatively low (KES 33,761.90) in relation to SRI (KES 40, 374.52). Furthermore, findings indicated that farmers practicing CF had more farming experience in years (8.1 years) than their counterparts doing SRI (6.2 Years).

With regard to the access of extension services, 92.66 and 69.52% of farmers practicing SRI and CF had access to extension respectively. These results implied that SRI farmers relatively had higher access to extension services. The findings also showed that 88.42% of SRI farmers were doing casual works as an off-farm income, while 91.43% of CF farmers were doing the same, showing that many CF farmers were undertaking off-farm activities to increase their income. Results indicated that 91.43% of farmers doing CF had access to credit services and only 33.98% of farmers practicing SRI had access to this service. The distance from the canal for SRI farmers was 5.4788 km and that of CF was found to be 4.1714 km, implying that long distance from canal called for water management practices (Table 1).

Table 1. Demographic and socio-economic characteristics of the respondents.

Variable	SRI	CF	Pooled mean	
	n=259	n=105	n=364	t/*Chi* value
Age (Mean age)	42	41	41	52.40(0.00***)
Gender				
Female (%)	22.39	31.43	38.1	3.25(0.001***)
Male (%)	77.61	68.57	73.09	
Education level				
Primary	56.33	43.67	43.41	
Secondary	79.17	20.83	46.15	159.60 (0.00***)
post-secondary	97.37	2.63	10.44	
Household size	4.12	5.2	5	48.08 (0.00***)
Farm size (Ha)	1.5	2.1	1.8	15.85 (0.00***)
Monthly Income (KES)	40374.5200	33761.9000	37,068.21	47.70 (0.00***)
Years in paddy farming	6.2	8.1	7.1	27.08 (0.00***)
Access to extension services	92.66	69.52	81.09	33.21 (0.00***)
	7.34	30.48	18.91	
Casual work	88.42	91.43	89.93	
Livestock keeping	3.86	7.57	5.72	11.36 (0.00***)
Others	7.72	1	4.36	
Credit access	33.98	51.43	42.71	98.65 (0.00***)
	66.02	48.57	57.3	
Distance to canal	5.4788	4.1714	4.83	3.59 (0.07 **)

***,** denote significance at 1 and 5%.

Analysis of gross margins for SRI and CF

The profits of the SRI and the CF farmers were determined. To determine the profitability levels of the two groups, attempts were made to estimate the gross margins from the rice farming. This is due to the fact that the fixed costs of the smallholder farmers were negligible. The mean cost of production in one hectare of rice was calculated as listed in Figure 1.

The SRI farmers used KES 1,282.83 on purchase of seeds for planting one hectare compared to CF farmers who used KES 2,276.66. Therefore, the SRI farmers saved KES 993.83 per hectare over CF with the application of fertilizer. SRI recorded the highest cost of KES 6,026.27 compared to conventional flooding where it was KES 2,639.49 therefore CF farmers saved KES 3386.78 per hectare. The cost of ploughing for the SRI farmers was KES 4797.16 while in CF it was KES 5797.16. The difference in the cost of ploughing was KES 1,000. The difference on cost of ploughing between the SRI and CF farmers was positively significant and therefore it had a positive influence on profitability.

In the CF method, the Cost of Agrochemicals was KES 300.61, while in SRI the cost was KES 613.50 per hectare. The CF farmers saved herbicides cost of KES 312.89 over the SRI farmers. The difference in the cost of herbicides was significant and this implied that herbicide cost had a positive influence on the profitability of rice farming.

Analysis of gross margins (Table 2) revealed that the average variable cost per hectare for the adopters of SRI was KES 54,564.07 and the gross revenue was KES 118,408 with gross margins of KES 63,843.93 per hectare per season. On the other hand, the variable cost for the CF was 44,252.42 with gross revenue of 74,784 and gross margins of KES 30,351.58 per hectare. The results revealed that SRI technology is more profitable by KES 41, 843 compared to CF in the study area. The result is in agreement with findings of Denkyirah (2015) who reported that SRI was more profitable than CF in Ghana.

Endogenous switching regression results for the factors influencing rice productivity

The coefficients of years spent in farming and marital status were negative but statically significant in influencing

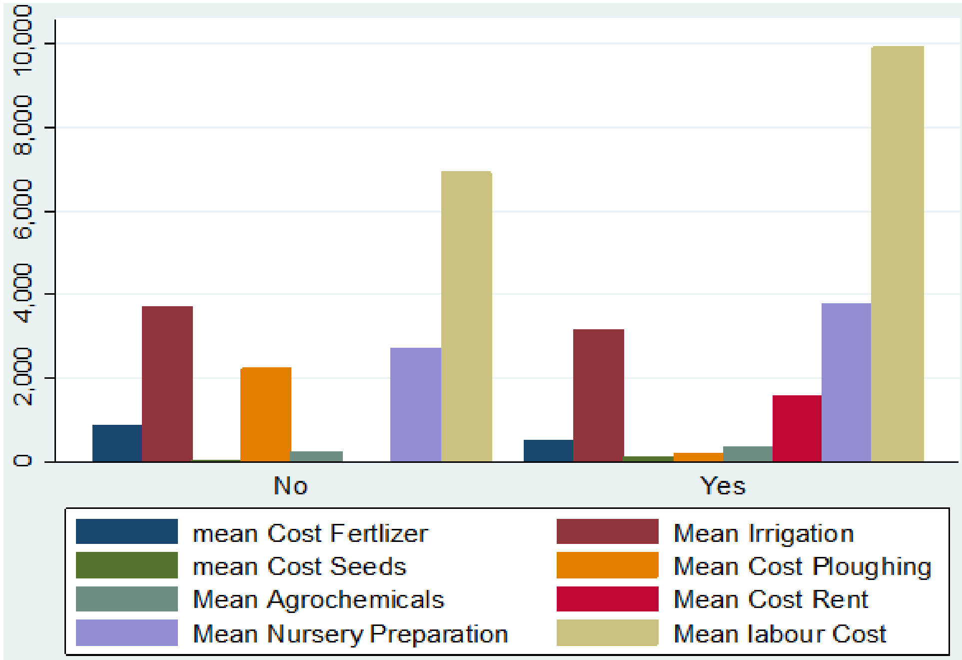

Figure 1. Mean of variable cost for CF and SRI.

Table 2. Gross margin analysis for SRI and CF.

Variable	SRI	CF	T test	P value
Bags harvested	19	12	-16.68	0.00***
Farm gate price(per bag)	6,232	6,232	-0.02	0.00***
Gross revenue	118,408	74,784	**121.56**	0.00***
Total variable cost (KES)	54,564.07	52,710.39	**21.23**	0.00***
Gross Margins Per Season (KES)	63,843.93	22,073.61	**20.23**	0.04**

rice productivity (Table 3). The plausible explanation for the negative relationship between years in rice farming and rice productivity is that experienced farmers were more satisfied with CF methods and thus finding it difficult to adopt SRI. Myint and Napasintuwon (2016) to the contrary reported that Paw rice adoption can be accelerated by promoting it to farmers with higher experience. Furthermore, Paudel et al. (2019) indicated that experience in rice farming positively has effects on rice productivity of adopters of improved technologies.

It was found that household size had a positive coefficient and statistically significant at 1% level. This shows that large households with labor endowment are important in increasing rice yields. Thus, more family labour is likely to be engaged in farm activities. This implies that an increase in household size increases output. These can be attributed to the fact that the production of rice requires more labor that is supplied from the family. Similar findings were reported by Amare et al. (2012) who found a positive relationship between the size of household and productivity of farmers in various technologies.

The results found that off-farm work was statistically significant at 1% level. The results showed that off-farm work positively affects rice productivity. This implies that the household's heads whose main job is farming are less likely to obtain more yields than the part-time farmers. This may be related to access to frequent contacts through off-farm activities and therefore access to information flow. Besides, the risky perceptions of farmers who entirely depend on farm incomes may be hesitant to adopt new technologies such as SRI. Similar results were reported by Poornima (2017) that farm activities have positive effects on rice yield and household income.

Access to credit by the smallholder farmers had a positive coefficient and significant at 1% level. Credit is an important factor in agricultural production. Farmers

Table 3. Endogenous switching regression results for rice productivity.

Variable	Selection equation		SRI Regime		CF Regime	
	Coeff	P>z	Coeff	P>z	Coeff.	P>z
Age	0.051	0.009***	-0.133	0.241	-0.005	0.919
Education	-0.179	0.501	2.266	0.175	-1.388	0.041
Marital status	-2.546	0.001***	5.197	0.090**	-4.352	0.001***
Household size	0.854	0.000***	0.639	0.583	1.709	0.000***
Average monthly income	-1.4E-05	0.434	0.0002	0.011**	5.83	0.083
Off- farm work	1.428	0.000***	-7.544	0.00***	3.782	0.000***
Farm size	0.336	0.231	0.627	0.662	1.602	0.004**
Years in rice farming	-0.144	0.014**	1.153	0.003**	-0.496	0.003**
Distance from the canal	0.740	0.00***	-2.292	0.003**	0.666	0.038
Extension services	2.639	0.00***	-5.704	0.096	3.832	0.008**
Credit access	4.024	0.00***	-13.299	0.000***	2.672	0.063
Labor use	3.901	0.00***	0.0003	0.000***	4.74	0.058
Wald Chi2 (13)	613.520					
Prob> chi2	0.000					
Log likelihood	-1250.870					
Rho1	-0.231					
Rho 2	0.210					
Sigma	7.5318					
Lambda	-1.7449					

LR test of indep. eqns. (rho = 0): chi2(1) = 2.06 Prob > chi2 = 0.1509,*** 1% level of significance, **5% level of significance.

with access to credit have a high likelihood of increasing production. Credit is accessed by having membership in co-operative or any other financial organization. The results agrees with the findings of Abdulai and Huffman (2014) who noted that access to credit has a positive relationship with the productivity of rice farmers. The distance from the canal influences rice production positively. It is significant at 10%. As the distance increases from the canals, farmers are keener to use resources efficiently such as water in the production of rice. Similar findings were found by Kamoshita and Dinh (2018) that increased distance from water sources affected rice productivity positively. Pede et al. (2018) further showed that the location of the farmer to water source affects the level of productivity.

Access to extension services has a positive coefficient and is statistically significant at 1% for the selection equation; the results imply that the value of providing farmers with skills and new production techniques improves on yields. Access to extension services positively affects rice productivity. This implies that farmers with access to extension services can acquire training on methods of rice production. In addition, farmers are usually informed of the existence and the effectiveness of new technologies such as SRI. The extension agents act as the links between the innovators and the users of the new technology. This helps to reduce the cost of a transaction when training on the new practices. Similar findings were reported by Kinuthia (2015), that access to extension affects the productivity of new varieties. Varma (2017) also reported that access to extension services positively affects rice productivity and income consecutively. Furthermore, Abdulai and Huffman (2014) indicated that access to extension positively affects the productivity of adopters and non-adopters of the new technologies.

The farm size is statistically significant at 5%. An increase in the farm size increases the probability of adopting water-saving technology and thus enhancing rice production. The small landholdings hinder the practice of new technology compared to large farm holdings. Farmers with large holdings can afford to devote their lands to try new practices such as SRI unlike those farmers with less farm size. Distance from the canal affects rice productivity positively. These results imply that farmers closer to the water source have better yields than farmers far from the canals. Farmers in far distances from water sources have the likelihood of experiencing water shortages which on the other hand reduces the output. Similar findings were found by Kamoshita et al. (2018) that distance from water sources negatively affected rice productivity. Pede et al. (2018) further showed that the location of the farmer to water source affects the level of productivity. Labour is statistically significant at 1%. Rice farming is regarded as labor intensive. Therefore labor is an integral variable to determine the productivity of the technology. The findings are consistent with the findings of Canon et al. (2018), Adesina and Zinnah (1993), Karki (2010) and Karubanga et al. (2019).

Conclusion

The study assessed the factors influencing rice productivity under SRI and CF. The results of econometric modeling showed that household size, involvement in off-farm work, farmer experience, distance from the canal, access to extension services, credit access and labor use affect rice productivity significantly. These findings suggest that paying attention to these factors is a good strategy to enhance rice productivity under SRI among smallholders in Mwea Irrigation Scheme. Additionally, the study compared the profitability of SRI and CF using Gross Margins (GM). It was established that both CF and SRI had higher returns than costs. Therefore it makes it profitable to use either SRI or CF. However, the returns of SRI outweigh the returns of CF thus making SRI more profitable. However, it was noted that SRI is more labour intensive as compared to CF. The labour requirement of SRI is high during the initial stages of land preparation and weeding.

Recommendations

(1) To improve rice productivity in Kenya, the government and development partners should work together to improve access to suitable agricultural credit. This can be realized by the formation of more farmer cooperatives in the study area.
(2) The government of Kenya together with research organizations should also play a role in providing training on SRI components and strengthen the field demonstration process for better adoption of SRI and improved returns.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Agronomic and economic evaluation of phosphate fertilizer use in maize-bean cropping systems in Western Kenya

Dorcus Ofuyo*, Peter Opala and George Odhiambo

Department of Applied Plant Sciences, School of Agriculture and Food Security, Maseno University, Kenya.

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Effects of phosphorus rate and crop arrangement on yields and economic benefits in maize-bean cropping systems were investigated for two seasons: Short rains (SR) of 2015 and long rains (LR) of 2016 in Western Kenya. A split plot design with five crop arrangements in the main plots; one row of maize and beans alternating (conventional), maize and beans planted in the same hole (SH), two rows of maize alternating with two of beans (Mbili), sole maize and sole beans, and three P rates; 0, 30, and 60 kg ha^{-1} in the subplots was used. There were no significant effects of crop arrangement on maize and bean yields in LR but bean yields increased with increasing P rate in both seasons. Within a crop arrangement, maize yields also increased with P rate in the SR. Conventional and Mbili arrangements had similar yields for both beans and maize which were superior to SH at 60 kg P ha^{-1} in SR. Sole beans significantly out-yielded intercropped ones. Intercropping was only beneficial (LER > 1) with adequate rainfall in SR but financial returns were too low for all the tested practices because of low yields coupled with high production costs and low producer prices.

Key words: Crop arrangements, intercropping efficiency, phosphorus.

INTRODUCTION

In western Kenya, increased population pressure has reduced per capita area of cropping land and most small scale farmers therefore own less than 0.2 ha of land (Vanlauwe et al., 2011). The key to increasing crop yields in this region, in order to feed the growing population, therefore lies with intensification that is, increasing yields per unit area rather than expansion of the cropping area.

However, most of these lands have over the years been depleted of plant nutrients especially nitrogen (N) and phosphorus (P) and crop yields are therefore low (Sanchez et al., 1997). Developing sustainable cropping systems to better exploit soil nutrients resources such as N and P in these soils is one of the research challenges. Therefore intercropping of cereals with legumes has been considered as one of the efficient cropping systems that increase use of such nutrients and as a means of

*Corresponding author. E-mail: mercyofuyo@gmail.com.

maximizing land use (Lithourgidis et al., 2011; Matusso et al., 2014). Intercropping is not a new concept, but because of the current threat to food security, there has been a renewed interest to better productivity of such systems in tropical agriculture (Mal´ezieux, 2009).

Intercropping maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) is widely practiced on small scale farms in western Kenya. In such systems, beans are supposed to fix N and therefore secure the nitrogen economy while increasing yield of maize (Giller, 2001). However widespread phosphorus deficiencies in western Kenya limit nitrogen fixation by beans consequently negating their usefulness as a component of maize-bean intercropping systems. For efficient nitrogen fixation by the legume, adequate phosphorous must be supplied in form of fertilizers because nitrogen fixing bacteria require high energy in the form of ATP (Attar et al., 2012).

Crop productivity in intercropping systems depends on many factors including the crop variety used, plant density, cropping seasons and agricultural practices like irrigation, fertilization etc. (Tsubo et al., 2003). One aspect that has received little attention in intercropping research is the spatial arrangement of crops within the cropping system yet it is one of the most important agronomic factors that determine whether an intercrop system will be advantageous or not with regard to yield gains (Natarajan and Shumba, 1999). Since plants stand still in the land, the way they are distributed greatly influence the ability of a crop to capture and use environmental resources (radiation, water, and nutrients), which are necessary for growth and yield (Satorre, 2013). There is evidence that crop arrangements may create different microclimates in the stands and therefore influence the efficiency with which the growth resources are utilized (Dolijanovic et al., 2013). An ideal spatial arrangement is the one which maximizes the complementarity between the component crops and enhances physiological efficiency of the intercropping system in a given environment (Natarajan, 1990). Hence agronomic manipulation of the spatial arrangements of the component crops can strongly affect growth and yields of the crops and hence determine whether an intercrop system will be advantageous or not with regard to yield gains (Natarajan and Shumba, 1999). There is however little understanding on how crop arrangements interact with fertilizer inputs to affect crop yield in intercropping systems (Mal´ezieux, 2009). There are also concerns that even when the agronomic effectiveness of certain technologies is well established, adoption of such technologies by farmers is sometimes dismal (Opala et al., 2010). A fact that is often overlooked is that adoption of any technology by a farmer is not only based on yield returns but also on the accruing economic benefits (Odendo et al., 2007; Tungani et al., 2003). The objective of this study is therefore to assess the interactive effects of crop arrangement and P fertilizer rates and associated economic benefits.

MATERIALS AND METHODS

Site description

The study was conducted at Bugeng'i (0°7'N, 34°24'E) in Busia County in western Kenya, at an altitude of 1298 m above sea level. The area has two rainy seasons with long rains (LR) from March to July while short rains (SR) are from August to December; the mean annual rainfall ranges between 1270 and 1790 mm. The mean annual maximum temperatures range from 26 to 30°C while the mean minimum temperatures vary between 14 and 18°C. The dominant soil types are the highly weathered ferrasols (Jaetzold et al., 2009).

Soil sampling and analysis

Soils for site characterization at the beginning of the study were obtained at a depth of 0 - 20 cm by randomly auguring several spots in the field and then bulking the soil to get one composite. The soil was analysed using standard laboratory procedures (Okalebo et al., 2002). Soil pH was determined in a soil-water (1:2.5) suspension with a pH meter. Organic carbon was determined by Walkley-Black method while exchangeable calcium, magnesium potassium and extractable P were determined by Mehlich double acid method. Total soil N was determined by Kjeldahl acid digestion method.

Experimental design and treatments

The study was conducted for two consecutive cropping seasons; the SR in September 2015 and LR in March 2016. A split-plot design with 15 treatments replicated three times was used. The main plots consisted of five levels of maize-bean cropping arrangement as follows; (i) one row of maize alternating with one row of beans (conventional) (ii) maize planted in the same hole with beans (iii) two rows of maize alternating with two rows of beans (Mbili) (iv) sole maize and (v) sole beans. These were combined in a factorial arrangement with three P fertilizer levels, that is, 0, 30 and 60 kg P ha^{-1} in the subplots.

Crop establishment and management

Land was prepared to a medium seedbed tilth and plots measuring 4.5 m × 3 m demarcated. Sole maize (variety Western Hybrid 505) and common beans (Rose coco variety) were planted at 75 cm by 30 cm (44, 444 plants ha^{-1}) and 30 × 15 cm (202,020 plants ha^{-1}) respectively at the onset of the rains in each season. Maize was planted at two seeds per hill and later thinned to one plant. In all crop arrangements two beans per hill were planted and thinned to one except for maize and beans in the same hole where three bean seeds were planted and later thinned to two to give a bean population of 88, 888 plants ha^{-1} in all the intercrops. Triple superphosphate and calcium ammonium nitrate were evenly broadcast in the appropriate plots and incorporated into the soil at planting. However, only a third of N fertilizer (20 kg N ha^{-1}) was applied at planting. The rest 40 kg N ha^{-1} was applied using spot application to all maize treatments at 6 weeks after planting (WAP). Sole bean treatments were not top dressed with N fertilizer because the beans were inoculated and were therefore expected to fix N for

Table 1. Values used for cost- benefit analysis in both seasons.

Parameter	Value
Input costs	**Ksh kg^{-1}**
Rose cocoa grains: Sole beans	250
WH 505 maize grains	200
TSP fertilizer	70
CAN fertilizer	60
Bio fix	1250
Labour costs	
Ploughing	9000
Harrowing	6000
1st and 2nd weeding sole maize	10000
1st and 2nd weeding sole beans and intercrops	15000
Top-dressing	2000
Harvesting sole crops	7500
Harvesting intercrops	12500
Output prices	
Maize grain	35
Bean grain	75
Maize stover	3

Ksh is Kenya Shilling.

their growth. The crops were managed using the recommended agronomic practices for the area and harvested at physiological maturity. The yields of both crops were determined at moisture content of 13.5%.

Land equivalent ratio

Land Equivalent Ratio (LER) was used to compare yield advantage obtained from different intercropping arrangements. It was calculated as follows:

LER= Partial LER maize + Partial LER beans (1)

$$LER = \frac{Yab}{Yaa} + \frac{Yba}{Ybb} \quad (2)$$

Where *Y*aa and *Y*bb are yields as sole crops and *Y*ab and *Y*ba are yields in intercrops (Mead and Willey, 1980).

Economic analysis

Economic analysis was conducted using cost-benefit analysis (CIMMYT, 1988). The prices of the fertilizer and seed inputs were determined through a market survey of the area (Table 1) while the labour cost was determined by observing how long it took to perform specific activities and valued using the mean market wage rates within the study area. Economic benefits were calculated by multiplying the crop yields with prevailing market prices. To evaluate the economic benefits of the treatments under consideration, the benefit: cost ratios (BCRs), calculated as the net benefit due to the treatment divided by the total cost associated with that treatment was used.

Data analysis

Yield data were subjected to analysis of variance using Genstat software (Genstat Release 7.22, 2010) and treatment means separated by Least Significant Differences of means (LSD) at $p < 0.05$.

RESULTS AND DISCUSSION

Soil physical and chemical properties

Soil properties prior to establishment of the experiments at the site are presented in Table 2. The soil was very acidic with a pH of 4.8. This is to be expected in this high rainfall area because most of the basic cations have been leached (Kisinyo et al., 2014). This is confirmed by the low levels of Mg and K at the site. However, Ca was not limiting. Available P was below the critical value of 20 mg kg^{-1} that is considered adequate for most crops therefore justifying the need for application of P fertilizers at this site. Similar low P levels across many parts of western Kenya have been reported (Opala et al., 2014; Nziguheba et al., 2002) and attributed mainly to the high

Table 2. Initial soil properties at Bugeng'i.

Soil property	Value
pH (1:2.5 Soil:H_2O)	4.8
Total Organic Carbon (%)	1.1
Total Nitrogen (%)	0.12
Available P (mg kg^{-1})	8
Exchangeable Ca (Cmol kg^{-1})	4.22
Exchangeable Mg (Cmol kg^{-1})	0.01
Exchangeable K (Cmol kg^{-1})	0.29
Sand (%)	34
Clay (%)	26
Silt (%)	40
Textural class	Clay Loam

Table 3. Bean yields as affected by crop arrangement and phosphorus rate at Bugeng'i.

	Phosphorus rate kg ha^{-1}							
	Short rains seasons			Mean	Long rains season			Mean
Crop arrangement	0	30	60		0	30	60	
Conventional	0.09	0.14	0.22	0.15	0.18	0.33	0.34	0.28
Mbili	0.14	0.17	0.25	0.19	0.23	0.4	0.42	0.35
Maize + beans (SH)	0.13	0.13	1.23	0.5	0.17	0.34	0.34	0.28
Sole beans	0.42	0.6	1.8	0.94	0.5	0.88	0.99	0.79
Mean	0.2	0.26	0.87	0.45	0.27	0.49	0.52	0.43
	Probabilities of the F test for the ANOVA for system and P rate							
CA	0.01				NS			
P rate	0.001				0.03			
CA × P rate	NS				NS			
LSD								
CA	0.24				NS			
P rate	0.07				0.02			
CA x P rate	NS				NS			

SH = same hole; LSD = Least significant difference of means; N.S = not significant; CA= crop arrangement.

P-fixation capacity of these soils and cropping with little or no P inputs which has depleted soil P stock (Buresh et al., 1997). Organic C and N were below the optimum values of 2 and 0.2% respectively (Okalebo et al., 2002) likely again due to continuous cropping with no appropriate soil fertility replenishment measures.

Bean yields

Effects of treatments on bean yields are presented in Table 3. There was no significant effect of crop arrangement on bean yields in the LR. However in the SR, when averaged across all P rates, sole bean crop had significantly higher grain yields than the other crop arrangements. The effect of P fertilizer on bean yield was significant in both seasons. In the SR, application of 60 kg P ha^{-1} gave significantly higher bean yields than at 0 and 30 kg P ha^{-1} while in the LR bean yields at application of 30 and 60 kg P ha^{-1} did not differ significantly but were however significantly higher than at 0 kg P ha^{-1}. This response to P application confirms that the initial available soil P (8 mg kg^{-1}) at these sites was deficient. These results are consistent with those of Kajumula and Muhammad (2012) in Tanzania who observed that under Low P availability, beans suffer from reduced rate of

Table 4. Maize yields as affected by crop arrangement and phosphorus rate at Bugeng'i.

	Short rains season				Long rains season			
	Phosphorus rate kg ha^{-1}							
Crop arrangement	0	30	60	mean	0	30	60	mean
Conventional	2.43	4.13	5.84	4.02	0.26	0.55	0.59	0.46
Mbili	2.13 1.82	5.4	5.5	4.35	0.18	0.49	0.51	0.39
Maize+ beans (SH)	1.55	2.32	3.33	2.49	0.4	0.46	0.49	0.45
Sole maize		2.24	3.18	2.32	0.67	0.77	0.78	0.74
Mean	1.98	3.52	4.37	3.29	0.41	0.56	0.57	0.51

Probabilities of the F test for the ANOVA for system and P rate

	Short rains season	Long rains season
CA	0.001	NS
P rate	<0.001	NS
CA x P rate	NS	NS
LSD		
CA	0.75	NS
P rate	0.46	NS
CA x P rate	0.98	NS

SH = same hole; LSD = Least significant difference of means; N.S = not significant; CA= crop arrangement.

photosynthesis therefore impacting negatively on yield.

The average bean yields (0.45 and 0.43 t ha^{-1} in the SR and LR respectively) were lower than the potential yield of 3 t ha^{-1} that was reported by Namugwanya et al. (2014). These poor yields are attributed to the adverse weather conditions during the study period. In the SR season, heavy rain physically damaged the bean leaves. In the LR, there was severe drought with no rain received during the critical flowering period. The highest bean yields were obtained in the sole bean crops mainly because of their higher plant population (202,020 plants ha^{-1}) compared the intercrops (88, 888 plants ha^{-1}) but also due to reduction in yields per plant due to competition in the intercrops. Other crop arrangements did not differ significantly in bean yields.

Maize grain yields

Maize grain yields were higher in the SR (mean of 3.29 t ha^{-1}) than the LR (mean of 0.51 t ha^{-1}) at (Table 4). The variation in maize grain yield observed between the two seasons is attributed mainly to the differences in rainfall. In the SR season, the rainfall was unusually high 1065 mm compared to 137.50 mm, the normal long term means and well distributed during the growing period of maize. However, in the LR season the rainfall was low and poorly distributed. Only 529 mm of rainfall was recorded in this season, with only 30 mm being received in the month of June at the critical stage when the maize was tasselling and no rainfall was recorded in July. There were no significant treatment effects on maize grain yields in the LR (Table 4) mainly due to severe drought. In the SR, there was no significant interaction between P rate and crop arrangement on maize grain yield but maize yields generally increased with increasing P rate within a crop arrangement (Table 4).

Crop arrangement significantly affected maize yields in this season where the mean yields for conventional and Mbili arrangements were statistically similar but were significantly higher than those of maize planted in the same hole with beans and sole maize. The better performance of these two intercropping arrangements compared to maize and beans planted in the same hole is attributed to the appropriateness of these crop arrangements that reduced interspecies competition for growth resources between maize and beans. Similar results were reported by Mattuso et al. (2014) and Mucheru-Muna et al. (2010) in the central highlands of Kenya, and Woomer et al. (2004) in western Kenya. While competition for nutrients and water is expected to be severe in maize and beans planted in the same hole therefore contributing to low maize yields, the low yield of sole maize compared to the other intercrops of Mbili and conventional was unexpected. Many other studies have reported that maize yields are usually depressed or not affected by the intercropped beans (Nassarya et al., 2020; Morgado and Willey, 2008).

Application of 60 kg P ha^{-1} gave significantly higher maize yields than at 0 and 30 kg P ha^{-1} during the SR season, when rainfall was not limiting, for most crop arrangements confirming. Since phosphorus was limiting at this site, the response to P was not entirely unexpected. Similar increases in maize yield have been

Table 5. Land equivalent Ratios for the intercrops at Bugeng'i.

Treatment	SR	LR
Conventional 0P	1.23	0.81
Conventional 30P	1.33	1.21
Conventional 60P	2.09	1.41
Mbili 0P	1.18	0.71
Mbili 30P	1.76	0.9
Mbili 60P	2.05	2.15
Maize, bean 0P	0.88	0.77
Maize, bean 30P	1.22	0.95
Maize, bean 60P	0.7	1.05

Table 6. Cost, benefits and cost - benefit ratios of treatments.

Treatment	Costs (Ksh)	Net benefits (Ksh)		BCR	
		SR	LR	S R	LR
Sole bean 0P	84069	-52069	-76569	-0.62	-0.91
Sole bean 30P	94520	-59471	-57020	-0.63	-0.6
Sole bean 60P	104971	-65373	-38971	-0.62	-0.37
Maize, bean 0P	94566	32484	-62836	0.34	-0.66
Maize, bean30P	105017	30042	-67887	0.29	-0.64
Maize, bean 60P	115468	59910	-71768	0.52	-0.62
Conventional 0P	98816	58374	-67546	0.59	-0.68
Conventional 30P	109276	97182	-63017	0.89	-0.58
Conventional 60P	119718	148400	-67868	1.24	-0.57
Mbili 0P	98816	51894	-73106	0.53	-0.74
Mbili 30P	109267	151892	-56087	1.39	-0.51
Mbili 60P	119718	161760	-62928	1.35	-0.53
Sole maize 0P	69333	4437	-37753	0.07	-0.54
Sole maize 30P	79784	-235	-43714	0	-0.55
Sole maize 60P	90235	23643	-52855	0.26	-0.59

demonstrated in many other studies in western Kenya (Nziguheba et al., 2016; Opala et al., 2012).

Land equivalent ratio

The total LER during the SR season showed yield advantage (LER >1) of intercropping maize and beans over component sole crops for all the intercropping arrangements and fertilizers rates except for maize and beans planted in the same hole at 0 kg P ha^{-1} (Table 5). However in the LR, only the conventional arrangement at P rates of 30 and 60 kg ha^{-1} and Mbili at 60 kg ha^{-1} had total yields of the intercrops being greater than the monocrops (LER >1). The better performance of the intercrop in the SR is attributed to more efficient resource use and resource complementarity under the prevailing favourable rainfall compared to sole cropping. Similar results were reported by Latati et al. (2013) and Tsubo et al. (2001). However, when fertilizer was applied and maize and beans planted in the same hole, the nutrients became limiting due to severe competition. The low LERs in the LR are attributed to competition for water by the component crops. The intercrops had higher plant water requirements and hence consumed more water than sole crops. The sole crops therefore performed better under the water stress than the intercrops in this season that received below average rainfall.

Cost - benefit analysis

Results of cost-benefit analysis for the 2015 SR and 2016 LR are shown in Table 6. The costs for the two seasons are similar because the same treatments were repeated. In both seasons, sole maize at 0 kg P ha^{-1} recorded the least cost (Ksh 69, 333) while conventional and Mbili both at 60 kg P ha^{-1} recorded the highest costs (Ksh 119,718) because of higher labour costs in these crop arrangements. There were negative financial returns across all the treatments in the LR mainly due to high costs of production that could not be compensated through the sale of the low yields of maize and beans in that season. However positive financial returns were recorded in the SR with Mbili arrangement at 60 kg P ha^{-1} recording the highest financial returns (Ksh 161,760). This was attributed to better yields that were achieved by this crop arrangement in this season. Similar results were reported by Mucheru-Muna et al. (2010) and Nekesa et al. (2005) in Central Kenya. However, all treatments recorded BCR values of < 2 with the highest BCR (1.27) obtained with Mbili at 30 kg P ha^{-1}. The general rule is that a BCR of at least 2 is attractive to farmers (FAO, 2006). None of the treatments in the present study is therefore likely to be adopted by rational farmers in the study area if the prevailing climatic and economic conditions prevail. Similar results that showed technologies having agronomic effectiveness but being economically unattractive have been reported by other workers in western Kenya (Nyambati and Opala, 2014; Jama et al., 1997).

Conclusion

The yields of component crops generally did not significantly differ among crop arrangements and P rates in the LR under drought conditions which limited growth. However, when rainfall conditions were more favourable, both bean and maize yields generally increased with increasing P rate in this P deficient soil. Among the crop arrangements, conventional and Mbili arrangements had similar yields but were superior to maize and beans planted in the same hole. In addition, during the SR season, sole beans recorded significantly higher bean yields than the intercropped ones. None of the treatments was economically attractive because of high costs of production coupled with low yields and low prices offered for the crops. Therefore, unless smallholder farmers are assisted by subsidizing fertilizer inputs and/or offered higher prices for their produce, the vicious poverty cycle prevailing in the region will continue as they must till their land to eke a living.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Leaf organic content variation of 2 durum wheat genotypes under water stress by applying phytohormone (IAA)

A. Bendjama[1*] and S. Ramdani[2]

[1]Department of Natural and Life Sciences, Faculty of Sciences, University of 20 august 1955 Skikda, Algeria.
[2]Scientific and Technical Research Center in Physico-Chemical Analysis (CRAPC), Tipaza, Algeria.

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Water stress is the most important problem in plant growth and development. Greenhouse trial is carried out on 2 durum wheat (*Triticum durum* Desf) genotypes (MBB and WAHA), to evaluate the effect of water stress on the biochemical content of leaves, and the possibility of reducing this effect by applying a growth regulator Indole-3-acetic acid (IAA) by seeds soaking and foliar spraying. Water stress causes a large accumulation of proline and soluble sugars and a decrease in the chlorophyll content of the leaves. The results obtained show that the effect of the hormone on the above-mentioned parameters is different according to the genotype and the mode of treatment, as well as the growth phase of the plant, without being able to promote application by soaking or spraying. The application of IAA has relatively reduced the effect of water stress by promoting the synthesis of proline and soluble sugars as osmotic regulators, and by increasing the chlorophyll content of the leaves. proline and soluble sugars concentrations showed negative and significant correlations with those of total chlorophyll. Our present study highlights some biochemical responses of plants to tolerate a water deficit and the possible involvement of exogenus application of IAA, as a phytohormone, in these regulatory mechanisms.

Key words: *Triticum durum* Desf, water stress, Indole-3-acetic acid (IAA), seed soaking, foliar spraying.

INTRODUCTION

Water is a vital element for the growth of plants. It is the most important factor that determines the growth and development of organisms. Plants are exposed to a variety of abiotic stresses in nature and exhibit unique and complex responses to these stresses depending on their degree of plasticity. When plants grow in a water-limited environment, they undergo morpho-physiological, phenological and biochemical modifications, to maintain

*Corresponding author. Email: bendjama45@gmail.com.

constant cellular water potential, considered as water deficiency adaptation mechanisms (Manavalan et al., 2009; Basu et al., 2016). Plants accumulate osmolytes or compatible solutes to protect the cellular machinery from various environmental stresses (Giri, 2011). The most well-known osmolytes are glycine betaine (GB), sugars (mannitol, sorbitol, and trehalose), polyamines, and proline. These osmolytes get accumulated under various abiotic stresses and confer tolerance to cell without interfering with the cellular machinery of the plant (Chen, 2002; Anjum et al., 2017).The accumulation of proline and sugars is a clear marker for environmental stress, particularly in plants under drought stress (Watanabe et al., 2000). Proline and sugars accumulation may also be part of the stress signal influencing adaptive responses in drought stress conditions by helping to maintain membrane stability, preventing and protecting membrane fusion and; keeping protein so as to remain functional (Maggio et al., 2002; Xonostle-Cazares et al., 2010; Arabzadeh, 2012). Drought stress caused a large decline in chlorophyll a, chlorophyll b content, and the total chlorophyll content in all sunflower varieties investigated Ommen et al. (1999) and Manivannan et al. (2007) reported that leaf chlorophyll content decreases as a result of drought stress. Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, affecting cholorophyll components and damaging the photosynthetic apparatus (IturbeOrmaetxe et al., 1998).

Phytohormones are the key regulators of plant growth and developmental processes and also crucial for biotic and abiotic stress response throughout their life cycle (Sah et al., 2016; Ullah et al., 2017). Phytohormones including ABA, jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) are involved in osmotic adjustment and other drought-related processes (Khan et al., 2015; Vishwakarma et al., 2017). Auxins are the group of phytohormones which play a significant role in plant growth, development and response to various stresses (Singh et al., 2017). IAA is an auxin that participates in many plant processes including oxidative stress defense. It has become more evident that adaptation to drought is accompanied by an increase in the IAA levels (Zholkevich and Pustovoitova, 1993). Indole acetic acid (IAA) plays a vital role in maintaining plant growth under stress conditions (Gulnaz et al., 1999; Iqbal and Ashraf, 2007).

In the present study, we investigated the effects of exogenous indole − 3-acetic acid (IAA) on biochemical changes of durum wheat under drought stress conditions. Two genotypes were selected for this study: WAHA, which is considered a relatively drought-resistant genotype, and MBB, which is on the other hand sensitive to water deficit Subjected to different levels of water stress while counteracting this constraint by soaking the seeds in the IAA solution before transplanting, and by spraying the plant leaves with the IAA solution at three growth stages. To study the plant responses, some biochemical parameters (the ratio of proline, dissolved sugars, chlorophyll) are evaluated, which are supposed to contribute to plant adaptation under abiotic stress conditions and that can be adopted in the selection of plant varieties programs for this purpose.

MATERIALS AND METHODS

Trials management

The study was conducted in a plastic house located in the compound of the lead section - University of Skikda, Algeria; its estimated temperature was between 9°C and 15°C at night and between 24°C and 42°C during the day; its humidity ranged between 75 and 100%. The plastic house opens daily during hot days. 3 levels of watering were used:

S_0 level = Field capacity watering.
S_1 level = 50% field capacity Watering.
S_2 level = 25% field capacity Watering.

The hormone is applied by seed soaking (7 ppm IAA solution) and leaf spraying (0.5ppm IAA solution). The experiment design was 2 factorials (3 water levels and 2 durum wheat genotypes (WAHA and MBB) and two types of IAA application laid in completely randomized design (CRD) with three replications. A total of 36 pots (28 cm deep with 25 cm diameter at top) were prepared with each 6 kg of Homogeneous agricultural soil which is relatively rich in organic matter.

Field capacity (100%) of the soils was determined through gravimetric methods before use.
Seeds of each cultivar were presoaked in hormonal solution (7ppm) in the dark for 48 h.

Proline accumulation in the fresh leaves was determined according to the method of Bates et al. (1973). Free proline was extracted using aqueous sulfosalicylic acid. The filtrate (1 ml) was mixed with equal volumes of glacial acetic acid and ninhydrin reagent (1.25 g ninhydrin, 30 ml of glacial acetic acid, 20 ml of H_3PO_4) and incubated for 1 h at 100°C. The reaction was stopped by placing the test tubes in cold water. The reaction mixtures were rigorously mixed with 3 ml toluene. The absorbance of toluene phase was estimated at 520 nm using a spectrophotometer. The proline concentration was determined using a standard curve.

Soluble sugars were determined based on the phenol-sulphuric acid method (Dubois et al., 1956). 0.1 g of dry leaves was homogenized with deionized water, filtered and the extract was treated with 1 ml of phenol (5%) and 5 ml of sulphuric acid (96%). The mixture was incubated at room temperature for 1h and then absorbance at 490 nm was read on a spectrophotometer. Contents of soluble sugar were determined by using glucose as a standard and expressed as µg/mg.

Chlorophyll pigments was extracted from 100 mg crushed fresh leaves in a sufficient volume of 80% acetone; chlorophylls a, b and total chlorophyll were estimated by following the method of Inskeep and Bloom (1985).

Table 1. Average proline concentration (µg / 100 mg) ± standard deviation.

IAA treatment	Stress levels	None		Seed soaking		Foliar spraying	
Growth phases		**WAHA**	**MBB**	**WAHA**	**MBB**	**WAHA**	**MBB**
	S_0	5.37±0.08	4.82±0.27	26.61±2.5	6.72±1.1	3.78±0.71	3.38±0.07
Vegetative phase	S_1	29.93±15.08	15.04±1.77	48.18±7.6	27.63±4.33	31.83±14.73	9.22±1.92
	S_2	53.14±7.14	41.94±13.18	80.59±15.06	41.32±13.93	29.48±4.57	20±2.25
	S_0	10.51±2.32	20.15±5.11	10.51±2.32	11.89±2.71	9.93±3.54	8.55±1.25
Heading	S_1	20.15±10.1	44.97±3.99	19.22±8.27	43.41±5.0	22.19±11.96	46.31±8.5
	S_2	36.1±5.65	68.54±4.8	53.99±14.55	72.24±2.17	75.91±1.01	75.05±3.88
	S_0	11.1±1.82	22.24±3.97	12.84±2.68	22.19±9.65	13.15±0.9	22.39±5.02
Anthesis	S_1	18.69±8.28	33.99±0.93	35.63±5.83	35.92±4.02	77.08±7.78	37.38±5.28
	S_2	124.77±25.31	113.87±23.17	152.71±41.71	122.63±23.18	160.06±19.78	187.16±6.21

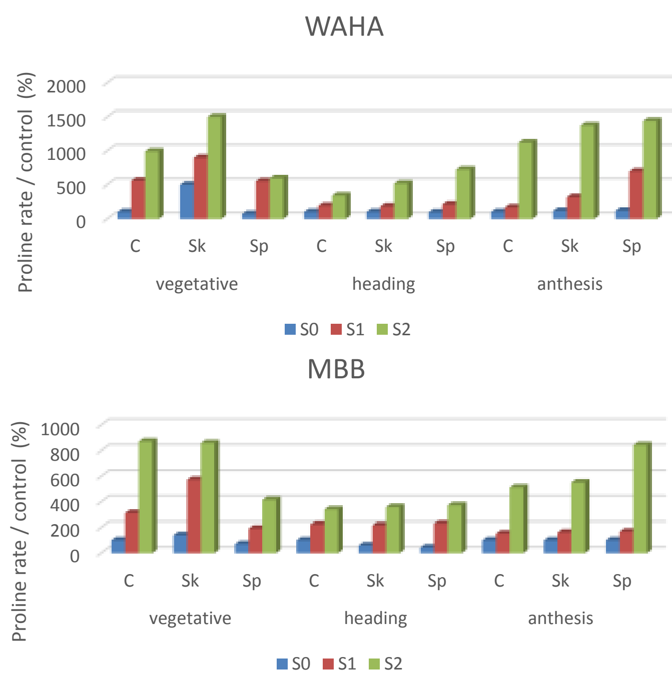


Figure 1. Effect of watering levels and hormone treatment on leaf proline content.

Statistical analysis

The data are presented as the mean values ± SE. Every treatment was replicated three times. Statistical analysis was performed using two-way ANOVA and differences between the mean values were compared using the LSD test at P ≤ 0.05.

RESULTS

Table 1 and Figure 1 show the effect of the genotype, hormone treatment and the irrigation levels on the average leaf content of proline during plant development. Results showed significant variation in free proline content within a variety as well as among varieties under different treatments. Both varieties have the ability to accumulate proline under osmotic stress to different degrees. The amount of free proline was high under osmotic stress compared to unstressed conditions. Comparing the results of the three phases, the of proline

Table 2. Analysis of variance for the concentration of proline.

Phases		Vegetative		Heading		Anthesis	
Source	DDL	F	Pr	F	Pr	F	Pr
G	1	47.94***	00	68.24***	00	0.05^{ns}	0.8
Irg	2	86.87***	00	283.98***	00	346.59***	00
Hrm	2	33.66***	00	4.34**	0.02	16.41***	00
G*Irg	2	3.63*	0.03	12.0***	00	1.74 ^{ns}	0.18
G*Hrm	2	6.3**	0.004	5.9**	0.006	0.7 ^{ns}	0.51
Hrm*Irg	4	4.52**	0.004	8.04***	00	4.71**	0.003

DDL: degrees of freedom, F: F test, P: P value.

increased by increasing the duration of water stress, Plants in the S_2 irrigation level recorded the highest proline content at each stage while those in the S_0 treatment had the lowest. Proline content of the two wheat genotypes increased significantly under drought treatment in all plant development stages. WAHA accumulates more proline than MBB during the vegetative phase. The effect of hormone treatment varied from one phase to another, where the seed soaking has a positive effect during the vegetative phase for WAHA. Spraying has negative effect on the two genotypes; whereas during the other two phases it is the spraying treatment that has a positive effect on the accumulation of proline in both genotypes.

Analysis of variance (ANOVA) shows that the main effects, genotype, irrigation and hormone treatment are generally significant in all phases (Table 2). This indicates that the two genotypes have the capacity to modulate their endogenous proline to avoid water deficit conditions and that the application of the hormone significantly affects this response. First order interactions are also generally significant which indicates that the effect of each treatment differs from the levels of the others; thus the effect of the hormone on the accumulation of proline is dependent on the genotype and the intensity of the water stress. In fact, the accumulation of proline by each genotype is the result of the main effects and their interactions. The NEWMAN-KEULS test at the limit (α = 0.05%) shows the presence of homogeneous groups for the second order interaction between effects: genotype, irrigation and hormone treatment (Figure 2).

Sugars

Table 3 and Figure 3 show the effect of the genotype, hormone treatment and irrigation levels on the average leaf content of sugars during plant development. The accumulation of soluble sugars increased by increasing the duration of water stress. The two genotypes significantly increase their sugar content under stress conditions during anthesis, in particular. Note that genotype MBB accumulates more soluble sugar than WAHA during vegetative and heading phases. The opposite is noted in the anthesis. The effect of hormone treatment varied from one phase to another, where both seed soaking and foliar spraying by IAA have a positive effect on the accumulation of sugars for the two genotypes. During the heading phase this effect is less, whereas during the anthesis phase, spraying application has a strong positive effect on the two genotypes.

Analysis of variance (ANOVA) shows that the main effects, genotype, irrigation and hormone treatment as well as those first order interactions are generally significant (Table 4; Figure 4) suggesting that the two genotypes accumulate soluble sugars at different levels in response to water stress and that IAA is involved in this response. Interactions are not significant at the vegetative stage. This indicates that the soluble sugars synthesis is dependent only on main effects.

The NEWMAN-KEULS test at the limit (α = 0.05%) shows the presence of homogeneous groups with the smallest significant difference (LSD) for the first and second order interaction between effects: genotype, irrigation and hormone treatment (Figure 4).

Chlorophyll

The results of the 3 stages of plant development (Table 5; Figure 5) show that the total chlorophyll content decreases significantly under stress conditions. Both hormonal treatments significantly improve the chlorophyll content of WAHA during the heading phase; and spraying application was shown to be the best.

ANOVA shows that the main effects, genotype, irrigation and IAA treatment as well as their first order interactions are significant at the heading and the anthesis phases (Table 6). This indicates that the chlorophyll synthesis is affected by water stress and IAA treatment. The NEWMAN-KEULS test at the limit (α =

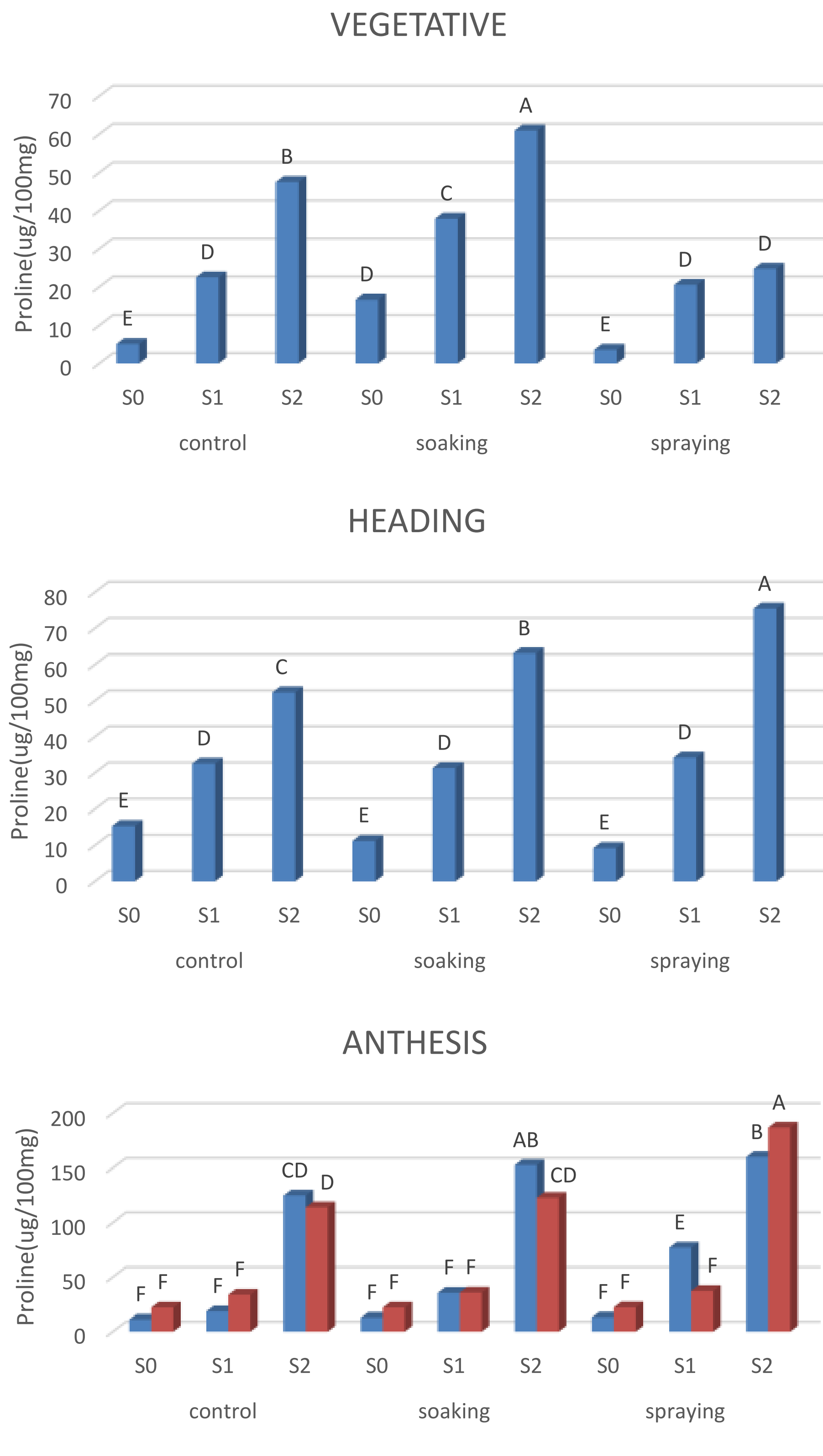


Figure 2. Proline content of two durum wheat genotypes grown under control and drought stressed conditions and exogenous IAA application. Values with different letters are significantly different at P=0.05.

Table 3. Average dissolved sugars concentration (µg / 100 mg) ± Standard deviation.

IAA Applic.	Stress levels	None		Seed soaking		Foliar spraying	
		WAHA	MBB	WAHA	MBB	WAHA	MBB
Vegetative phase	S_0	0.32±0.14	0.41±0.16	0.33±0.10	0.54±0.1	0.14±0.03	0.42±0.08
	S_1	0.48±0.07	0.62±0.27	0.50±0.05	0.72±0.05	0.47±0.12	0.61±0.11
	S_2	0.69±0.13	0.77±0.11	0.95±0.44	1.16±0.28	0.84±0.06	0.93±0.05
Heading	S_0	15.54±1.37	19.43±1.22	15.54±1.25	15.21±1.86	13.01±2.57	15.62±1.54
	S_1	17.28±1.30	23.79±0.91	15.67±2.65	16.68±3.76	16.32±1.57	25.62±5.72
	S_2	16.31±1.93	31.10±3.04	18.97±1.4	32.60±2.02	20.94±8.38	37.97±6.26
Anthesis	S_0	83.2±15.35	12.26±3.07	85.36±6.8	9.55±2.61	91.76±2.34	10.03±1.41
	S_1	122.07±33.94	17.39±2.04	176.92±33.36	14.21±0.38	196.53±65.56	15.52±3.0
	S_2	219.76±54.66	77.02±47.44	358.58±53.0	144.76±21.48	452.67±60.72	339.01±54.08

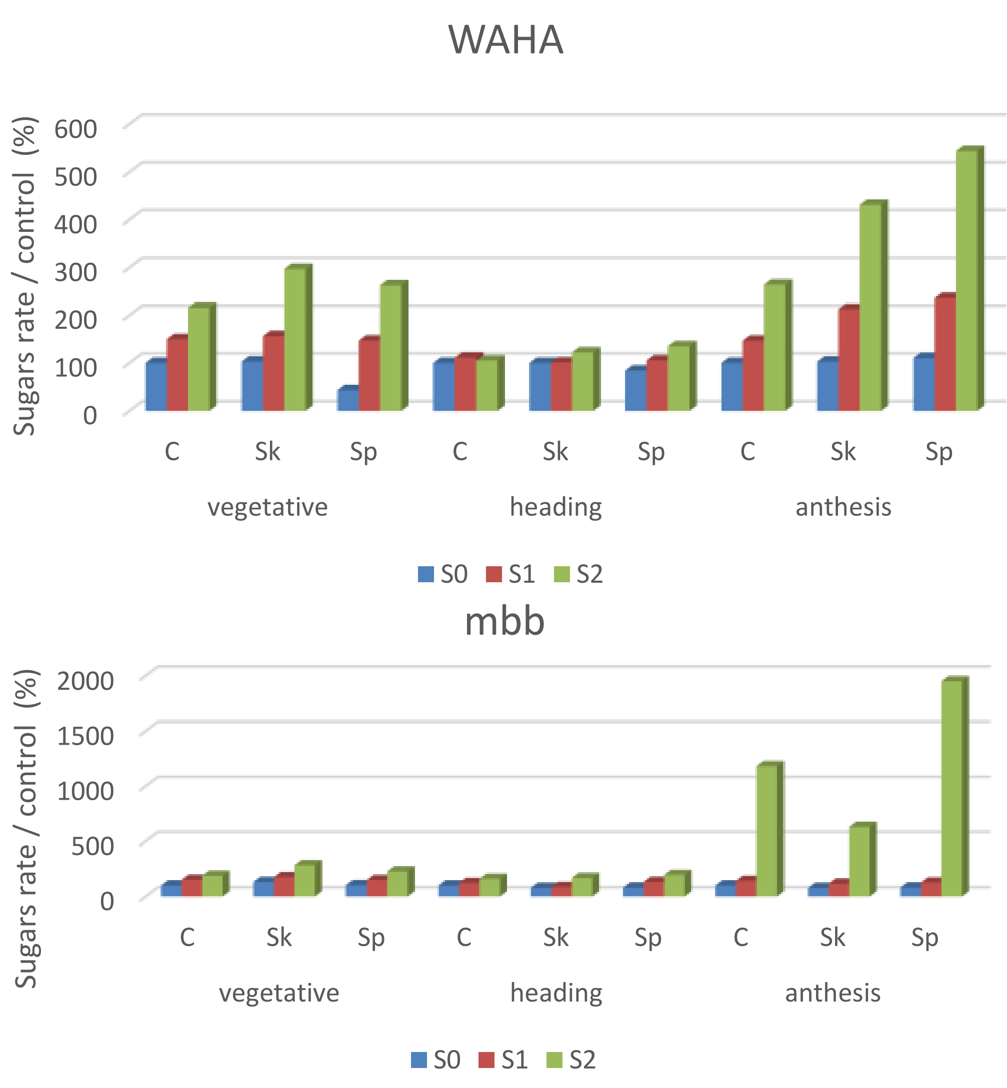


Figure 3. Effect of watering levels and hormone treatment on leaves sugars content.

Table 4. Analysis of variance for the concentration of dissolved sugars.

Phases		Vegetative		Heading		Anthesis	
Source	**DDL**	**F**	**Pr**	**F**	**Pr**	**F**	**Pr**
G	1	13.19***	0.001	68.64***	00	179.4***	00
Irg	2	47.43***	00	46.04***	00	194.5***	00
Hrm	2	4.52**	0.017	2.44 ns	0.09	28.5***	00
G*Irg	2	0.2 ns	0.8	18.14***	00	7.31**	0.002
G*Hrm	2	0.52 ns	0.6	2.54 ns	0.09	7.44**	0.002
Hrm*Irg	4	1.47 ns	0.2	3.56**	0.015	18.75***	00

*** very highly significant, DDL: degrees of freedom, F: F test, P: P value.

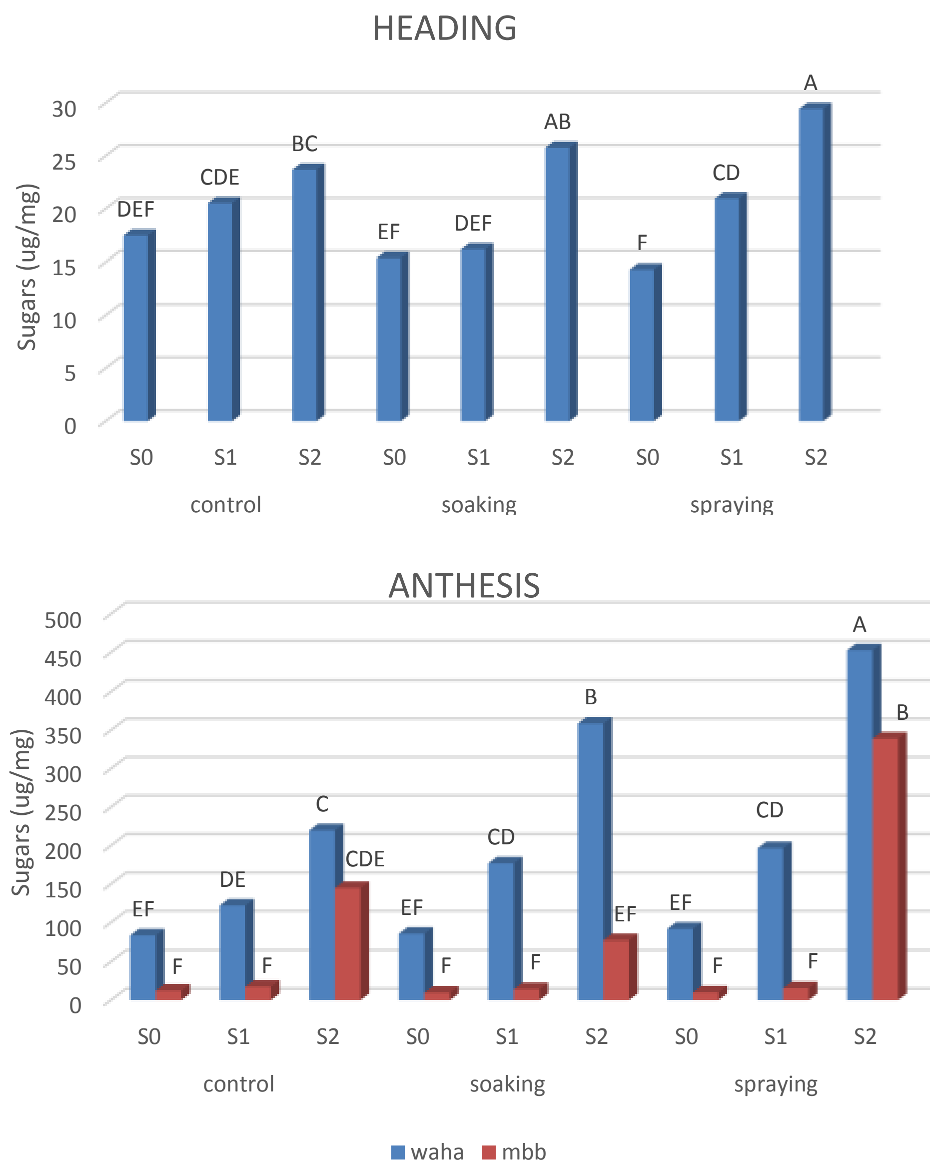
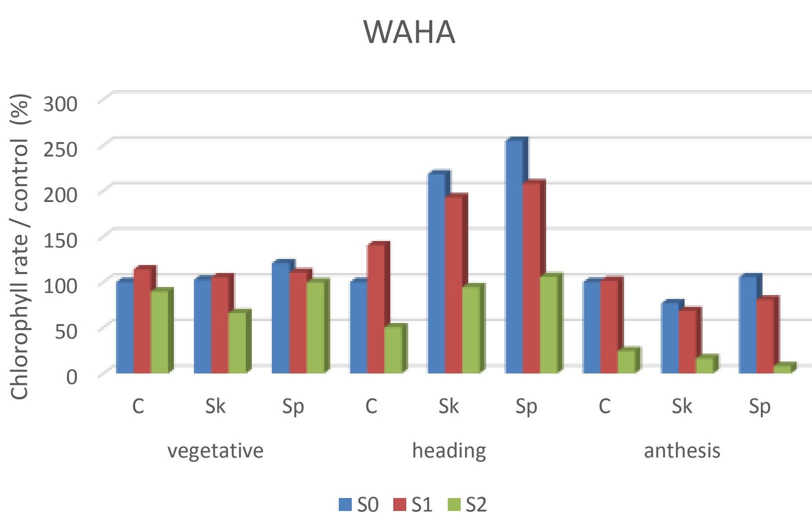


Figure 4. Soluble sugars content of 2 durum wheat genotypes grown under control and drought stressed conditions and exogenous IAA application. Values with different letters are significantly different at P=0.05.

Table 5. Average chlorophyll concentration (µg / 100 mg FM) ± Standard deviation.

IAA Applic.	Stress levels	None		Seed soaking		Foliar spraying	
Growth phases		WAHA	MBB	WAHA	MBB	WAHA	MBB
	S_0	13.62±4.40	15.51±1.43	13.97±2.31	11.83±1.87	16.44±7.88	14.04±1.49
Vegetative phase	S_1	15.52±1.08	11.06±1.4	14.32±1.46	14.33±1.4	15.05±0.9	13.76±3.11
	S_2	12.19±3.27	10.24±2.7	8.98±1.76	11.63±2.33	13.58±1.03	11.90±3.9
	S_0	10.67±3.69	12.44±1.46	23.28±5.14	30.29±1.78	27.2±5.49	19.68±1.91
Heading	S_1	14.99±4.06	11.99±0.92	20.56±3.6	13.8±0.72	22.19±1.79	13.82±0.94
	S_2	5.35±1.14	4.2±2.17	10.06±1.43	3.91±1.64	11.29±3.96	3.31±0.90
	S_0	24.16±1.65	12.49±1.99	18.51±0.14	13.13±5.98	25.44±3.28	18.70±1.99
Anthesis	S_1	24.53±5.21	10.10±3.59	16.49±0.80	8.58±0.36	19.46±4.14	9.58±0.28
	S_2	5.96±1.24	9.52±0.53	3.99±2.23	6.78±0.97	1.92±0.85	7.41±0.51



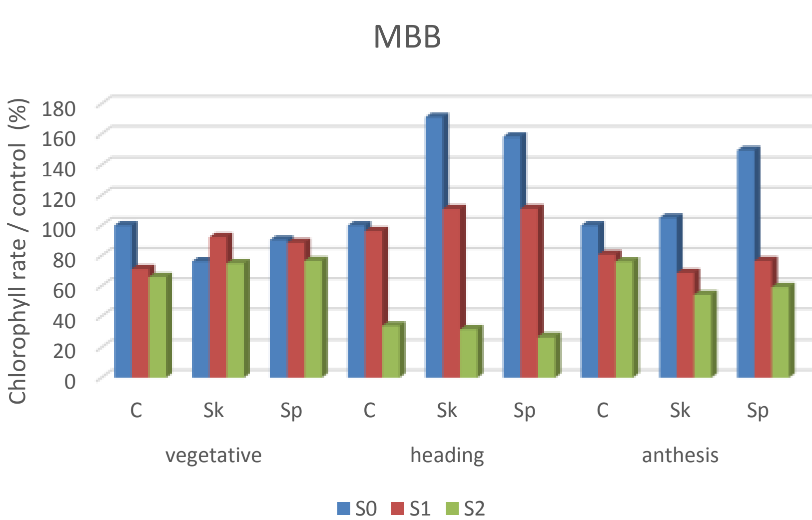


Figure 5. Effect of irrigation levels and hormone treatment on leaves total chlorophyll content.

Table 6. Analysis of variance for the concentration of dissolved sugars.

Phases		Vegetative		Heading		Anthesis	
Source	**DDL**	**F**	**Pr**	**F**	**Pr**	**F**	**Pr**
G	1	1.71ns	0.19	21.92***	00	47.23***	00
Irg	2	5.91**	0.006	122***	00	112.5***	00
Hrm	2	1.43ns	0.25	34.37***	00	7.45***	0.002
G*Irg	2	0.69ns	0.51	6.98***	0.002	39.34***	00
G*Hrm	2	0.59ns	0.56	8.46***	0.001	3.33*	0.04
Hrm*Irg	4	0.79ns	0.54	10.24***	00	4.22**	0.006

*** very highly significant, DDL :degrees of freedom, F : F test, P : Pvalue.

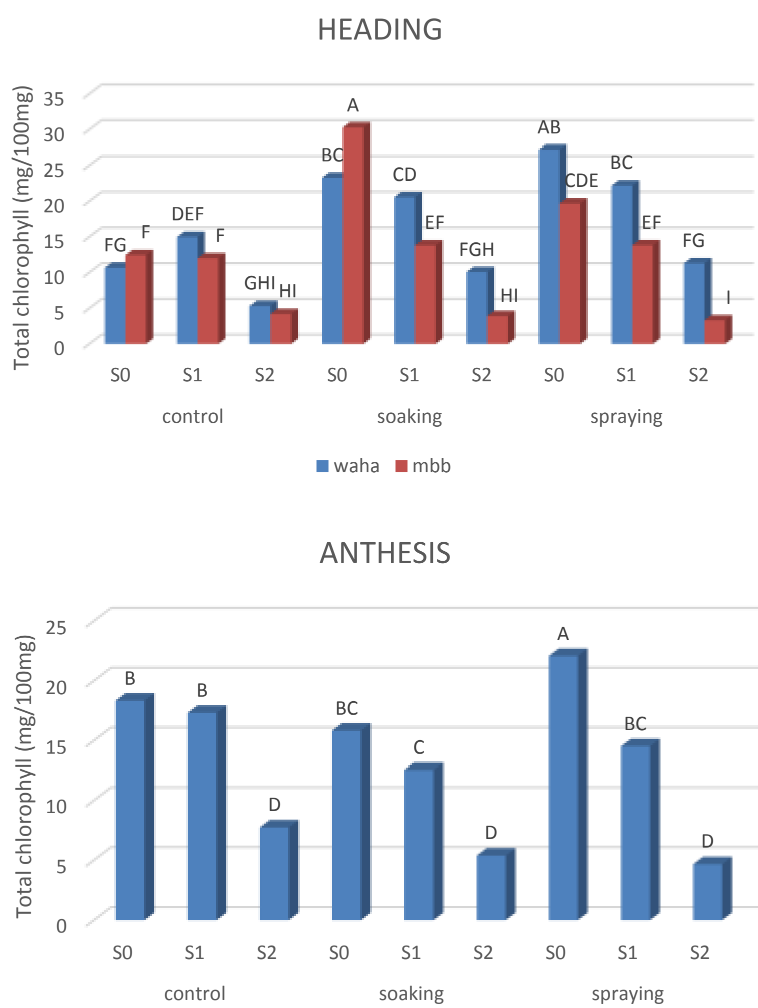


Figure 6. Total chlorophyll content of two durum wheat genotypes grown under control and drought stressed conditions and exogenous IAA application. Values with different letters are significantly different at P=0.05.

0.05%) shows the presence of homogeneous groups with the smallest significant difference (LSD) for the second order interaction between effects: genotype, irrigation and hormone treatment (Figure 6).

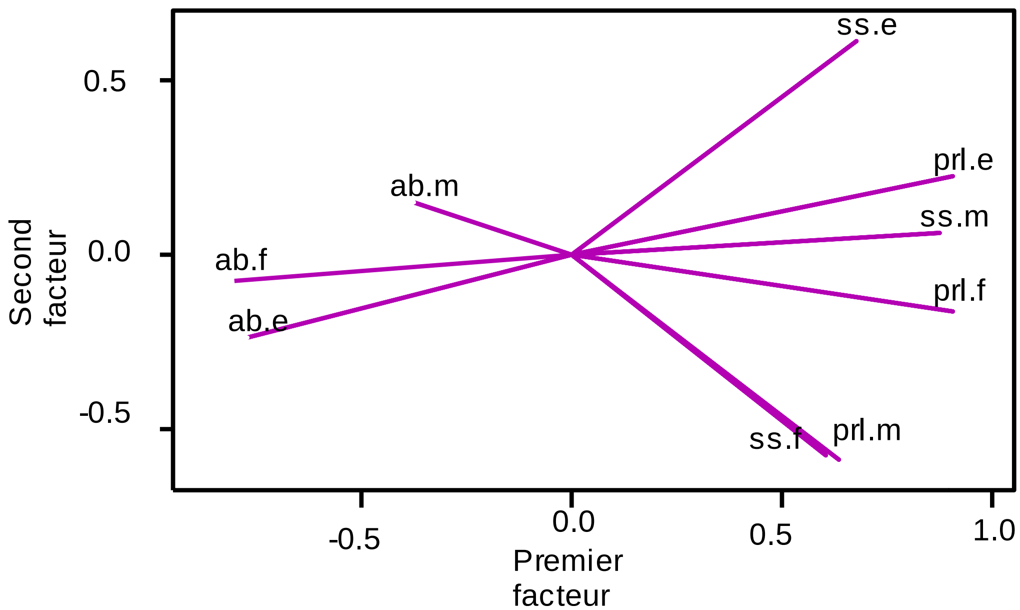


Figure 7. Correlation diagram between leaf proline, soluble sugar and chlorophyll contents.
pr.m, ss.m, ab.m(proline, soluble sugars, chlorophyll at vegetative stage respectively)
pr.e, ss.e, ab.e (proline, soluble sugars, chlorophyll at heading stage respectively)
pr.f, ss.f, ab.f (proline, soluble sugars, chlorophyll at anthesis stage respectively).

To examine the relationships between the different measurements made, we performed a PCA (Figure 7). The percentage of information for axis 1 was 56.5% and 13.5% for axis 2, that is, 70%. Prol.e / prl.f, prl.e / ss.m and prl.f / ss.f where the correlation coefficient was (0.80), 0.78) and (0.76) respectively, noting that the ss.e / prl.m and ss.e / ss.f correlations were weak. Correlations between chlorophyll ratios were low except for ab.e / ab.f which were significant (0.54). The correlations between proline and dissolved sugar concentrations and total chlorophyll concentrations were negative and significant, the most important being: (-0.75), (-0.73) and (-0.70) for ab.f / prl. e, ab.f / ss.m و ab / prl.e, respectively.

DISCUSSION

The concentration of proline and soluble sugars in leaves of the two wheat genotypes significantly increased at the different growth stages in stressed plants. The highest levels of accumulation were in the anthesis phase. These results suggest that the osmotic adjustment is a common response of plants under drought conditions irrespective of the growth stage of the plant. Proline, a compatible osmolyte, is known to accumulate under abiotic stress (Sandhya et al., 2010). Osmotic adjustment through the accumulation of cellular solutes, such as proline, has been suggested as one of the possible means for overcoming osmotic stress caused by water loss (Caballero et al., 2005). According to Umezawa et al. (2006), plants have the ability to accumulate non-toxic compounds such as proline which protects cell damage due to low water potential of cells, which is a way of plant adaptation to drought stress tolerance. The accumulation of glucose in different wheat varieties was observed under the conditions of water deficit (El-jaafari, 1993; Brinis, 1995). Several studies have also shown a positive correlation between the accumulation of proline and sugars and the severity of water stress (Berllinger et al., 1991; Gorham, 1993).

The results show that the IAA treatment mostly led to a greater accumulation of both proline and sugars in the two varieties in the anthesis and heading stages in particular, suggesting that IAA had a positive effect in improving drought tolerance of wheat. Studies have shown that plants pre-treated with IAA exhibited enhanced drought resistance (Almazroue, 2014). Foliar spraying with growth regulators (IAA and GA3) showed significant effect on plant, to the extent of reducing the hurt effect of salinity on the vegetative measurements and some physiological components of plant (Gherroucha et al., 2011). Shalaby and Kishk (1986) confirmed the increase of proline accumulation in the presence of growth regulators; stimulating hormones, especially quinine, regulate the synthesis and accumulation of dissolved sugars in growing plants in saline media. Chlorophyll content decreased significantly under water stress in both wheat genotypes; various reports have noted that the water deficit decreases considerably, depending on the intensity and duration of the stress, the total chlorophyll content in different plant species (Mafakheri et al., 2010; Gholamin and Khatnezhad, 2011; Din et al., 2011; Mouradi et al., 2016). Exogenous IAA appears to have significant beneficial effects on chlorophyll content; Zhang et al. (2020), in a study on white clover, noted that the chlorophyll content of leaves increased significantly when treated with exogenous IAA. Negative and significant correlations between proline and dissolved sugar concentrations and total chlorophyll concentrations were found. These results are in accordance with other researches (Schonfeld et al., 1988; Bayoumi et al., 2008; Rad et al., 2012).

Conclusion

From the present experiment, it can be concluded that water deficit significantly increases the leaf content in proline and soluble sugars and decreases their chlorophyll content in both genotypes used in this study. The IAA application considerably counteracts these effects by improving the biosynthesis of these components. The effect of hormone treatment varies from one phase to another where the leaf spraying treatment was shown to be the best.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Biofortified bean genotypes under integrated soil fertility management across sub-humid agro-ecological zones of The Democratic Republic of Congo

Kanyenga L. Antoine[1,2*], Kizungu V. Roger[3,5], Kasongo L. Emery[2], Kalonji M. Adrien[4] and Rowland M. Chirwa[5]

[1]CIAT-HarvestPlus Program P. O. Box 1860, Bukavu, South Kivu, D.R. Congo.
[2]Department of Crop Sciences, Faculty of Agronomy, University of Lubumbashi (UNILU), P. O. Box 1825, Lubumbashi, D.R. Congo.
[3]National Institute for Agricultural Research and Studies (INERA), B.P. 2037 Kinshasa 1, D.R. Congo.
[4]Department of Plant Pathology, Faculty of Agronomy, University of Kinshasa (UNIKIN), Kinshasa, R.D. Congo.
[5]CIAT –Malawi, Lilongwe, Malawi.

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This study was implemented to evaluate the performance of biofortified bean under different integrated soil fertility management (ISFM) options and agro-ecological conditions in Democratic Republic of Congo (DRC). A Split-plot design with eight genotypes as main factors and four ISFM options as secondary factors was carried out in eight production zones distributed across South-Kivu, North-Kivu, and Katanga provinces. The application of lime + manure + NPK increased the bean yield by 173% in Lohutu. Compared to local variety in Lohutu, the CODMLB001 variety under the same option increased the yield by 252%. The same ISFM option allowed best response in terms of micronutrient content of bean in Rutshuru, inducing up to 80.3 mg.kg^{-1} Fe, representing increase 41%. For Zn, the best response was obtained with lime + NPK applied in Kipopo that induced up to 32.2 mg.kg^{-1} Zn. Lime + manure + NPK fertilizer option reduced root rot severity by 17.8% compared to the control. This study confirmed the potential of increasing bean productivity, micronutrient and reducing the severity of major diseases through application of soil fertility management options, which will vary with the bean genotype and the environment under which bean is cultivated.

Key words: Integrated soil fertility management, bean yield, micronutrient content, disease control, *Phaseolus vulgaris* L.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for direct consumption in the world (HarvestPlus Iron-bean, 2009; FAO, 1999; Juhi et al., 2010). The annual global bean production is

*Corresponding author. Email: a.k.lubobo@cgiar.org, kanyengalub@gmail.com. Tel: +243997028562.

approximately 12 million metric tons, with 2.5 million metric tons in Africa (Welch, 2001). Because of their high protein, mineral and fiber content, beans are consumed instead of meat in developing countries (Wortmann et al., 1998). In DRC, common bean is among the most widely consumed and cultivated food crops particularly in the provinces of North Kivu, South Kivu, and former Katanga; where bean consumption has been estimated up to 300 g per capita per day (HarvestPlus DRC, 2009).

Like in most of Sub-Saharan countries, bean yield in Democratic Republic of Congo (DRC) is generally low (Bationo, 2010; Lubanga et al., 2012) even if a large literature has reported the introduction of improved high yield varieties (Kanyenga et al., 2016). In bean-based agrosystems of DRC, bean yields range between 400 and 800 kg.ha^{-1} (Bouwmeester et al., 2009) whereas, in research station, bush bean yield may reach 3,000 and up to 6,000 kg.ha^{-1} for climbing beans (Kanyenga et al., 2012). Low yield in bean-based agrosystems is attributed to many biotic and abiotic factors; bio-aggressors, limited use of improved varieties, and low soil fertility– (Wortmann et al., 1998; Kimani et al., 2001). Bean agronomic performance and nutritional value (iron and zinc) – are function of several factors including genetic variability, environmental conditions and technical itineraries (Kanyenga et al., 2016). Therefore, in most sub Saharan countries like DRC, food is often sufficient in fat and carbohydrates but poor in protein, especially vitamins and minerals (Díaz de la Garza et al., 2007). Obviously, vitamin A, Fe and Zn deficiency likely cause malnutrition, irreversible blindness, anaemia, poor body and cognitive development, as well as increased vulnerability to life-threatening diseases (Gepts et al., 2008). Nowadays, extended literature is devoted to the research of sustainable practices and solutions for improving both bean grain yield and nutritional value (Welch and Graham, 2004; CIAT, 2008; Lunze et al., 2012; Marles, 2017). On one hand, to overcome low soil fertility challenge in bean production, previous studies suggested an integrated soil fertility management (ISFM) (Vanlauwe et al., 2015). This is a composite technology, including the use of improved germplasm, judicious mineral fertilizer application and improved organic matter management (Lambrecht, 2013). These strategies combine technologies that include crop and kitchen residues, manure, compost, biomass transfer, green manures, cover crops, liming, phosphate rock and mineral fertilizers in different combinations (Lunze et al., 2012).The ISFM techniques are recognized to not only improve soil fertility but also increase and stabilize bean yield in a sustainable way (CIALCA 2009). On the other hand, to overcome the deficiency in minerals, the International Centre for Tropical Agriculture (CIAT), through its HarvestPlus programme, developed different market class of dry beans. Generally, common beans contain 3.14-12.07 mg.kg^{-1} Fe and <1.89-6.24 mg.kg^{-1} Zn (Marles, 2017), whereas, biofortified genotypes contain 40-90 mg.kg^{-1} Fe and 10-35 mg.kg^{-1} Zn (CIAT, 2008). According to Welch and Graham (2004), biofortified crops are also likely to have a positive indirect impact on agriculture, with a higher mineral content that provides better protection against pests, diseases and environmental stresses besides increasing yields. Dissemination of such seeds to poor rural households is one of the best ways to improve their diet and to combat malnutrition.

In Democratic Republic of Congo (DRC), the promotion of biofortified crops including bean by HarvestPlus programme and its partners (Institut National pour l'Etude et la Recherche Agronomique – INERA) is a great step in improving nutrition as well as food security. Most previous studies devoted to bean performance in contrasting soil and climatic conditions have admitted that micronutrient content in bean, in addition to its genetic component, may be influenced either directly by soil fertility status or indirectly by external inputs and the application of ISFM techniques (Beebe et al., 2008; Blair et al., 2008; Blair et al., 2009). In DRC, the knowledge on the agronomic and nutritional performance of biofortified bean genotypes across highly differing soil and climate types is still limited.

Therefore, soil and climate on the performance of biofortified bean are unknown. Actually, there is a need to improve the understanding on the coupling between ISFM and pedoclimatic conditions and their effects on Fe and Zn content in biofortified bean genotypes as well as their grain yield. This understanding of edaphic and climatic adaptation of biofortified bean in RDC will be a great step in attempt to disseminate high micronutrient source and contribute to nutritional security of the population. The purpose of this study is to evaluate the iron and zinc content, yield, and resistance or tolerance to disease of biofortified bean genotypes cultivated under ISFM and agroecological conditions of DRC.

MATERIALS AND METHODS

Characteristics and description of the study area

This study was conducted in eight bean production zones distributed across three provinces of DRC, namely South-Kivu, North-Kivu, and former province of Katanga. The South Kivu is characterized by a humid tropical climate influenced by the altitude, with two seasons: the dry season, from June to August, and the rainy season, September to May; with the average annual temperature of 19°C in the north and 10°C in the south, and the average annual precipitation ranging between 1200 and 1700 mm (Ellen, 2008; Pypers et al., 2010). During the rainy season, there is a short dry season from mid-January to mid-February dividing the rainy season in two: the long rainy season from September to February (known as season A) and the short rainy season (called season B) from February to June. The sites in Katanga belong to CW6 climate type according to the Köppen classification. This climate type is characterized by a well-marked dry season of six months (May-October), alternating with six months of rainy season (November-April). Their annual temperatures range between 11 and 28°C for an annual average of 20°C (Malaisse, 1983). According to Sys and Schmitz (1959), soils are heavily weathered and belong to the ferralitic group with a red, ochre-red, and yellow-coloured foliar horizon categorized as zonal, intra- zonal, and azonal. In North Kivu, four seasons are observed: two wet

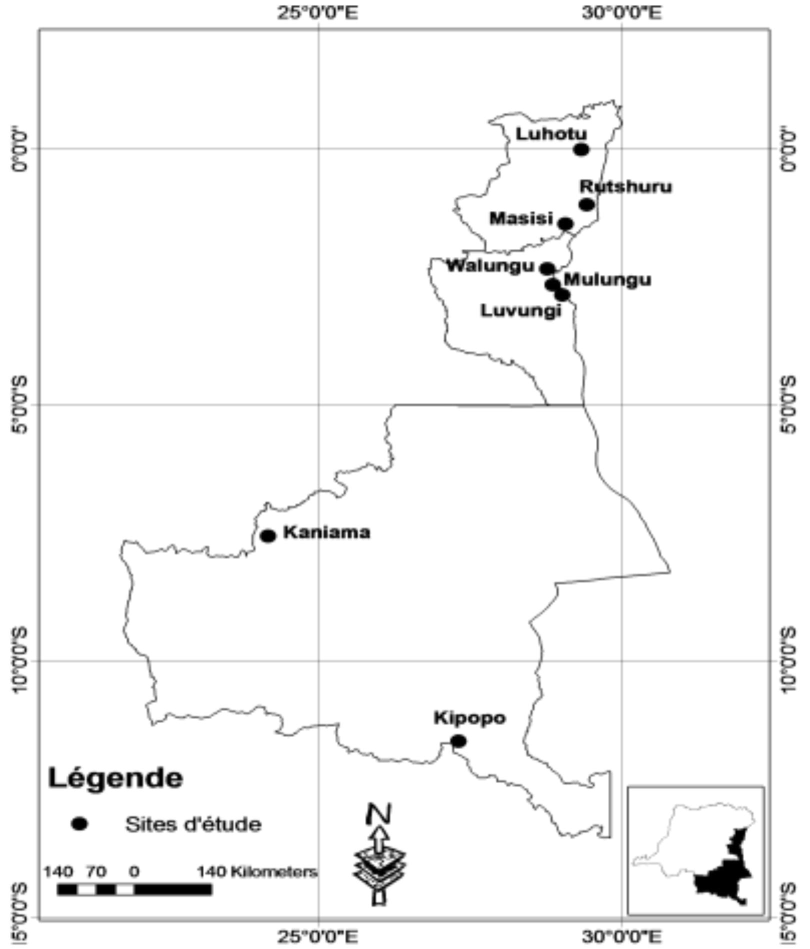

Figure 1. Localisation of study sites. Mulungu, Walungu and Luvungi are located in South-Kivu, Rutshuru, Masisi, and Lohutu are located in North-Kivu, whereas, Kipopo and Kaniama are located in the former province of Katanga.

seasons and two dry seasons. The first wet season is between mid-August and mid-January and the second is practically from mid-February to mid-July. As for the two dry seasons, they are very short. The soils of North Kivu could be divided into three great classes: recent volcanic soils (from lava flows from volcanoes), soils of alluvial plains (these soils are found in the plains of Semliki and come from lacustrine deposits, the Semliki River and its tributaries), and soils of ancient rocks: these soils are very deep and rich in humus. Three experiment sites are located in highland called Walungu, Mulungu, and Lohotu; two in midlands, Kipopo and Masisi, and three others in lowlands of Kaniama, Luvungi and Rutshuru (Figure 1). Soil characteristics (Table 1) as well as annual rainfall vary in these areas (Table 2).

METHODOLOGY

Set-up and implementation of experiments

Undisturbed soil samples were collected on each experimental site. In each study site, a Split-plot design with three replicates was installed. The treatments consisted of eight bush bean genotypes, CodMLB001, Hm1-7, RWK10, LSA144, Maharagi Soja, K132, NGWAKUNGWAKU, agronomically described by Kanyenga et al. (2016), local variety coupled to eight different options of ISFM with :10 $t.ha^{-1}$ farm manure, 2.5 $t.ha^{-1}$ lime, 0.2 $t.ha^{-1}$ NPK 10-20-10, manure + NPK, manure + lime, lime + NPK, lime + manure + NPK, and a control. The different ISFM options were applied according to the doses, the timing and the method of application to each specific technique. Thus, lime was applied in depth of 10-15 cm in 2 weeks before planting. One-year-old cattle manure was applied at the same time and depth with lime and incorporated to the soil. NPK 10-20-10 fertilizers were applied on the day of planting. The planting density of 250.000 plants per hectare was performed and two to three manual weeding was done, and no treatment to pest or disease was applied.

The plant parameters were observed as, severity of the bean stem maggot (BSM) *Ophiomyia spp*., bean root rot (BRR) of the genus *Fusarium* and *Pythium,* and the angular leaf spots (ALS) caused by *P. griseola* scored according to the CIAT rating scale (Van Scoonhoven and Voyset, 1991), as well as bean grain yield. At harvest, 15 pods were harvested from 15 plants on the two central rows removing the plants from the borders.

Chemical analyses of soils and micronutrient analysis in bean grains

Soil samples from each experimental site were analyzed and following parameters were determined: pH, organic carbon, total nitrogen and phosphorus, total iron and zinc. Contents of Fe and Zn in bean grains were analyzed by spectrometry of X-ray fluorescence and determined as $mg.kg^{-1}$ as described in the protocol developed by Stangoulis et al. (2010).

Table 1. Some chemical proprieties of soils in different sites.

Sites	pH_{water}	N (%)	OC (%)	P ($mg.kg^{-1}$)	CEC ($Cmol.kg^{-1}$)	Fe ($mg.kg^{-1}$)	Zn ($mg.kg^{-1}$)
Kipopo	5.5	0.8	19.82	32	10.2	84.74	1.98
Kaniama	5.4	0.1	22.26	202	11.15	270	4.88
Walungu	4.5	1.33	12.9	118	15.61	469.7	34.55
Mulungu	6.5	0.22	11.47	165	39.2	270.9	10.9
Luvungi	4.1	0.93	8.2	105	11.07	307.7	10.61
Rutshuru	5.8	0.3	3.13	73.6	42.3	124.6	3.56
Masisi	6.4	0.48	5.391	56.2	45.6	189	3.14
Lohotu	6.5	0.16	2.347	138.8	24.7	213.6	1.14

Source: University of Rwanda, Butare Campus, Soil laboratory.

Table 2. GPS coordinates, rainfall and landscape of experimental locations.

Sites	Altitude (mm)	Longitude (degree)	Latitude (minute)	Rainfall (mm)	Rainfall (days of rain)	Landscapes
Kipopo	1300	27°22' 32,7"	11°34'42,0"	1147	96	Midland
Kaniama	779	24°10' 30,9"	07°40' 18,0"	1425	131	Lowland
Walungu	2045	28°42' 39,1"	02°43' 03,4"	1779	165	Highland
Mulungu	1640	28°58' 04,5"	02°45' 32,1"	1803	187	Highland
Luvungi	907	28°01' 11,4"	02°51' 16,6"	899	128	Lowland
Rutshuru	1091	29°43' 58,7"	01.09.222	1009	142	Lowland
Masisi	2022	29°04' 04,8"	01.47.351	1648	190	Midland
Lohotu	2448	29°20' 29,3"	00.00.539	1850	204	Highland

Statistical analysis

To detect the combined and individual effects of genotypes, ISFM techniques and environments one-way and multi-group analysis of variance (ANOVA) were applied. Correlation matrix (Pearson coefficient) was used to highlight the relationship between Fe and Zn contents and bean yield. All statistical analyses were performed using R 3.3.0 (The-R-Core-Team, 2016) and GenStat 17th considering $P < 0.05$ as the level of significance.

RESULTS

Effects of ISFM options on bean grain yield of studied genotypes

Comparing ISFM techniques in North Kivu, (Table 3) the highest yield was obtained when lime is combined to mineral fertilizer NPK 10-20-10 in Lohotu, whereas, in Masisi and Rutshuru, the highest bean grain yield is obtained when lime is in combination with manure and mineral fertilizer NPK 10-20-10. Whatever the studied site, the lowest mean yield was obtained with the control, as well as, whatever the experimental site and the applied ISFM technique, the most productive bean genotype was CODMLB001 and the least productive genotype was the local variety. The best combination for North-Kivu of ISFM technique x bean genotype was lime + manure + NPK applied to CODMLB001 (Table 3). Based on bean yield disregarding ISFM and genotypic variability, the studied sites in North Kivu can be classified as follows: Masisi > Lohotu > Rutshuru. At Luhotu application of lime + manure + NPK to CODMLB001 genotype compared to control, increased the yield by 173%, as well as, in comparing with local variety the biofortified CODMLB001 under the application of lime + manure + NPK, increased the yield by 252%.

Comparing ISFM techniques in South Kivu (Table 4), independent of the bean genotype, the highest bean yield was obtained when lime was combined to mineral fertilizer NPK 10-20-10 in Mulungu, whereas, in Luvungi and Walungu, the highest yield was obtained when lime was in combination with manure and mineral fertilizer NPK 10-20-10. Whatever the studied site, the lowest yield was obtained with the control, as well as, independent of experimental site and applied ISFM technique, the most productive bean genotype was CODMLB001, while the least productive was the local variety. The best combination ISFM technique x bean genotype in South Kivu was lime + manure + NPK applied to CODMLB001. Based on the obtained average bean yield disregarding applied ISFM and genotypic variability, the studied sites in North Kivu can be classified as follows: Mulungu > Walungu > Luvungi.

Results with and without liming from Mulungu, the Hm 21-7 genotype yield under liming improved to 65.5%. However, for local variety in Luvungi, lime induced a reduction of grain yield (Table 4). At Mulundu, application of lime + manure + NPK to CODMLB001 genotype compared with control increased the yield by 163% while, compared to local variety the biofortified

Table 3. Effects of ISFM options on the yield (kg.ha $^{-1}$) of different bean genotypes in North Kivu. C: Control; L: Lime; N: NPK; M: Manure.

	Genotypes	ISFM options								Mean
		C	L	L+N	L+M	M	N	L+M+N	M+N	
Lohotu CVC0. 05 (88.5±45.0)	CODMLB001	953.9	1530.9	2404	1713.2	1838.3	2066.3	2604	2167	1909.7^{a}
	Hm21-7	788.3	1318.2	2197.2	1308.3	1708.3	1895.7	2292.1	1928.8	1679.6^{b}
	K132	676.6	1119.8	1645	1091.2	1148	1175.2	1766.8	1363.7	1248.3^{d}
	LSA144	669.6	822.6	1268.8	1112.3	986.8	896.4	1473.1	1502.4	1091.5^{f}
	MAHARAGISOJA	582.2	825.3	1601.8	1113	994.2	1018	1096.4	1179.8	1051.3^{g}
	NGWAKUNGWAKU	595.4	864.6	1867.1	990.1	1421	1223.8	1223.8	1208	1174.2^{e}
	RWR10	870.6	1513.7	2092.3	1245.2	1692.3	1353.9	2135.7	1724	1578.5^{c}
	Local variety	566	732	858.7	756.3	799.4	674.6	739.4	885.9	751.5^{h}
	Mean	712.8^{h}	1090.9^{g}	1741.9^{a}	1166.2^{f}	1323.6^{d}	1288.0^{e}	1666.4^{b}	1494.9^{c}	1310.6
Masisi CVC0. 05 (82.1±41.7)	CODMLB001	1239.9	2103.7	2208.1	2110.3	2462.1	2544.6	2677	2187.4	2191.6^{a}
	Hm21-7	1126.3	1904.1	2057.9	1924.8	2140	2185.8	2101.6	2018.7	1932.4^{b}
	K132	1042.8	1349.6	1436.8	1510.3	1592.7	1736.6	2122.3	1858.2	1581.2^{c}
	LSA144	915	1085.2	1386.7	1452.7	1262.6	1394.2	1821.1	1972.3	1411.2^{d}
	MAHARAGISOJA	704.2	995.6	1817.6	1227.6	1269	1330.8	1389.6	1526.7	1282.6^{e}
	NGWAKUNGWAKU	636	1100.4	1500.3	1190.9	1652	1253.4	1182.7	1449	1245.6^{f}
	RWR10	1168.2	1848.8	2079.2	1626.6	2188.4	1971.8	2548.8	2028.7	1932.6^{b}
	Local variety	823.7	871.1	1024.4	950.3	963.7	753.4	1141.7	1035.8	945.5^{g}
	Mean	957.0^{g}	1407.3^{f}	1688.9^{c}	1499.2^{e}	1691.3^{c}	1646.3^{d}	1873.1^{a}	1759.6^{b}	1565.3
Rutshuru CVC 0. 05 (114.5±59.2)	CODMLB001	1184.7	990.9	1506.4	1479.9	1390.4	1177	1963.2	1636.3	1416.1^{a}
	Hm21-7	949.4	878.7	1352.4	1323.6	1128	930.2	1798	1566.6	1240.9^{b}
	K132	743.1	769.7	1085.1	985.1	775.9	947.3	1232.2	1085.8	953.0^{d}
	LSA144	650.2	795.4	878.6	1046.6	642.7	831.4	987.4	929.7	845.3^{e}
	MAHARAGISOJA	621.7	632.7	893.2	817.6	627.1	740	1136.9	1043.6	814.1^{e}
	NGWAKUNGWAKU	713.9	479.2	894.6	855.8	553.3	638.4	1086.6	958.9	772.6^{f}
	RWR10	941.8	861.3	1220.2	1232.8	932.8	1023.7	1554.3	1302.7	1133.7^{c}
	Local variety	519.7	448.2	661.7	934.6	526.4	708.9	870.7	812.8	685.4^{g}
	Mean	790.6^{e}	732.0^{f}	1061.5^{c}	1084.5^{c}	822.1^{e}	874.6^{d}	1328.7^{a}	1167.0^{b}	982.6

Means with the same letter in line and column were not significantly different

Table 4. Effects of ISFM options on the yield (kg.ha $^{-1}$) of different bean genotypes in South Kivu. C: Control; L: Lime; N: NPK; M: Manure.

Sites	Genotypes	ISFM options								Mean
		C	L	L+N	L+M	M	N	L+M+N	M+N	
	CODMLB001	1160.2	1003.9	1479.2	14799	1251	1320.2	1896.6	1447.4	1379.8^{a}
	Hm21-7	949.4	934.7	1266.3	1323.6	1087.1	1028	1486.9	1299.9	1172^{b}
	K132	743.1	769.7	1085.1	996.1	775.9	957.4	1183.9	1069.1	947.5^{d}
	LSA144	650.2	776.8	878.6	968.8	664.9	903.7	987.4	929.7	845e
Luvungi CVC$_{0.05}$ (77.8±39.5)	MAHARAGI SOJA	621.7	743.8	893.2	850.9	738.1	797.8	988.8	1043.6	834.7e^{f}
	NGWAKUNGWAKU	713.9	654.8	894.6	855.8	720	770.7	975.4	927.8	814.1^{f}
	RWR10	941.8	891.2	1197.1	1021.3	917.2	1025.9	1121	1141.2	1032.1^{c}
	Local variety	519.7	501.6	661.7	882.8	537.6	742.2	722.9	812.8	671.6^{g}
	Mean	787.5^{f}	784.5^{f}	1044.5^{c}	1047.4^{c}	836.5e	943.2^{d}	1170.4^{a}	1083.9^{b}	962.2
	CODMLB001	1007.8	1561.3	2405.2	1714.4	1839.6	2096.8	2647.2	2204.3	1934.6^{a}
	Hm21-7	815.3	1348.7	2209.6	1309.6	1709.6	1926.1	2335.3	1166.1	1702.5^{b}
	K132	648.4	1150.2	1646.2	1081.3	1149.2	1205.7	1810	1401	1261.5^{d}
	LSA144	624.8	797.4	1270	1113.6	936.9	904.7	1516.3	1539.8	1091.3^{f}
Mulungu CVC$_{0.05}$ (79.9±40.6)	MAHARAGI SOJA	500.3	833.6	1603	1114.2	995.4	1048.4	1139.7	1217	1056.5^{g}
	NGWAKUNGWAKU	465.6	906.1	1868	991.3	1422.2	1254.2	1267	1245.3	1177.5e
	RWR10	859.1	1544.1	2093.6	1246.4	1669.4	1383.3	2178.3	1761.3	1592.2^{c}
	Local variety	544.7	762.4	859.9	757.6	800.7	693.9	782.7	923.2	765.6^{h}
	Mean	683.3^{g}	1113.0^{f}	1744.5^{a}	1166.1e	1318.8^{d}	1314.3^{d}	1709.6^{b}	1532.3^{c}	1322.7
	CODMLB001	1308.3	1259.6	1608.2	1581.7	1624.2	1392.4	2017.2	1698.8	1568.6^{a}
	Hm21-7	1096.2	1147.3	1454.2	1425.3	1337.2	1145.7	2003.4	1629	1404.8^{b}
	K132	889.9	1038.3	1186.9	1086.9	985.1	1140.8	1374	1148.2	1105.3^{d}
	LSA144	797	1064.1	980.3	1148.3	851.9	1024.9	1109.2	992.1	996e
Walungu CVC$_{0.05}$ (77.5±39.4)	MAHARAGI SOJA	768.4	901.3	995	919.3	858.6	933.4	1229.3	1106	963.9^{f}
	NGWAKUNGWAKU	838.4	747.9	996.3	957.6	726.6	887.2	1113.7	1021.3	915.6^{g}
	RWR10	1088.6	1130	1322	1334.6	1142	1239.1	1709.8	1365.1	1291.4^{c}
	Local variety	666.4	667.7	763.4	1036.3	713.3	825.7	928.9	875.2	910.9^{h}
	Mean	931.7^{g}	994.5^{f}	1163.3^{c}	1186.3^{c}	1034.4e	1074.9^{d}	1442.9^{a}	1229.5^{b}	1132.2

Means with the same letter down a column are not significantly different.

Table 5. Effects of ISFM options on the yield (kg.ha $^{-1}$) of different bean genotypes in Katanga. C: Control; L: Lime; N: NPK; M: Manure.

Sites	Genotypes	ISFM options								Mean
Kaniama		C	L	L+N	L+M	M	N	L+M+N	M+N	
$CVC_{0.05}$ (77.8±39.6)	CODMLB001	1221.6	1130	1119.4	1096	1495	1263	1451.4	1142.1	1239.7^{a}
	Hm21-7	1007.3	1018	984.7	1136	1208	1016	1356.6	1277.2	1125.4^{b}
	K132	754.1	908.7	696.7	876.1	855.4	1011	1116.7	996.4	901.9^{d}
	LSA144	771.3	934.4	820.3	822.4	722.2	895.2	1030	1018.8	876.8^{d}
	MAHARAGI SOJA	611.8	771.7	751.7	889.8	706.7	803.8	1142.2	861.3	817.4^{e}
	NGWAKUNGWAKU	713.7	618.2	682.2	933.7	632.9	724.2	872.4	986.1	770.4^{f}
	RWR10	964.2	1000	800.1	909.8	1012	1109	1256.9	1063.2	1014.5^{c}
	Local variety	653.4	538	575.2	718.8	583.7	706	973.6	774.3	690.4^{g}
	Mean	837.2^{f}	864.9^{e}	803.8^{g}	922.8^{cd}	901.9^{d}	941.1^{c}	1150^{a}	1014.9^{b}	929.6
Kipopo	CODMLB001	1239.9	2104	2797	2110	1824	2412	3132.6	2723	2292.8^{a}
$CVC_{0.05}$ (77.4±39.4)	Hm21-7	1126.3	1904	2182.3	1925	1157	2119	2764.9	2458.4	1954.3^{a}
	K132	1042.8	1350	2403.4	1510	754.7	1684	2224.6	1858.2	1603.5^{d}
	LSA144	915	1085	1708.9	1453	732.4	1394	1921.1	1827.9	1379.7^{e}
	MAHARAGI SOJA	704.2	995.6	2049.7	1228	1095	1331	1389.6	1526.7	1289.9^{f}
	NGWAKUNGWAKU	636	1100	1987.8	1191	707.6	1231	1388.4	1449	1211.4^{fg}
	RWR10	1168.2	1849	2472.6	1627	1221	1905	2671	2028.7	1867.7^{c}
	Local variety	535	662	982.2	950.3	465.1	711.4	936	913.6	769.5h
	Mean	920.9^{g}	1381.2^{e}	2073^{a}	1499.2^{d}	994.3^{f}	1598.5^{c}	2053.5^{a}	1848.2^{b}	1546.1

Means with the same letter down a column are not significantly different.

CODMLB001 variety under the application of lime + manure + NPK, increased the yield by 238%.

In Katanga, comparing the ISFM techniques (Table 5) independently of bean genotype, the highest yield was obtained when lime was combined to manure and mineral fertilizer NPK 10-20-10 in both Kaniama and Kipopo. In Kaniama, the lowest yield was obtained when lime is combined with mineral fertilizer N PK 10-20-10, whereas, in Kipopo, the lowest yield was obtained with control. Whatever the experimental site and the applied ISFM technique, the most productive bean genotype was CODMLB001, although in Kipopo, it did not differ from Hm21-7, while the least productive was the local variety. The best combination ISFM technique x bean genotype for Katanga was lime + manure + NPK applied to CODMLB001. Bean yield in Kipopo is higher compared to Kaniama. In Kipopo, application of lime + manure + NPK to CODMLB001 genotype increased the yield by 153%, while compared to local variety, CODMLB001 variety under the application of lime + manure + NPK, increased the yield by 235%.

Effects of ISFM options and genotype on micronutrient content in bean grains

The Fe content indicates that, in studied agroecological zones, the highest concentration was obtained in Rutshuru, whereas, the lowest is obtained in Kaniama. Considering ISFM, the highest Fe content was obtained with lime combined with manure and chemical fertilizer NPK 10-20-10 and the lowest was obtained with the untreated control. Based on applied ISFM techniques in relation to agroecological

Table 6. Effects of ISFM techniques on iron and zinc content (mg.kg $^{-1}$) in bean grains in studied agro-ecological zones.

Micronutrients	ISFM options	Kaniama	Kipopo	Luhotu	Luvungi	Masisi	Mulungu	Rutshuru	Walungu	Mean
Fe	Control	52.2	52.3	53.6	56	55.3	60.1	57.1	55.6	55.3
	Lime	50.7	54.6	58.5	57.9	59.1	55.1	56.3	54.9	55.9
	Lime + NPK	58	64	63.4	67.6	59.6	62.3	68.7	67.3	63.9
	Lime + manure	58.3	57.5	59.8	62.6	57	63.5	63.1	62	60.5
	Manure	55	55.7	59.8	59.8	60	58.6	59.4	58	58.3
	NPK	55	56.7	61.4	60.7	61.2	59.5	60	58.9	59.2
	NPK + lime + manure	64.4	68.4	73.2	72.5	61.2	64.2	80.3	78.4	70.3
	NPK + manure	61.1	64.3	68.4	67.1	58.6	63.6	72	71.2	65.8
	Mean	56.8	59.2	62.3	63	59	60.9	64.6	63.3	61.1
Zn	Control	26.3	25.2	26.8	25.7	28.4	28.4	27.6	28.4	27.1
	Lime	27.9	27	26.8	27.4	27.7	27.7	28.3	27.7	27.6
	Lime + NPK	28.2	32.2	29.5	29.2	31.8	31.8	28.9	31.8	30.4
	Lime + manure	27	30.4	28.7	28.8	30.4	30.4	30.2	30.4	29.5
	Manure	27.6	27.9	27.8	27.5	28	28	28.2	28	27.9
	NPK	28.7	28.4	28.4	27.8	28.4	28.4	28.9	28.4	28.4
	NPK + lime + manure	29.8	30	29.7	30.3	30.3	30.3	30.3	30.3	30.1
	NPK + manure	30.1	29.8	20	29.1	30.9	30.9	30.9	30.9	30.3
	Mean	28.2	28.9	28.5	28.2	29.5	29.5	29.2	29.5	28.9

zones, the best response was obtained with lime + manure + NPK applied in Rutshuru. Compared to control, the application of NPK + lime + manure increased Fe content in Rutshuru (Table 6).

Based on Zn content in bean grain, the highest mean content was obtained in Masisi, Mulungu, and Walungu. Considering ISFM options, the highest mean Zn content (> 30 mg.kg^{-1}) is obtained with lime + NPK, Manure + NPK, and lime + manure + NPK, whereas, the lowest Zn mean content is obtained with the control. Based on applied ISFM techniques in relation to agroecological zones, the best response was obtained with lime + NPK applied in Kipopo (Table 6). Contents of Fe and Zn in bean genotypes were influenced by ISFM techniques (Table 7). The highest Fe content was obtained with lime + manure + NPK, while the highest content among genotypes was obtained on CODMLB001 under lime + manure + NPK. The influence of ISFM on Zn content in grains was observed with the highest values obtained on application of three treatments, lime + NPK fertilizer, manure + NPK fertilizer and lime + manure + NPK fertilizer, while the best genotype was CODMLB001 under lime + manure + NPK supply (Table 7).

Effect of ISFM options and genotypes on the severity of diseases

The incidence of BRR among agroecological zones was lower in Luhotu and higher in Rutshuru (Table 8). The incidence of BRR in relation to applied ISFM techniques was higher with NPK alone and control. Comparing ISFM techniques in studied sites in relation to BRR, the highest incidence was observed in Rutshuru (with lime + NPK treatment) and Walungu (with lime + NPK and NPK alone). This incidence is similar to what was observed on control. Comparing ISFM techniques, the highest incidence of ALS was observed with control and lime treatment, whereas, the lowest was observed with NPK + lime + manure. Comparing agroecological zones, the highest incidence of ALS was observed Walungu and the lowest in Luhotu. The comparison of ISFM in relation to BSM incidence indicates that the highest mean incidence was observed with the control, whereas, the lowest was observed with lime + NPK and NPK + lime + manure. Table 9 shows the incidences of BRR, ALS and BSM in relation to bean genotypes and agroecological zones. Among the evaluated bean genotypes, the lowest BRR incidence was observed with CODMLB001; the same observation was made for ALS and BSM. The lowest incidence of BRR and ALS was observed in Luhotu, and Masisi for BSM. The combined effects of ISFM and bean genotype are given in Table 10. The lowest incidence of BRR, BSM and ALS was observed when lime + manure + NPK was applied to CODMLB001. Local varieties were characterized by high incidence whatever the diseases and applied ISFM technique.

Correlations between yield, micronutrients and the severity of diseases

The effect of ISFM options revealed the existence of correlations either negatively or positively between the severity of all bio-aggressors and the yield; between bio-aggressors and micronutrient content; among the bio aggressors and among the micronutrients (Table 11).

In respect to yield and bio-aggressors, there were negative and significant correlations with BRR, BSM

Table 7. Effects of ISFM techniques on iron and zinc content (mg.kg $^{-1}$) in bean genotypes. C: Control; L: Lime; N: NPK; M: Manure.

Micronutrients	Genotypes	ISFM options								Mean
		C	L	L+N	L+M	M+N	L+M+N	M+N		
Fe (mg.kg^{-1})	CODMLB001	66	68.5	84	75.3	68.4	71.8	91.5	79.5	75.6[a]
	Hm21-7	62.3	66.2	76.7	68.7	65	66.5	79.4	77.4	70.3[b]
	K132	52.2	54.2	55	56.8	61.2	55	64.8	59.7	57.4[ef]
	LSA144	55.7	62.6	63.8	57.1	61.3	62.4	62.6	59.7	60.6[d]
	MAHARAGI SOJA	51.8	47.4	62.3	52.9	54.6	52.1	61.4	58.2	55.1[fg]
	NGWAKUNGWAKU	51.4	46.8	53.4	52.5	49	49.3	62.4	64.7	53.7[gh]
	RWR10	55.6	67.2	68.1	72.2	64.5	68.8	74	71	67.7[c]
	Local variety	49.8	46.1	50.1	44	49.5	51.2	59.1	58.7	51.1[h]
	Mean	55.3[e]	55.9[de]	63.9[b]	60.5[c]	58.3[cd]	59.2[c]	70.3[a]	65.8[b]	61.1
Zn (mg.kg^{-1})	CODMLB001	30.6	27.8	30.6	31.2	31.1	30	33.6	32.3	30.9[a]
	Hm21-7	29.1	27.2	30.9	32	28.5	28.6	29.7	31.6	29.7[ab]
	K132	24.6	27.6	27.8	29.1	26.1	28	30.3	27.4	27.6[d]
	LSA144	25.6	28.3	31.3	29.7	28.9	31	24.7	26.8	28.3[c]
	MAHARAGI SOJA	25.4	27.2	30.8	26.5	25.7	26.8	31.4	28.4	27.8[d]
	NGWAKUNGWAKU	25.3	24.6	29.2	27.9	28.1	27.7	29.6	29.8	27.7[d]
	RWR10	27.7	32.2	30	30.5	29.7	28.3	32.9	35.3	30.8[a]
	Local variety	28.4	25.8	32.6	29.4	24.9	26.9	28.8	31	28.5[c]
	Mean	27.1[e]	27.6[de]	30.4[a]	29.5[b]	27.9[cd]	28.4[c]	30.1[a]	30.3[a]	28.9

Means with the same letter down a column are not significantly different.

(r = -31%) and ALS disease (r = -21%). The same trend was observed between iron and diseases, 36% with BRR, -24% with BSM and -19% with ALS. Positive and significant correlations were obtained between Fe content and yield (r = 72%), also bio-aggressors between them 20% between ALS disease and BRR, 38% between BSM and BRR and 35% between BSM and ALS disease.

DISCUSSION

Effects of ISFM options on bean grain yield of studied genotypes

Globally, the results of this study showed that, compared to control and local varieties, the application of lime + manure + NPK to CODMLB001 genotype significantly increased the yield. The obtained yields vary with genotypes, environments, and applied ISFM. These observations indicated the crucial role of both bean genotype and ISFM in increasing bean yields. Obviously, liming is an effective and dominant practice to raise soil pH and reduce acidity-related constraints to improve crop yields (Fageria and Baligar, 2008). Benefits of liming include increased

Table 8. Effects of ISFM options on the incidence of Bean Root Rot (BRR), Angular Leaf Spot (ALS) and Bean Steam Maggot (BSM) in different study sites.

Diseases	ISFM	Environments							Mean
		Kipopo	Luhotu	Luvungi	Masisi	Mulungu	Rutshuru	Walungu	
BRR	Check	5.0	3.7	4.5	4.1	4.8	4.0	5.1	4.5
	Lime	3.8	3.4	4.2	4.1	4.5	5.1	4.6	4.2
LSD	Lime + NPK	3.3	3.4	4.1	4.1	4.8	5.1	5.1	4.3
E : 0.20	Lime + Manure	3.6	3.5	4.0	4.8	4.8	4.0	3.8	4.1
I x E : 0.33	Manure	3.6	3.7	4.6	3.8	3.0	4.6	3.6	3.9
	NPK	4.7	3.4	4.6	4.1	4.8	5.0	5.1	4.5
	NPK+ Lime + Manure	3.0	3.3	4.0	3.0	3.7	4.6	4.2	3.7
	NPK + Manure	3.6	3.9	4.4	4.3	3.0	4.6	4.6	4.1
	Mean	3.8	3.5	4.3	4.0	4.2	4.6	4.5	4.1
	$LSD_{0.05}$	0.23	0.22	0.39	0.25	0.23	0.25	0.33	0.10
ALS	Check	4.9	4.9	4.0	4.8	4.9	4.8	4.0	4.6
	Lime	5.5	4.2	4.6	4.6	4.1	4.2	5.1	4.6
LSD	Lime + NPK	4.4	4.8	5.1	4.6	3.0	4.5	5.1	4.5
E : 0.15	Lime + Manure	4.6	4.5	4.0	4.6	3.0	3.6	4.0	4.1
I x E : 0.28	Manure	3.8	3.4	4.6	4.1	4.8	5.0	4.6	4.3
	NPK	4.3	3.0	4.5	4.8	4.5	4.5	5.0	4.4
	NPK + Lime + Manur	3.7	3.7	4.6	4.1	3.0	3.8	4.6	3.9
	NPK + Manure	3.7	3.0	4.6	4.1	4.5	5.1	4.6	4.2
	Mean	4.4	3.9	4.5	4.5	4.0	4.4	4.6	4.3
	$LSD_{0.05}$	0.22	0.26	0.27	0.23	0.22	0.32	0.27	0.10
BSM	Check	5.0	4.1	5.0	3.5	4.9	4.9	4.8	4.6
	Lime	4.8	4.8	3.8	3.4	4.2	4.1	4.2	4.2
LSD	Lime + NPK	5.1	5.1	3.3	3.4	4.8	3.0	4.5	4.2
E : 0.15	Lime + Manure	4.0	4.0	3.6	3.5	4.5	3.0	3.6	3.8
I x E : 0.29	Manure	5.1	4.6	3.6	3.9	3.4	4.8	5.0	4.4
	NPK	4.2	4.6	4.7	3.5	3.0	4.5	4.5	4.1
	NPK + Lime+Manur	5.1	4.6	3.0	3.4	3.8	3.0	3.8	3.8
	NPK + Manure	5.1	4.6	3.6	3.9	3.0	4.5	5.1	4.3
	Mean	4.8	4.5	3.8	3.6	4.0	4.0	4.4	4.2
	$LSD_{0.05}$	0.31	0.28	0.23	0.20	0.26	0.22	0.32	0.10

LSD: Least Significant Difference, E: Environment, I x E: Interaction between disease Incidence and Environment.

availability of essential nutrients (Ca, P, Mo) and the decreased solubility of toxic elements Al and Mn (Haynes and Ludekeet, 1981; Buni, 2014). In addition, liming also improves biological N_2 fixation in acid soils and enhances net mineralization of organic N (Edmeades and Ridley, 2003; Moreira and Fageria, 2010). However, liming experiments in plateau and hills of Rwanda showed that addition of lime alone is insufficient to rehabilitate poor or depleted soils; therefore, the best practice is one that combines lime, organic manure and inorganic fertilizers (Mukuralinda, 2007). Indeed, chemical fertilizer NPK 10-20-10 likely played an important role in rapidly releasing essential nutrient to plants, and manure application was essential in improving nutrient supply to plant. Manure is considered a source of major plant nutrients such as N, P and potassium (K) (Murmu et al., 2013). It can also provide many of the secondary nutrients that plants require. Benefits of manure amendments added to soil include pH depressing and faster infiltration rate due to enhanced soil aggregation (Liang et al., 2011). Moreover, bean yield increase because of manure application has been observed by extended number of authors (e.g. Nabahungu, 2003; Alley and Vanlauwe, 2009; Bekunda et al., 2010; Vanlauwe et al., 2015; Musaninkindi, 2013; Khaim et al., 2013; Ndengu et al., 2017).

This study showed that independently to sites and applied ISFM technique, the most productive bean genotype is CODMLB001 and the least productive genotype was the local variety. Interesting results have been also obtained with RWK10 (2548.8 $kg.ha^{-1}$) in Masisi and Hm21-7 in Mulungu (2335.4 $kg.ha^{-1}$). Comparing the results obtained in Mulungu with and without liming, the Hm 21-7 genotype was able to improve its yield from 815 to 1348.7 $kg.ha^{-1}$ with lime application. This was an increase of 65.4% that is greater than that reported by Lunze et al. (2007) where

Table 9. Effects of genotypes on the incidence of Bean Root Rot (BRR), Angular Leaf Spot (ALS) and Bean Steam Maggot (BSM) in different study sites.

Diseases	Genotypes	Environments							Mean
		Kipopo	Luhotu	Luvungi	Masisi	Mulungu	Rutshuru	Walungu	
BRR	CODMLB001	1.8	3.0	3.6	2.0	3.0	4.1	2.8	2.9
	Hm21-7	2.8	3.6	4.3	3.3	3.7	4.5	4.7	3.8
LSD	K132	4.3	3.0	4.4	4.2	4.6	4.9	4.1	4.2
E : 0.20	LSA144	4.8	3.7	4.4	5.0	4.4	4.6	4.7	4.5
G x E : 0.32	MSOJA	4.6	3.8	4.4	5.2	4.2	4.4	5.0	4.5
	NGWAKU	4.0	3.7	4.7	4.5	4.2	4.5	4.0	4.2
	RWR10	3.3	3.6	3.9	4.2	4.0	4.7	4.5	4.0
	Local variety	5.0	3.9	4.7	3.8	5.5	5.4	6.1	4.9
	Mean	3.8	3.5	4.3	4.0	4.2	4.6	4.5	4.1
	$LSD_{0.05}$	0.23	0.23	0.39	0.23	0.26	0.27	0.27	0.10
ALS	CODMLB001	2.3	3.0	3.6	2.4	2.5	2.8	4.1	3.0
	Hm21-7	3.4	3.3	4.3	3.7	3.3	4.3	4.5	3.8
LSD	K132	5.1	4.2	4.9	4.1	4.6	4.5	4.9	4.6
E : 0.15	LSA144	5.2	4.3	4.5	5.7	4.3	5.0	4.6	4.8
G x E : 0.29	MSOJA	4.7	4.2	4.4	5.3	4.3	4.9	4.4	4.6
	NGWAKU	4.5	4.1	4.5	4.8	3.9	3.8	4.5	4.3
	RWR10	4.1	3.5	4.7	4.4	3.8	4.2	4.7	4.2
	Local variety	5.6	5.0	5.3	5.2	5.3	5.8	5.4	5.4
	Mean	4.4	3.9	4.5	4.5	4.0	4.4	4.6	4.3
	$LSD_{0.05}$	0.23	0.26	0.27	0.25	0.27	0.26	0.27	0.10
BSM	CODMLB001	2.8	3.8	1.8	2.7	3.0	2.5	2.8	2.8
	Hm21-7	4.0	4.4	2.8	4.0	3.3	3.3	4.3	3.7
LSD	K132	5.3	4.9	4.3	3.0	4.2	4.6	4.5	4.4
E : 0.15	LSA144	5.8	4.6	4.8	3.7	4.3	4.3	5.0	4.6
G x E : 0.28	MSOJA	4.8	4.4	4.6	3.8	4.2	4.3	4.9	4.4
	NGWAKUGWAKU	5.2	4.5	4.0	3.8	4.1	3.9	3.8	4.2
	RWR10	4.4	4.6	3.3	3.6	3.5	3.8	4.2	3.9
	Local variety	6.1	5.3	5.0	4.0	5.0	5.3	5.8	5.2
	Mean	4.8	4.5	3.8	3.6	4.0	4.0	4.4	4.2
	$LSD_{0.05}$	0.21	0.28	0.23	0.24	0.27	0.27	0.26	0.10

LSD: Least Significant Difference, E: Environment, G x E: Interaction between Genotype and Environment.

Hm21-7 showed grain yields ranging from 1317 to 1455 $kg.ha^{-1}$. To the local variety in Luvungi, lime induced a reduction in grain yield, from 519.7 $kg.ha^{-1}$ in control to 501.6 $kg.ha^{-1}$ with lime. This was similar to the results obtained by Ngongo et al. (2000), FAO (2006) and CIALCA (2009). The difference in bean yield under the same treatment of ISFM should be due to the inherent genetic potential of each variety to produce and respond to it, as well as to the both edaphic and climatic conditions (Ndengu et al., 2017). The low yield obtained with the control likely indicates the low natural ability of studied soils to induce bean yield. Within a same province, the difference in bean yield is likely a result of the combination between soil and climate, for example, in North Kivu soils in Rutshuru have been found moderately acid, poor in organic matter and with high CEC, contrary to Masisi where soils have been found slightly acid but rich in organic matter. In the same province, Rutshuru is the most enriched in phosphorus, but with low CEC. Globally, results showed that biofortified varieties tended to yield better than local varieties. Furthermore, soil fertility improvement resulted in positive yield increases in most of the cases. This was similar to the observations of many other authors (Vanlauwe et al., 2015; Musaninkindi, 2013; Sebuwufu, 2013; Khaim et al., 2013; Ndengu et al., 2017).

Effects of ISFM options and genotype on micronutrient content in bean grains

The Fe content in different agroecological zones in relation with ISFM was higher in Rutshuru and lower in Kaniama. This study showed that the best genotypes under the best ISFM option were CodMLB001, Hm 21-7, and RWK 10. Notably, there is a wide genetic variability in seed mineral composition and their concentration is partly controlled by the environment in which beans are

Table 10. Effects of the interaction genotype x ISFM options on disease incidence of Bean Root Rot (BRR), Angular Leaf Spot (ALS), and Bean Steam Maggot (BSM). C: Control; L: Lime; N: NPK; M: Manure.

Diseases	Genotypes	ISFM options								Mean
		C	L	L+N	L+M	M	N	L+M+N	M+N	
BRR	CODMLB001	3.0	2.5	3.1	3.3	2.9	3.7	2.2	2.5	2.9
	Hm21-7	4.3	4.1	3.9	3.9	3.5	4.1	3.1	3.8	3.8
$LSD_{0.05}$	K132	4.4	4.2	4.4	4.0	4.3	4.0	3.9	4.3	4.2
G : 0.10	LSA144	4.9	4.4	4.3	4.7	4.2	4.8	4.1	4.7	4.5
I : 0.10	MAHARAGISOJA	4.6	4.9	4.8	3.9	4.4	5.1	4.1	4.5	4.5
G x I : 0.29	NGWAKUNGWAKU	4.7	4.1	4.6	4.2	3.9	4.7	3.5	4.3	4.2
	RWR10	4.8	4.4	3.9	3.8	2.8	4.9	3.7	3.6	4.0
	Local variety	5.0	5.2	5.1	4.9	4.8	4.7	4.9	4.7	4.9
	Mean	4.5	4.2	4.3	4.1	3.9	4.5	3.7	4.1	4.1
ALS	CODMLB001	3.3	2.9	3.2	2.8	3.6	3.0	2.4	2.5	3.0
	Hm21-7	4.0	4.3	4.2	3.3	3.9	3.5	3.5	3.9	3.8
$LSD_{0.05}$	K132	4.8	4.3	5.0	4.6	5.1	4.5	4.3	4.1	4.6
G : 0.10	LSA144	5.6	5.4	4.4	4.4	4.2	4.9	4.4	5.0	4.8
I : 0.10	MAHARAGISOJA	4.2	5.4	4.9	4.2	4.5	4.7	4.7	4.3	4.6
G x I : 0.28	NGWAKUNGWAKU	4.2	4.8	5.1	3.9	4.4	4.4	3.5	4.2	4.3
	RWR10	5.2	4.1	3.7	3.5	3.9	5.0	3.8	4.5	4.2
	Local variety	5.6	5.7	5.7	5.9	5.2	5.0	4.7	5.1	5.4
	Mean	4.6	4.6	4.5	4.1	4.3	4.4	3.9	4.2	4.3
BSM	CODMLB001	3.4	2.5	2.8	2.5	3.7	2.5	2.3	2.6	2.8
$LSD_{0.05}$	Hm21-7	4.2	3.8	3.6	3.3	4.0	3.6	3.5	3.9	3.7
G : 0.10	K132	4.6	4.1	4.7	4.3	4.8	4.2	4.1	4.5	4.4
I : 0.10	LSA144	5.2	5.0	4.2	4.0	4.5	4.6	4.6	4.9	4.6
G x I : 0.27	MAHARAGISOJA	4.4	4.8	4.5	3.9	4.7	4.7	4.1	4.4	4.4
	NGWAKUNGWAKU	4.5	4.5	4.5	3.7	4.2	4.3	3.5	4.3	4.2
	RWR10	5.0	3.5	3.6	3.5	3.7	4.4	3.3	4.3	3.9
	Local variety	5.3	5.4	5.5	4.9	5.3	4.9	5.1	5.3	5.2
	Mean	4.6	4.2	4.2	3.8	4.4	4.1	3.8	4.3	4.2

LSD: Least Significant Difference, E: Environment, I x E: Interaction between disease Incidence and Environment.

planted (Beebe et al., 2000; Gelin et al., 2007; Blair et al., 2009). Under these conditions, only genotypes selected for their physiological ability to adapt to acid soils such as Hm 21-7 can be considered as a prototype (Lunze et al., 2012; Beebe et al., 2008). The performance of Hm 21-7 was higher than reported by CIALCA (2009) in Walungu with 62 $mg.kg^{-1}$, but corroborate with Lunze et al. (2012) for both Hm 21-7 and LSA144. Considering ISFM options, the highest Fe content was obtained with lime combined with manure and chemical fertilizer NPK 10-20-10, and the lowest was obtained with the control. Macronutrient fertilizers containing N, P, and K can have significant effects on the accumulation of nutrients in edible plant products (Allaway, 1986; Grunes and Allaway, 1985). However, providing more Fe to plants than required for maximum yield does little to further increase the Fe in edible seeds and grains (Bouis and Welch, 2010). Among factors pointed out to influence Fe uptake by plants, liming is cited due to its pH increasing effect, which limits the availability of Fe to plants. According to this statement, this study should have obtained lower Fe content with the application of lime (Elgala et al., 1976; Bohn et al., 1979). This contrasting result should be due to many reasons including: the high potential of tested bean genotype to uptake Fe, initial high Fe content in soils, high buffering capacity of soils, etc. The Fe availability is dictated by the soil redox potential and pH, thus, in soils on aerobic conditions or high pH, Fe is readily oxidized, and is predominately in the form of insoluble ferric oxides. At lower pH, ferric-Fe is released from the oxide, and becomes more available for uptake by roots (Morrissey and Guerinot, 2009).

The result of this study showed that Zn content in bean grains in relation with ISFM was higher in Masisi, Mulungu, and Walungu compared to other sites. Considering ISFM options, the highest Zn content was obtained with lime + NPK, Manure + NPK, and lime + manure + NPK, whereas, the lowest Zn mean content is obtained with the control. Increasing Zn levels via Zn fertilization has been shown for navy beans (*Phaseolus vulgaris* L.), as well as other crops such as rice (Moraghan, 1980; Welch, 1986). It is stated that Zn content of grain was affected by its content in soils,

Table 11. Correlations between diseases of Bean Root Rot (BRR), Angular Leaf Spot (ALS) and Bean Steam Maggot (BSM), ISFM, grain yield, Fe, and Zn content.

Diseases	ISFM	Yield	Fe	Zn	BSM	BRR
ALS		-0.25	-0.29	-0.04ns	0.23	0.26
	Check	-0.17	-0.24	0.04ns	0.27	0.23
	Lime	-0.13	-0.32	-0.08ns	0.42	0.30
	Lime + NPK	-0.28	-0.40	-0.11ns	0.22	0.18
	Lime + Manure	-0.18	-0.34	-0.17	0.26	0.19
	Manure	-0.32	-0.31	-0.03ns	0.08ns	0.28
	NPK	-0.23	-0.23	0.07ns	0.14	0.23
	NPK + Lime + Manure	-0.37	-0.28	-0.04ns	0.17	0.33
	NPK + Manure	-0.32	-0.15	0.03ns	0.22	0.27
BRR		-0.24	-0.20	-0.03ns	0.23	
	Check	-0.16	-0.21	-0.07ns	0.26	
	Lime	-0.23	-0.19	0.01ns	0.25	
	Lime + NPK	-0.27	-0.23	-0.07ns	0.14	
	Lime + Manure	0.02ns	-0.05ns	-0.07ns	0.18	
	Manure	-0.27	-0.26	-0.02ns	0.28	
	NPK	-0.19	-0.09ns	0.17	0.13ns	
	NPK + Lime + Manure	-0.49	-0.27	-0.01ns	0.13	
	NPK + Manure	-0.29	-0.16	-0.05ns	0.44	
BSM		-0.19	-0.22	-0.04ns		
	Check	-0.33	-0.11	-0.07ns		
	Lime	-0.24	-0.36	-0.11ns		
	Lime + NPK	-0.25	-0.29	-0.09ns		
	Lime + Manure	-0.17	-0.24	-0.16		
	Manure	-0.14	-0.23	0.02ns		
	NPK	-0.32	-0.27	0.00ns		
	NPK + Lime + Manure	-0.14	-0.26	-0.01		
	NPK + Manure	-0.20	-0.22	-0.03ns		

Legend: ALS = Angular Leaf Spots, BRR= Bean Root Rots, BSM= Bean Steam Maggots.

accordingly, when the amount of Zn increases in soil, the amount of Zn in grain also increases (Cakmak et al., 1989). The Zn absorption capacity is reduced by high P utilization and Zn in plant and soil has an antagonism state with P –negative interaction– (Mousavi, 2011). This statement indicated that the application of chemical fertilizers NPK or manure as a source of P could affect Zn uptake by plants and consequently reduce its content in grains. Furthermore, Zn availability decreases with increasing soil pH, because the minerals solubility reduced and Zn uptake increases with soil colloidal particles such as clay minerals, iron and aluminium oxides, organic matter and calcium carbonate (Irmak et al., 2008; Salimpour et al., 2010; Khorgamy and Farnis, 2009). The best genotypes under the best ISFM option for Zn are CodMLB001, RWK10, and Maharagi Soja. Nevertheless, some influence of the manure-NPK combination on Zn content with RWK10 was observed, likely meaning that this genotype has a better ability to accumulate Zn. Globally, manure-NPK combination allowed an increase of 27.4% of Zn. Lime alone has increased the Zn content for all sites, which was similar to the results reported by other researchers (e.g. CIALCA, 2009; Sanginga et al., 2009). Based on applied ISFM techniques in relation to agroecological zones, the best response is obtained with lime + NPK applied in Kipopo which induced up to 32.2 $mg.kg^{-1}$ Zn.

Low Fe and Zn content observed in other locations such as Kaniama, should be a consequence of antinutrient substances. Antinutrients can depress Fe and Zn bioavailability (e.g. phytate and certain polyphenolics); there are current challenges in increasing micronutrient content in bean grains (Bouis and Welch, 2010). It has been shown that the Fe and Zn content; although dependent on genetic diversity with significant positive correlations (Tryphone and Nchimbi-Msolla 2010; Gepts et al., 2008; Blair et al., 2008), but environmental conditions (soils and climates) and farming practices could modify more or less their concentration in bean grains (Pereira et al., 2014; Beebbe et al., 2000). Obviously, physicochemical and biological properties of the soil as well as the rainfall determine the performance and stability of biofortified bean genotypes (Kanyenga et al., 2016).

Effect of ISFM options and genotype on the severity of diseases

The incidence of BRR in agroecological zones was lower in Lohotu and higher in Rutshuru (Table 7). The incidence of BRR related to ISFM, was higher with NPK alone and control. Comparing ISFM in studied sites in relation to BRR, the highest incidence was observed in Rutshuru (with lime + NPK treatment) and Walungu (with lime + NPK and NPK alone). This incidence was similar to that observed on control. Comparing ISFM techniques, the highest incidence of ALS was observed with control and lime treatment, whereas, the lowest was observed with NPK + lime + manure. Comparing agroecological zones, the highest incidence of ALS was observed in Walungu and the lowest in Luhotu. The comparison of ISFM in relation to BSM incidence indicates that the highest mean incidence was observed with the control, whereas, the lowest was observed with lime + NPK and NPK + lime + manure. The lowest incidence of BRR, BSM and ALS was observed when lime + manure + NPK was applied to CODMLB001. Local varieties were characterized by high incidence whatever the diseases and applied ISFM technique. Lime-Manure-NPK Fertilizer option reduced root rot severity by 17.8% from 4.5 with control treatment to an incidence of 3.7 according to the CIAT rating scale (Van Schoonhoven, 1992). Furthermore, Lime + manure + NPK Fertilizer option induced the decrease of the severity of angular spots of beans by 15.3% from 4.9 to 3.9, and bean stem maggot by17.4% from 4.6 to 3.8. This observation corroborates the statement according to which ISFM techniques enhance the natural resistance capacity of bean genotypes to diseases (Tolera et al., 2005). However, observation from Ochilo (2013) showed that, the addition of soil amendments had no influence on the levels of infestation of the bean stem maggot and the black bean aphid, and their associated plant mortality.

The best genotype allowing less severity for all bio-aggressors at all sites was CodMLB001 which, compared to the local variety, showed less severity with a reduction of 40.8% for root rot, 44.5% angular spot disease in Lohutu, 46.2% for the bean stem maggot in Masisi. This states that the use of more or less resistant genotypes as one of the least effective ways to use in the integrated fight against bio-aggressors by reducing the severity of diseases such as angular spots (Kanyenga et al., 2016), root rot (Otsyula et al., 2005), and bean stem maggot. Given that rainfall was generally abundant and well distributed in Masisi, this would contribute to the reduction of the severity of the bean maggot (Wortmann et al. 1998) while the drought would increase its severity in other sites.

Correlations between yield, micronutrients and the severity of diseases

The effect of ISFM revealed the existence of correlations negative and positive for the severity of all bio-aggressors and the yield; bio-aggressors and micronutrient content; among the bio aggressors and among the micronutrients.

Yield and bio-aggressors had negative and significant correlations with root rot (r = -33%), bean stem maggot (r = -31%) and angular spot disease (r = -21%). The same trend is observed between iron and diseases 36% with root rot, -24% with bean maggot and -19% with angular spots. This means that when the severity of bio-aggressor increases, the yield and Fe content decreases in the same proportions. This observation should be explained by plant resource allocation principle. Indeed, the allocation of energy for resisting vis-à-vis of diseases reduces the amount of energy that should be allocated to other plant functions and consequently affecting nutrient uptake from soil. Positive and significant correlations were obtained between Fe content and yield (r = 72%), also bio-aggressors between them. This states that the increase of one parameter leads directly to the increase of the other in the same proportions. These correlations between yield and bio-aggressors varied between -33% for BRR, -31% for BSM and -21% for ALS, and are lower than those reported by Jesus Junior et al. (2001). The correlations between different bio-aggressors are similar to the results cited by other researchers (Saettlers, 1991; PABRA, 2008; CIAT, 2010). The degree of severity of ALS disease is more often influenced by genetic, environment, production pathways (Kanyenga et al., 2012), specific strains and breeds of bio-aggressors (Van Scoonhoven and Voyset, 1991). CodMLB001, Hm 21-7 and RWK 10 genotypes remain genotypes with genetic resistance and are therefore a source of varietal resistance in integrated strategies to control diseases for farmers in developing countries (CIAT, 2003; Chataika et al., 2010). The beneficial effects of inputs of inorganic fertilizers and organic amendments have also been reported by several other authors (e.g. Davet, 1996; Isaac, 1992; Mulongoy et al., 1992) but whose effects on the physicochemical and biological properties of the soil and the plant, sometimes inhibit beneficial interactions between beneficial and pathogenic microorganisms. These beneficial effects also depend more on the degree of genotype improvement (Havlin et al., 2005), for example, hybrids respond better than local varieties and environmental conditions (Durrieu, 1993; Tolera et al., 2005; Rui Yu-kui et al., 2009; Lucas and Kunzovas, 2014).

The local variety is everywhere the most sensitive genotype, the least productive, the least performant with regard to Zn and Fe content (except at Kipopo), and the most sensitive to the angular spots disease as previously reported by Kanyenga et al. (2012). This justifies the relevance of ISFM in bean cropping system in particular and crop production in general, as confirmed by Vanlauwe et al. (2015). However, Kankwatsha et al. (2008) and Thung and Rao (1999) consider that the application of organic and humic amendments requires huge quantities that are not always available and within reach of most producers who are essentially small farmers.

Conclusion

The potential of increasing bean productivity, micronutrient content and reducing of the disease severities is possible through promotion of appropriate soil fertility management options and genotype of the biofortified bean according to the environment under cultivation. Use of high-performing bean genotypes under integrated soil fertility management, like the combination of lime, manure and mineral fertilizers (NPK 10-20-10); in almost all agro-ecological zones, has significantly increased yield, micronutrient content (iron and zinc) and reduced the severity of major diseases (root rot and angular spots) and pests (bean stem maggot) that are with low soil fertility and rainfall disturbances, factors limiting the production of beans in the DRC. However their technical efficiency (that is aspects related to the availability of large quantities of inputs to cover the needs of crops, and their incorporation in the soil), economic (that is cost of production compared to profitability and return on investment cost) and ecological (that is synergy and antagonism between decomposing microorganisms, pathogens and nitrogen-fixing agents), their application and adoption at the level of small farmers still pose some problems and require a lot of research for their adaptation, dissemination and adoption among producers.

To maximize the production of biofortified beans, and in addition to improve malnutrition due to iron and zinc deficiency; the selection and use of genotypes with good genetic potential (combining high yield with high micronutrient content) and the application of improved soil fertility management techniques should be recommended to farmers. However, this can only be possible in locations with high production potential showing a good response to integrated soil fertility management options and where rainfall variations, even if unpredictable, have only a limited influence. This makes it possible to contribute effectively and in a sustainable manner to the improvement of food security and the reduction of malnutrition due to Fe and Zn deficiency among vulnerable populations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Response of eight sorghum varieties to plant density and nitrogen fertilization in the Sudano-Sahelian zone in Mali

Joseph Sékou B. DEMBELE[1,3], Boubacar GANO[1,3], Michel VAKSMANN[4,6], Mamoutou KOURESSY[2], Léonce Lamine DEMBELE[2], Mohamed DOUMBIA[2], Niaba TEME[2], Diaga DIOUF[3] and Alain AUDEBERT[5,6*]

[1]Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS), Institut Sénégalais de Recherches Agricoles (ISRA), Route de Khombole, BP 3320, Thiès, Sénégal.
[2]Institut d'Economie Rurale (IER), LABOSEP de Sotuba, BP 262, Bamako, Mali.
[3]Laboratoire Campus de Biotechnologies Végétales, Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop (UCAD), BP 5005, Code postal 10700, Dakar-Fann, Dakar, Sénégal.
[4]CIRAD, UMR AGAP, BP 1813, Bamako, Mali.
[5]CIRAD, UMR AGAP, BP 3320, Thiès, Sénégal.
[6]AGAP, University of Montpellier, CIRAD, INRA, Montpellier SupAgro, Montpellier, France.

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This work was conducted to study the performance of eight sorghum varieties that contrasted with intensified practices in the Sudano-Sahelian zone of Mali. Two experiments were carried out in 2018 and 2019 rainy seasons at Sotuba Agricultural Research Station in Mali. The experimental design used was a Split-split-plot with three replications and three factors including two plant densities (D1: 26666 plants ha^{-1} and D2: 53333 plants ha^{-1}) as the main plot, three nitrogen levels (0, 41 and 82 kg N ha^{-1}) as the subplot and varieties as the sub-subplot. Measurements focused on growth and physiological parameters, grain yield and yield components. The results showed that sorghum grain yield was positively correlated with straw yield, leaf area index, grain number per panicle, panicle number per m^2, panicle weight per m^2 in N0D1 (0 kg N ha^{-1} and 26666 plants ha^{-1}) and N2D2 (178 kg N ha^{-1} and 53 333 plants ha^{-1}). Furthermore, straw yield was positively correlated with the leaf area index and panicle weight m-² in N0D1 and in N2D2. Analysis of variance showed that plant density, nitrogen and variety effect on grain and straw yields were significant. The interaction density x nitrogen x variety effect was also significant on grain and straw yields. Grain and straw yields were high in the N2D2 treatment for eight varieties compared to the N0D1 treatment. GRINKAN, C2_075-15 and C2_007-03 varieties had the highest grain and straw yields in N0D1. These caudatum-type varieties could be recommended in less intensive sorghum production areas in Mali. The FADDA variety produced high grain and straw yields in N2D2. Guinea-type hybrid FADDA may be recommended for grain and straw production in intensive sorghum production areas in Mali.

Key words: Mali, intensification, varieties, Nitrogen, planting density, yields.

INTRODUCTION

Sorghum is one of the staple cereals grown in the semi-arid and arid regions of Africa and Asia (Srinivas et al., 2009; Borrell et al., 2014). It ranks fifth in the world in terms of production and growing area and the fourth most cultivated cereal in Mali during the raining season for human consumption and animal feeding. Despite its importance, sorghum yield remains low with less than one ton per hectare at national scale (Trouche et al., 2001). This low yield is mainly attributed to spatial and temporal variability in rainfall, poor soil fertility and extensive traditional agronomic management practices (Trouche et al., 2001; Leibman et al., 2014). Until now, to meet the food demand of the growing population, increase in production has been mainly achieved by expanding the areas dedicated to crop cultivation (Hanak-Freud, 2000). This strategy is limited by urbanization and the saturation of the rural space leading the farmers to use intensification method (Brocke et al., 2002; Xie et al., 2019). In addition, sorghum cultivation is highly competitive by potentially productive maize in areas of intensification in Mali (Bazile et al., 2008; Vaksmann et al., 2008). Presently, it is well documented that grain yield depends both on crop genetic potential and agronomic practices such as plant density and mineral fertilization (Moosavi et al., 2013; Kondombo et al., 2017). Numerous studies have shown the importance of plant density and nitrogen fertilization on sorghum production (Bayu et al., 2005; Akmal et al., 2010; Arunakumari and Rekha, 2016). In addition, previous studies reported that the optimum plant density depends on each crop (Biswas and Ahmed, 2014), beyond which the competition between plants for light, water and nutrients becomes important and can lead to decreased crop yields (Berenguer and Faci, 2001; Çalifikan et al., 2007; Li et al., 2016). Nitrogen is also one of the most important nutrients which must be used in an optimal quantity depending on plant density as its lack or excess can reduce crop productions (Fischera and Wilsonb, 1975; Ferraris and Charles, 1986; Tajul et al., 2013; Sher et al., 2016).

Recently in Mali, to increase sorghum production, mineral fertilization studies were experimented and diffused on sorghum varieties (Kouyate and Wendt, 1991; Zougmoré et al., 2003; Coulibaly et al., 2019). However, plant density of 25.000 hills/ha and the doses of 100 kg ha^{-1} diammonium phosphate (DAP) at sowing and 50 kg ha^{-1} urea before the panicle initiation were recommended for sorghum cultivation (Kouyate and Wendt, 1991; Coulibaly et al., 2019). These agronomic practices advised in the growing areas were disseminated separately either on local sorghum or on improved sorghum. Nowadays, little research has been done to understand the performance of newly improved sorghum varieties to respond to plant density and nitrogen fertilization to intensify grain and straw production. A better knowledge on the effect of these techniques on sorghum productivity (grain and straw) should contribute to a better understanding of the constraints related to sorghum intensification in the Sahel. The objective of this study was to identify sorghum varieties that respond to plant density and nitrogen fertilization, and to determine agro-morphological and physiological traits involved in the variation in plant density and nitrogen fertilization.

MATERIALS AND METHODS

Experimental site and growing conditions

Two field trials were conducted in the rainy seasons of 2018 and 2019 at the Sotuba Agricultural Research Station in Mali (12°39' N and 07°56' W). The climate of this area is Sudano-Sahelian type with an average annual rainfall of 866 mm on the period 1981-2010. The cumulative annual rainfall recorded in 2018 was 840 mm against 1158 mm in 2019 (Figure 1). The average annual temperatures during the 2018 and 2019 experiments were 27 and 28°C, respectively. In 2018, trial was conducted on sandy-silty soil (96.84%) with low clay content (3.85%), water pH (5.75), organic matter (0.37%), nitrogen (0.12%), assimilable phosphorus (10.77 ppm) and exchangeable potassium (0.13 meq/g). However, in 2019, the experimentation was carried out on sandy-silty soil (80%) with a clay content (20%), water pH (6.20), organic matter (1.46%), nitrogen (0.25%), assimilable phosphorus (14 ppm) and exchangeable potassium (0.40 meq/g). Sampling of the experimental soils was done at a 0-40 cm depth.

Plant materials

Eight contrasting sorghum varieties for different agro-morphological and physiological traits were assessed: hybrid varieties FADDA and PABLO (hybrid varieties) and open pollinated varieties SOUMBA, GRINKAN, WILIBALI (C2_007-03), WASSALEN (C2_075-15) and TINSAMBA (A12-79). The local variety TIEBILE (CSM335) was the control in this study (Table 1). These varieties represent the diversity of improved sorghum grown in Mali.

Experimental design and crop management

A split-split-plot design was used to study three factors including two plant densities (D1: 26666 plants ha^{-1} and D2: 53333 plants ha^{-1}) as the main plot, three nitrogen levels (0, 41 and 82 kg ha^{-1}) as the subplot and varieties as the sub-subplot with three replications. The dose of nitrogen recommended in Mali for the sorghum cultivation is 41 kg ha^{-1} (Kante et al., 2017). A total of 144 treatments were used. The area for each elementary plot was 18 m^2 (6 lines 4.5 m long and 4 m wide). The seeding spacing was 0.75 m x 0.5 m for the low density (D1) and 0.75 m x 0.25 m for the high density (D2). The soil was ploughed to a depth of about 30 cm. Sowing was done on June 18th, 2018 and on July 5th, 2019 after a good rainfall (25 mm) at a rate of 5 to 6 seeds per hill. Around 15 days after emergence, the plots were thinned to one plant per hole in wet condition. Nitrogen was applied in the form of urea in two fractions, three weeks after thinning (50%) of the plants and before

*Corresponding author. Email: alain.audebert@cirad.fr.

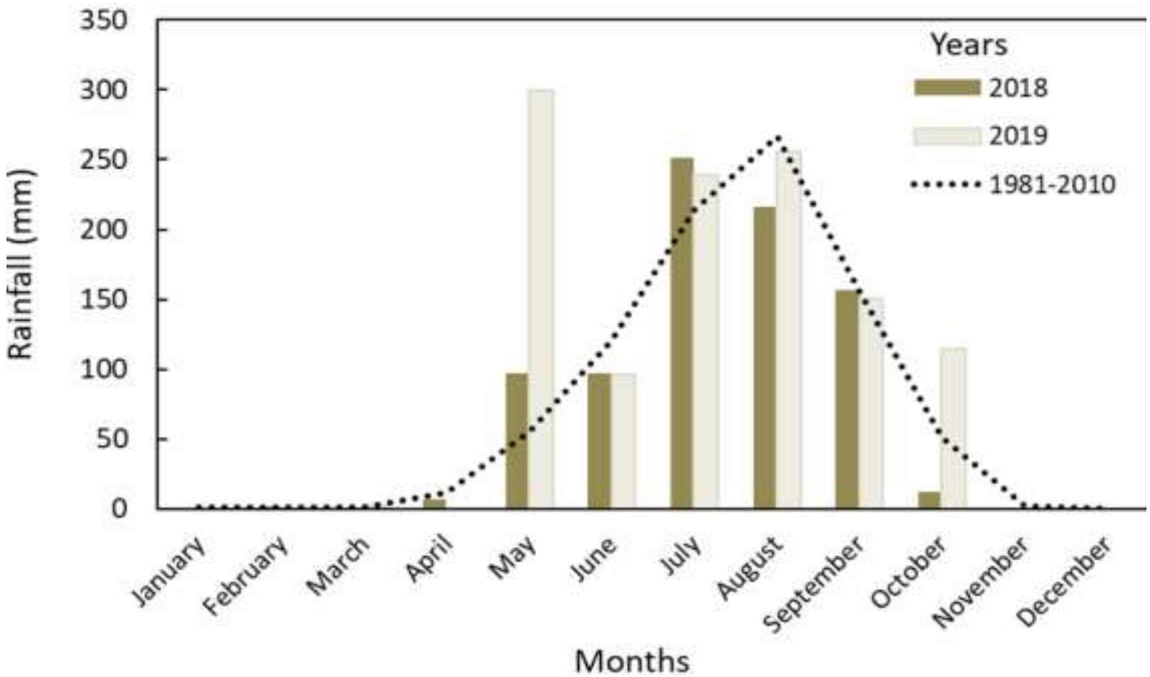

Figure 1. Monthly rainfall for 2018, 2019 and the last 30 years (1981-2010) recorded in Sotuba of January to December.

Table 1. Characteristics of eight sorghum varieties used in the 2018 and 2019 experiments.

Variety	Origin	Race	Cycle duration (days)	Plant height (cm)	Grain yield (t ha^{-1})	Isohyete (mm)
FADDA	Mali	Guinea (hybride)	117	300	4	700-1000
PABLO	Mali	Guinea (hybride)	115	400	4	700-1000
GRINKAN	Mali	Caudatum	125	200	2.8	800-1000
SOUMBA	Mali	Caudatum	110	240	2.8	600-800
TIEBILE	Mali	Guinea	125	360	2.5	800-1000
C2_007-03	Mali	Guinea-Caudatum	130	210	3.3	800-1000
C2_075-15	Mali	Guinea-Caudatum	130	170	3.3	800-1000
A12-79	Mali	Durra-Caudatum	135	175	4	800-1000

the panicle initiation (50%). Basal application of Phosphorus (46 kg P_2O_5) was homogeneously made in all plots before sowing as "phosphate naturel de Tilemsi" (PNT) granulated (31% P_2O_5). Two manual weeding were carried out in each experiment and crop ridging was performed after the second nitrogen application. Breakouts were realized between blocks and between replications to reduce nitrogen exchange. All plots were treated with EMACOT 050 WG insecticide in the vegetative phase of the crops according to the infestation level to control the attacks of legionary caterpillars (Spodoptera exempta).

Measurements and sampling

The phyllochron was calculated according to the ratio of number of days necessary for the appearance of the flag leaf to the total number of leaves that appeared on the main stem. Its measurement was carried out on three plants randomly selected from each elemental plot.

Physiological measurements were realized at flowering and carried on the leaf area index and chlorophyll estimation. The leaf area index was estimated with a Sunscan Septometer (Delta-T Device Ltd) equipped with an external BF5 sensor on plots (1 m^2) delimited in each elementary plot. The chlorophyll estimation was done with a SPAD-502 device. It was carried on the 3rd ligulated leaf of the main stalk from the top of the plant and was performed on the three plants used for the phyllochron determination. An area of 2.25 m^2 was used for plant height measurements, yield components, straw biomass and grain yield at physiological maturity. The seedlings of 6 hills for the low density and 12 hills for the high density were collected. Plant height was measured with a ruler and the panicule number per m^2 was assessed by manual counting. After harvesting (panicle and dry straw) and sun drying for one month, panicle weight per m^2 and straw yield were determined.

The dried panicles were threshed to determine grain yield. The 1000-grains weight was obtained by counting with an electric counter (NUMIGRAL) and the grain numbers per panicle was also calculated according to the ratio of average panicle weight to grain weight.

Data statistical analysis

The combined variance analysis of the two trials was performed with the "agricolae" package for the environment (Venables, Smith and the R Core Team, 2019) according to the split-split-plot model developed by Carmer et al. (1989). Shapiro-Wilk normality and Bartlett homogeneity tests were performed to identify and exclude aberrant data induced by soil heterogeneity for different measured variables. The Tukey test (smallest significant difference, LSD) at the 5% threshold was used to compare the means of the studied factors. Pearson correlation analyses were performed with the "Hmisc" package and the principal component analyses (PCA-biplot) were performed with the "FactoMineR" and "factoextra" packages in the same R software.

RESULTS

Grain yield and straw yield

The analysis of variance showed significant effects of the year, density, nitrogen dosage and variety on grain and straw yields. The interaction effects between year x density and year x nitrogen were also significant on grain and straw yields; and the year x variety interaction was significant for straw yield (Table 2). The interaction effects between nitrogen x variety and density x nitrogen x variety were likewise significant on grain yield and straw yield.

The highest grain yield was recorded in 2019 (3644 kg ha^{-1}) compared to 2018 (3322 kg ha^{-1}). Grain yield increased from 2975 kg ha^{-1} in D1 (26 666 plants $ha^{-1)}$ to 4013 kg ha^{-1} in D2 (53 333 plants ha^{-1}). Nitrogen application increased grain yield from 2918 kg ha^{-1} in N0 (0 kg Nha^{-1}) to 3935 kg ha-1 in N2 (178 kg Nha^{-1}). Straw yield was also higher in 2019 (18054 kg ha^{-1}) than 2018 (10510 kg ha^{-1}). It increased from 12785 kg ha^{-1} in D1 to 15995 kg ha^{-1} in D2; and from 12413 kg ha^{-1} in N0 to 16151 kg ha^{-1} in N2 (Table 2). Under different plant density and nitrogen combinations, high grain and straw yields were observed in the N2D2 treatment (178 kg N ha^{-1} and 53 333 plants ha^{-1}) for all varieties than in the N0D1 treatment (0 kg N ha^{-1} and 26 666 plants ha^{-1}). In N2D2, FADDA variety performed with a grain yield of 6241 kg ha^{-1}. It is followed by A12-79 (5342 kg ha^{-1}) while SOUMBA variety produced the lowest grain yield of 3193 kg ha-1 in N2D2 (Figure 2B). The same remark was made for straw yield, where FADDA (23511 kg ha^{-1}) was the best performing variety in N2D2. The C2_075-15 variety had the lowest straw yield, 14370 kg ha^{-1} (Figure 2D). In N0D1 (0 KgN ha^{-1} and 26666 plants ha^{-1}), GRINKAN, C2_075-15, C2_007-03, TIEBILE and SOUMBA varieties produced the highest grain yield with an average of 2694 kg ha^{-1}. Variety A12-79 recorded the lowest grain yield of 1806 kg ha^{-1} (Figure 2A). For straw yield, GRINKAN was the best performing variety (14474 kg ha^{-1}) in N0D1 and FADDA, TIEBILE, A12-19 and PABLO varieties produced the lowest straw with an average of 9234 kg ha^{-1} (Figure 2C). These results indicate that the increase in grain and straw yields is related to the increase in plant density and nitrogen application.

Agronomical and physiological parameters

The analysis of variance showed significant effects of the year, density, nitrogen and variety on the measured parameters (Table 3). The density x variety and nitrogen x variety interactions effects were significant on panicle number m-², panicle weight m^{-2} and plant height. The interaction effect between nitrogen x variety was also significant on SPAD value.

The panicle number m-² increased significantly with the increasing in plant density and nitrogen application. FADDA and TIEBILE varieties obtained on average 6.81 panicle m^{-2} in D2 (53 333 plants ha^{-1}). In addition, FADDA variety produced 7.41 panicle m^{-2} in N2 (178 kg Nha^{-1}) compare to the other varieties. FADDA variety obtained a panicle weight m^{-2} of 675 g in D2 and 7.94 g in N2 than the other varieties. FADDA statistically had the same panicle weight m^{-2} as PABLO and A12-79 in D2. For 1000WG, PABLO and TIEBIELE varieties recorded on average 24 g and the A12-79 variety was the least performing. The D1 density produced 457 more grains per panicle than the D2 density. It varied from 2803 grains in N0 to 3165 grains in N1 (89 kg N ha^{-1}) (statistically equal to N2). A12-79 variety had significantly the highest grain per panicle (3475). For plant height, PABLO variety was the longest in D2 (437 cm). It varied from N0 (269 cm) to N1 (279 cm) (statistically identical to N2).

The leaf area index value was 2.33 and 2.99 respectively in D1 and D2. Nitrogen input increased the vegetation cover from 2.49 in N0 to 2.89 in N2. FADDA had significantly the highest leaf area index (2.99) and A19-79 obtained lower value of 2.27. PABLO and FADDA varieties performed with SPAD values of 49.9 and 49.7 respectively in N2. It was 44.77 in D1 and 43.45 in D2. Phyllochron ranged from 3.01 days in D1 to 3.20 days in D2. Nitrogen application shortened phyllochron from 0.20 days in N2 to 0.08 days in N1. FADDA and PABLO varieties recorded a short phyllochron with an average of 2.96 days than the C2_007-03 and TIEBILE varieties, which averaged 3.23 days.

Contribution of variables to grain and straw production in sorghum

To better understand the variable contributions to the increase in grain yield and straw yield, a correlation

Table 2. Analysis of variance of the factors year (Y), plant density (D), nitrogen (A) and their interactions on grain yield (GY) and straw yield (STY) in 2018 and 2019.

Treatment	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Year		
2018	3322^{b}	10510^{b}
2019	3644^{a}	18054^{a}
Density (Plants ha^{-1})		
D26666 (D1)	2975^{b}	12785^{b}
D53333 (D2)	4013^{a}	15995^{a}
Nitrogen (Kg N ha^{-1})		
N0	2918^{c}	12413^{c}
N89 (N1)	3647^{b}	14597^{b}
N178 (N2)	3935^{a}	16151^{a}
Variety (V)		
A12-79	3338^{bc}	13307de
C2_007-03	3575^{b}	15453^{b}
C2_075-15	3391^{bc}	12228^{e}
FADDA	4473^{a}	17656^{a}
GRINKAN	3358^{bc}	14384^{bcd}
PABLO	3578^{b}	13784^{cd}
SOUMBA	2958^{c}	13528de
TIEBILE	3396^{bc}	15050^{bc}
Source of variation		
Year (Y)	***	***
Density (D)	***	***
Nitrogen (N)	***	***
Variety (V)	***	***
Y x D	*	*
Y x N	***	**
D x N	NS	NS
Y x V	NS	***
D x V	NS	NS
N x V	***	**
Y x D x N	NS	NS
Y x D x V	NS	NS
Y x N x V	NS	NS
D x N x V	**	*
Y x D x N x V	NS	NS

Values in the same column followed by different letters are significantly different at $p < 0.05$. *, **. *** Significantly different at 5, 1 and 0.1% respectively; NS, non-significant. D26666 (D1: 26666 plants ha^{-1}) and D53,333 (D2: 53333 plants ha^{-1}); N0 (0 kg N ha^{-1}), N89 (N1: 89 kg N ha^{-1}) and N178 (N2: 178 kg N ha^{-1}).

matrix was carried out on the average of N0D1 (Table 4) and N2D2 (Table 5) treatments. Grain yield was significantly and positively correlated with the leaf area index, grain number per panicle, panicle number per m^2, panicle weight per m^2 and straw yield in N0D1 and N2D2; and negatively correlated to phyllochron in N2D2. Straw yield was significantly and positively correlated to leaf area index, grain number per panicle, panicle number per m^2 and panicle weight per m^2 in N0D1 and N2D2. It was negatively correlated with plant height in N0D1 and

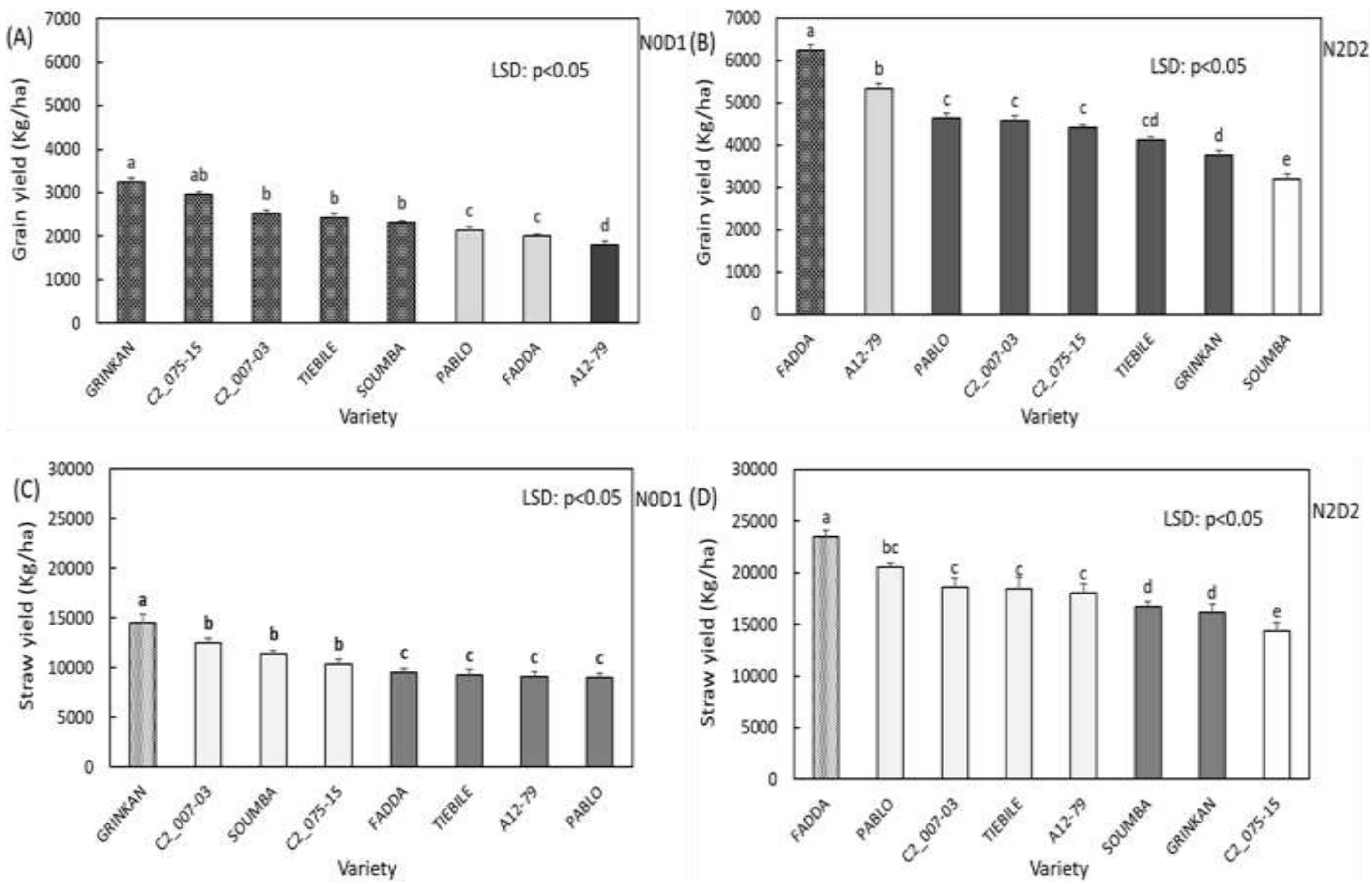

Figure 2. Grain yield (A) and (B); Straw yield (C) and (D) of eight sorghum varieties under different plant densities and nitrogen fertilization levels. N0D1 (0 kg N ha^{-1} and 26666 plants ha^{-1}), N2D2 (178 kg N ha^{-1} and 53333 plants ha^{-1}).

Table 3. Analysis of variance of the effects year, density, nitrogen variety and their interactions on the variables measured in 2018 and 2019.

Source of variation	panicle number per m^2	panicle weight per m^2	1000-Grain weight (g)	Grain number per panicle	Plant height (Cm)	Leaf area index	SPAD value	Phyllochron
Year (Y)	***	***	***	***	***	***	***	*
Density (D)	***	***	*	***	***	***	*	***
Nitrogen (N)	*	***	NS	*	***	**	***	***
Variety (V)	***	***	***	***	***	**	***	***
Y x D	*	NS	NS	NS	NS	NS	NS	NS
Y x N	*	***	NS	NS	NS	NS	NS	NS
D x N	NS	NS	NS	NS	NS	NS	NS	NS
Y x V	***	NS	***	NS	***	***	***	NS
D x V	**	*	NS	NS	***	NS	NS	NS
N x V	**	**	NS	NS	***	NS	*	NS
Y x D x N	NS	NS	NS	NS	NS	NS	NS	NS
Y x D x V	NS	NS	NS	NS	NS	NS	NS	NS
Y x N x V	NS	NS	NS	NS	NS	NS	NS	NS
D x N x V	NS	NS	NS	NS	NS	NS	NS	NS
Y x D x N x V	NS	NS	NS	NS	NS	NS	NS	NS

*, **. *** Significantly different at 5, 1 and 0.1% respectively; NS, non-significant.

Table 4. Correlation between agronomical traits and physiological parameters measured in N0D1.

Variable	Grain yield	Leaf area index	grain number per panicle	Plant height	Phyllochron	Panicle number per m²	Panicle weight per m²	SPAD value	Straw yield
Grain yield	1								
Leaf area index	0.84**								
Grain number/panicle	0.66**	0.24							
Plant height	-0.28	0.13	-0.76**						
Phyllochron	0.27	0.47*	-0.04	0.12					
Panicle number m-²	0.62**	0.72**	0.21	0.42*	0.4*				
Panicle weight m-²	0.93***	0.79**	0.58*	-0.1	0.14	0.73**			
SPAD value	0.08	-0.15	0.15	-0.33	-0.47*	-0.42*	-0.1		
Straw yield	0.75**	0.69**	0.57*	-0.48*	0.02	0.19	0.58*	0.35	
1000-grain weight	0.15	0.5*	-0.6**	0.7**	0.04	0.34	0.21	0.03	0.12

*, **. *** Significantly different at 5, 1 and 0.1% respectively. (0 kg N ha^{-1} and 26666 plants ha^{-1}).

Table 5. Correlation between agronomical traits and physiological parameters measured in N2D2.

Variable	Grain yield	Leaf area index	Grain number per panicle	Plant height	Phyllochron	Panicle number per m²	Panicle weight per m²	SPAD value	Straw yield
Grain yield	1								
Leaf area index	0.79**								
Grain number/panicle	0.44*	0.24							
Plant height	0.21	0.41*	-0.13						
Phyllochron	-0.54*	-0.32	-0.2	-0.13					
Panicle number m-²	0.66**	0.47*	-0.13	-0.12	-0.52*				
Panicle weight m-²	0.96***	0.86***	0.38	0.3	-0.66**	0.64**			
SPAD value	0.24	0.71**	-0.01	0.03	0.01	0.24	0.36		
Straw yield	0.71**	0.85***	-0.02	0.66**	-0.26	0.44*	0.75**	0.38	
1000-grain weight	-0.16	0.2	-0.41*	0.66**	0.49*	-0.4*	-0.13	0.21	0.49*

*, **. *** Significantly different at 5, 1 and 0.1% respectively. N2D2 (178 kg N ha^{-1} and 53 333 plants ha^{-1}).

positively correlated with plant height in N2D2. The variables correlated to grain and straw yields will be used in variety Characterization.

Characterization of eight varieties for the traits studied

Principal Component Analysis (PCA-biplot) based on eight sorghum varieties in treatments N0D1 (Figure 3A) and N2D2 was conducted. In N0D1, the ACP-biplot shows three homogeneous groups. Dimensions 1 and 2 explain respectively 47.6% and 29.9% of total variation. Group 1 includes SOUMBA, FADDA and A12-79 varieties. It is characterized by less important variables. Group 2 is determined by a plant height and 1000-grain weight and is composed of TIEBILE and PABLO. Group 3 involves the varieties of type caudatum GRINKAN, C2_075-15 and C2_007-03 and is characterized by grain yield, straw yield, leaf area index, weight per m^2 and Grain number per panicle raised. In N2D2, the ACP-biplot indicates four homogeneous groups. Dimensions 1 and 2 explain respectively 49.2% and 26.6% of total variability (Figure 3B). Group 1, which includes the GRINKAN, SOUMBA and C2_007-03 varieties is characterized by a long phyllochron. Group 2, which involves the TIEBILE and PABLO varieties is defined by a large 1000-grain weight and plant height. Varieties of Group 3 are characterized by high grain number per panicle and consist of C2_075-15 and A12-79. Group 4, which is a single FADDA variety of guinea type is characterized by better grain yield, straw yield, leaf area index, panicle number per m^2 and panicle weight per m^2.

DISCUSSION

The study on the performance of eight sorghum varieties

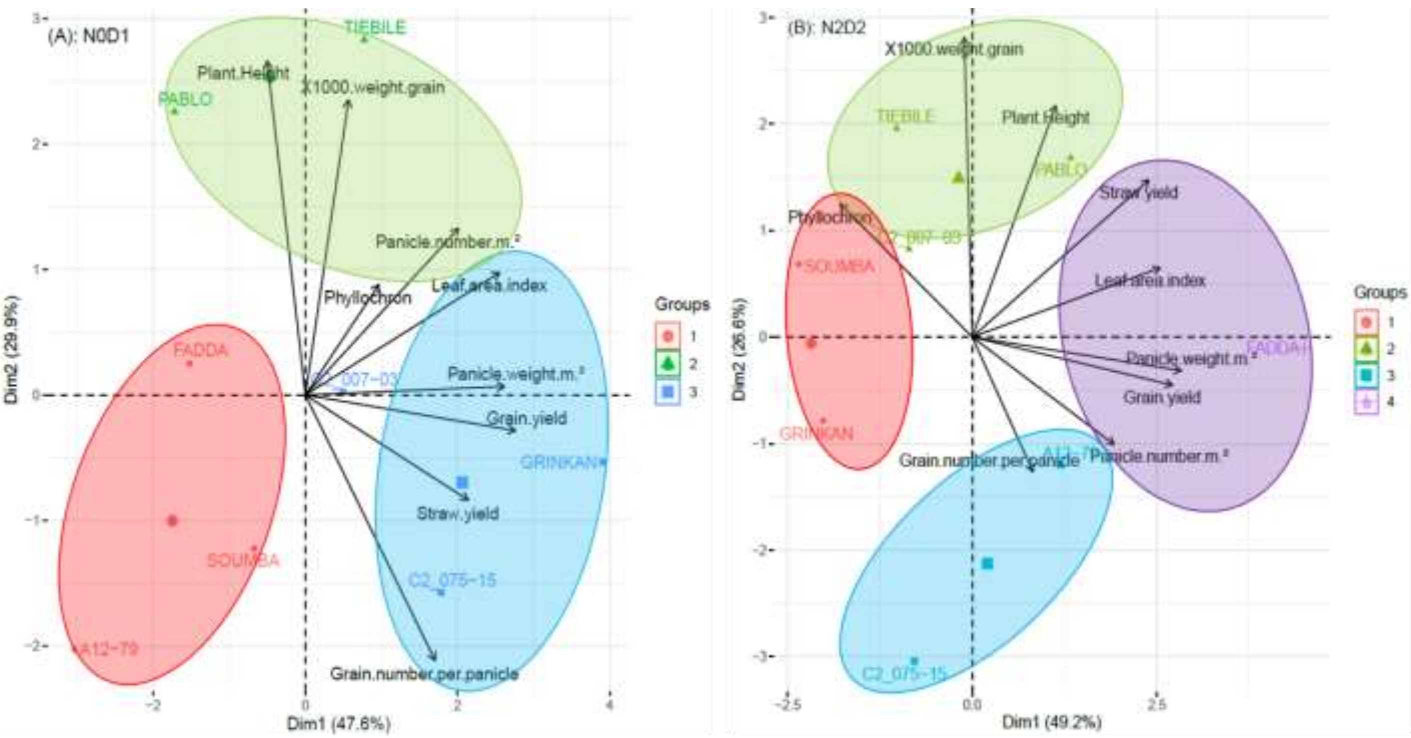

Figure 3. Principal component analysis (A: N0D1 and B: N2D2) with combination of variables and sorghum varieties at different planting densities and nitrogen fertilization. N0D1 (0 kg N ha^{-1} and 26666 plants ha^{-1}); N2D2 (178 kg N ha^{-1} and 53 333 plants ha^{-1})

at different plant densities and nitrogen fertilization enabled an understanding of the effect of intensification factors on sorghum grain and straw production in the Sudan-Sahelian zone in Mali. The response of the studied factors during the two-year trials may be due to rainfall distribution (Turgut et al., 2005; Oikeh et al., 2009) and soil heterogeneity. This could explain the decrease in grain yield (by 10%) and straw yield (by 72%) in the first trial as compared to the second. In this study, grain and straw yields increased with increasing plant density from D1 ((26666 plants ha^{-1})) to D2 (53 333 plants ha^{-1}) for all varieties tested. Nitrogen application also increased grain and straw yields from N0 (0 kg N ha^{-1}) to N2 (178 kg N ha^{-1}). Our results are similar to those reported by Moosavi et al. (2013) and Shrestha et al. (2018). However, the results also showed that grain yield and straw yield varied for all varieties at different plant density and nitrogen fertilization combinations. They also increased from N0D1 (0 KgN ha^{-1} and 26,666 plants ha^{-1}) to N2D2 (178 kg N ha^{-1} and 53 333 plants ha^{-1}). The highest grain yield under less intensive conditions (N0D1) was obtained with GRINKAN, C2_075-15, C2_007-03, TIEBILE and SOUMBA varieties than FADDA, PABLO and A12-19 varieties, which have been the lowest performing variety. GRINKAN variety of caudatum type produced the highest straw yield than FADDA, TIEBILE and PABLO varieties of guinea type except A12-79 in N0D1. Under intensive N2D2 treatment, FADDA variety recorded the highest grain yield (Figure 2B) and straw yield. These results suggest that response of varieties to different plant densities and nitrogen fertilization for grain and straw yields is highly variable and could be genetic. This shows that each variety or group of varieties needs an optimum nitrogen level and plant density to produce maximum grain and straw. Our results are consistent with studies conducted by Zhou et al. (2019). Shahrajabian et al. (2011) and Soleymani et al. (2011) also confirmed these findings in their study on sorghum.

Grain yield depends on the variety and growing conditions, in particular plant density and nitrogen fertilization. Its improvement depends on its components but also on physiological and growth traits. In this study, the panicle weight per m^2, leaf area index, straw yield and grain number per panicle parameters were most expressed in N0D1 (Table 4) and N2D2 (Table 5). These results clearly show that through these traits it is possible to increase grain yield under less intensive (N0D1) and intensive (N2D2) conditions. Researches conducted by Ogunlela and Okoh (1989), Buah et al. (2009), and Ajeigbe et al. (2018) reported similar results. But in N2D2, grain yield was strongly explained by panicle number per m^2. Moosavi et al. (2013) believed that at high plant density, emphasis should be placed on panicle number per m^2, because at high plant density, the grain number per panicle decreases even if grain yield per unit area increases. Straw yield in N0D1 and N2D2 was positively explained by leaf area index and panicle weight m^{-2}. There was also a positive correlation between straw yield, plant height and panicle number per m^2 in N2D2, but it was positively correlated by grain number per panicle in N0D1. This finding show that a selection made in favor of these traits can help us increase production of sorghum straw. According to Sahu et al. (2018), nitrogen application increases plant height and leaf area index in sorghum, which would be involved in increasing straw yield.

The variability observed under N0D1 and N2D2 treatments enabled the classification of eight varieties according to the traits studied. In N0D1, GRINKAN and C2_075-15 and C2_007-03 caudatum varieties (short size) produce the highest grain yield and are characterized by panicle weight per m^2, leaf area index, straw yield and grain number per panicle. In N2D2, FADDA (large size) guinea hybrid variety is the most performing and is characterized by grain yield, straw yield, leaf area index, panicle number m^{-2} and panicle weight per m^2. One of the specificities of this variety is its capacity to valorize nutrients and to develop an important tillering, a trait probably inherited from its parent Lata3. This would explain an increase in his traits in FADDA in N2D2. According to Lafarge et al. (2002) and Zand and Shakiba (2013), tillering is an important trait that leads to increased grain and straw yields in sorghum. In general, this trait has not been of much interest to the sorghum selection programs. However, it should now be one of the priorities of breeding programs to develop productive varieties with large tillering to intensify the crop sorghum.

Conclusion

This study showed that plant density and nitrogen fertilization on sorghum varieties significantly increased grain yield and straw yield. Grain yield in N0D1 and N2D2 was associated with panicle weight per m^2, leaf area index, straw yield, panicle number per m^2 and grain number per panicle. GRINKAN, C2_075-15 and C2_007-03 varieties produced maximum production of grains and straws in N0D1 (0 kg N ha^{-1} and 26666 plants ha^{-1}). These caudatum-type varieties may be recommended in less intensive sorghum production areas in Mali. FADDA variety produced the highest of grains and straws in N2D2 (178 kg N ha^{-1} and 53 333 plants ha^{-1}). Indeed, FADDA being a guinea-type hybrid variety could be recommended to the farmers for grain and fodder production because it is the variety that was better adapted to intensification.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Challenges in greenhouse crop production by smallholder farmers in Kisii County, Kenya

Francis Obuoro Wayua[1*], Vincent Ochieng[2], Violet Kirigua[3] and Lusike Wasilwa[3]

[1]Non-ruminant Research Institute, Kenya Agricultural and Livestock Research Organisation, P. O. Box 169-50100, Kakamega, Kenya.
[2]Food Crops Research Institute - Muguga, Kenya Agricultural and Livestock Research Organisation, P. O. Box 30148-00100, Nairobi, Kenya.
[3]Department of Crops Research, Kenya Agricultural and Livestock Research Organisation, Headquarters, P. O. Box 57811-00200, Nairobi, Kenya.

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Despite the success in greenhouse farming by large scale horticultural farmers in Kenya, smallholder greenhouse farming is beset by many challenges leading to over 30% failure rates. Reasons for the high failure rate are not clearly understood. The objective of this study was to assess the challenges in greenhouse crop production by small-scale farmers in Kisii County, Kenya and provide recommendations for sustained profitability. Data was collected through structured interviews with 138 greenhouse farmers, and analysed using descriptive statistics, t-test and chi-square. Overall, 48.6% of the greenhouses were non-functional, due to pests and diseases, inadequate supply of water, high investment costs, and insufficient knowledge on greenhouse crop farming. Other challenges included lack of market for the produce, and group dynamics challenges. Most of the abandoned greenhouses were owned by institutions (60.5%) and groups (55.6%), and lesser by individuals (38.2%). Number of years of operation significantly influenced functionality of the greenhouses (p=0.04). Greenhouses owned by individuals had significantly higher probability of being functional, than those owned by groups and institutions (p=0.05). Interventions for enhancing contribution of greenhouses to food security of small-scale farmers should include capacity building of farmers on greenhouse crop production, and linking them to appropriate sources of funding.

Key words: Challenges, greenhouse, small-scale farmers, crops, food security, Kenya.

INTRODUCTION

A greenhouse is a structure with walls and roof made of transparent material, such as glass or plastic, in which plants requiring regulated climatic conditions are grown (Smitha et al., 2016). Greenhouse crop farming has the advantage of offering year-round production of crops, crop protection, increased yields, vegetable production in limited land sizes and superior quality product (Wachira et al., 2014; Nordey et al., 2017). In Kenya, greenhouses

*Corresponding author. E-mail: francis.wayua@gmail.com. Tel: +254-710-629683.

are commonly and successfully used by large-scale horticultural farms (Justus and Yu, 2014), and are gradually being adopted by small-scale farmers to grow vegetables for food security (Omoro et al., 2014; Sanzua et al., 2018; van der Spijk, 2018).

Despite the success in greenhouse farming achieved by large scale horticultural and floricultural farmers for many years, smallholder greenhouse farming is beset by many challenges leading to the abandonment of some of these greenhouses. High failure rate of greenhouses have been reported in Kenya, ranging from 30 to 70% (Sanzua et al., 2018). Reasons for abandonment are postulated to be many challenges such as inappropriate structures, lack of information and skills in crop and greenhouse environment management, poor postharvest handling and marketing issues. However, the exact reasons for the high rate of abandonment of the greenhouses by smallholder farmers are not clearly understood. There is, therefore, a growing interest amongst development agents to identify and address the barriers currently preventing smallholder greenhouse farming for sustainably enhancing the food security for the poor farmers in Africa. The objective of this study was, therefore, to assess the challenges and constraints in greenhouse crop production by small-scale farmers in Kisii County, Kenya. Understanding the bottlenecks faced by farmers will provide insights on sustainable interventions needed for smallholder greenhouse crop production to increase the production and income of farmers.

MATERIALS AND METHODS

Study area

The study was conducted in Kisii County (Table 1), which lies between latitude 0° 40’ 38.4” South, and longitude 34° 34’ 46° 61” East (Kisii CIDP, 2018). Kisii County exhibits a highland equatorial climate resulting into a bimodal rainfall pattern with an average annual rainfall of 1,500 mm. The long rains are between March and June while the short rains are received from September to November; with the months of January and July being relatively dry. The maximum temperatures in the County range between 21 and 30°C, while the minimum temperatures range between 15 and 20°C. The high and relatively reliable rainfall patterns coupled with moderate temperatures are suitable for growing crops like tea, coffee, maize, beans, bananas, dairy farming and horticultural farming (Kisii CIDP, 2018). The human population is estimated at 1,260,509 (KNBS, 2019). Most people own small parcels of lands, with farm sizes between 0.2 to 2.1 acres (ASDSP, 2014). Greenhouse crop farming, therefore, has potential for enhancing food security in the County due to the small land holdings.

Sample size and sampling

A sampling frame was developed that gave an indication of where the greenhouses (whether in use or not) were located. This was done in collaboration with the Ministry of Agriculture extension staff in the study site. The list so obtained was enriched further through snow-balling technique, whereby managers of the selected greenhouses were asked to name any other greenhouses they knew of. With approximately 209 greenhouses in Kisii County (Omoro et al., 2014), sample size was determined based on the following equation (Yamane, 1967):

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

where n is the suggested sample size, N is the total number of greenhouses and e is the level of precision, set at 5% for the study. Hence the sample size (n) was set at 138 greenhouses.

Purposive sampling and snowballing technique was used to locate the greenhouses, based on accessibility. Key informants (agricultural extension officers and village elders) assisted in locating the greenhouses. Of the 138 greenhouses, 67 were owned by individual farmers, 20 by farmers groups and 43 by institutions (schools, vocational training centres and religious institutions).

Data collection

Data was collected using a structured questionnaire uploaded on the Open Data Kit (ODK) tool. Enumerators were recruited and trained to map and administer the questionnaires. The data detailed the (i) constraints and challenges in greenhouse farming, (ii) abandoned greenhouses, and (iii) proposed strategies to address the challenges. After detailing the constraints and challenges, the same respondents were asked to suggest probable solutions, based on their experiences.

Data analysis

Quantitative data were analysed using descriptive statistics (frequencies, means, totals and measures of dispersion)-continuous and categorical variables being reported as mean±standard errors and percent, respectively. Chi-square and independent sample t-test were used to compare variables between greenhouses in use and those abandoned, and relationship between abandoned greenhouses and type of ownership. A probability of 5% was considered significant for all statistical analyses. All analyses were done in SPSS (SPSS, 2011). The results are presented in tables, graphs and charts. Qualitative data were summarised into themes. The results are presented under the sub-topics (i) constraints and challenges in greenhouse farming, (ii) abandoned greenhouses, and (iii) proposed strategies to address the challenges.

RESULTS AND DISCUSSION

Constraints and challenges in greenhouse farming

All the respondents reported experiencing constraints in greenhouse crop production. Overall, the main constraints in greenhouse farming were pests and diseases (27.1%), inadequate supply of water (23.1%), high costs of inputs (17.2%) and lack of information on greenhouse farming technology (Table 2).

Pests and diseases

Pests and diseases emerged as the major challenge in

Table 1. Study sites and interviewed respondents in Kisii County, Kenya.

Sub-county	Number of respondents		
	Male	Female	Total
Nyaribari Chache	30	8	38
Bonchari	14	3	17
Bobasi	9	5	14
Kitutu Chache South	6	7	13
Nyamache	7	3	10
Nyaribari Masaba	6	4	10
South Mugirango	6	2	8
Kitutu Chache North	7	1	8
Bomachoge Chache	2	5	7
Gucha	5	1	6
Kenyenya	3	1	4
Bomachoge Borabu	2	1	3
Total	**97**	**41**	**138**

Table 2. Ranked challenges in greenhouse farming, in Kisii County, Kenya.

Constraint	% ranking			
	Individual	Groups	Institutions	All
Pests and diseases	26.3	33.3	24.4	27.1
Inadequate supply of water	22.6	22.2	24.4	23.1
High cost of inputs	19.7	20.4	12.2	17.2
Lack of scientific information on greenhouse farming	9.5	5.6	12.2	9.5
Lack of market for produce	8.7	7.5	2.4	6.6
High investment cost	5.1	1.9	1.2	3.7
Group dynamics challenges	1.5	7.4		2.2
Inability to regulate temperature in the greenhouse			6.1	1.8
Poor quality of construction material	0.7	1.9		0.7
Unavailability of quality planting material	0.7		3.7	1.5
Inability to restrict entry into the greenhouse	0.7		3.7	1.5
Non availability of skilled labour	2.2		2.4	1.9
Lack of irrigation piping material	1.5			0.7
Theft and insecurity			2.4	0.7
Poor soils			1.2	0.4
Soil testing			1.2	0.4
Pump maintenance			1.2	0.4
Maintenance of polythene			1.2	0.4
Destruction by wind	0.7			0.4
Total	100.0	100.0	100.0	100.0

smallholder greenhouse crop production in the study area. The pests and diseases varied with the type of crop grown in the greenhouse. The main crop grown in greenhouses in the study area was tomato. Major tomato pests included tomato leaf miner, white flies, aphids, cutworms, thrips, spidermite and African bollworms. Major diseases of tomatoes in Kenya are late blight, early blight, bacterial wilt, Fusarium wilt, bacterial spot and powdery mildew, among others. Previous studies in Kenya also identified pests and diseases to be a major challenge in greenhouse crop production by small-scale farmers in Kenya (Anon, 2011). This, however, contrasts studies in other countries which found pests and disease to be the least problem in greenhouse crop production (Senthilkumar et al., 2018).

Generally, pathogens and insects can be established in a greenhouse very fast. Compared to open field cultivation where many environmental factors come into play, pathogens and insects inside a greenhouse are difficult if not impossible to control. The best strategy is prevention. For greenhouses that are covered with plastic, the use of ultraviolet-absorbing plastics can reduce insect problems (Wambui, 2012). Good management includes the use of resistant varieties and biological pesticides that are used in organic production. However, this study also found that these are expensive for the smallholder farmer.

Inadequate supply of water for irrigation in the greenhouse

Inadequate supply of water for irrigating the greenhouse crops was the second most important challenge for greenhouse crop production. Whereas greenhouse crop production is destined to use less water compared to open field vegetable cultivation (Wambua and Mutua, 2014), farmers in the study region experienced serious water problems in their greenhouse operations. The main source of water used in the greenhouses were borehole (32.3%), harvested rain water (32.3%) and river (27.9%). The farmers did not take the irrigation water for testing for pathogens and chemical contaminants. This could be another source of the soil-borne pathogens such as bacterial wilt and Fusarium wilt (Aloyce et al., 2017; Stewart-Wade, 2011).

High cost of inputs

The high cost of inputs was another challenge faced by farmers. This included the high upfront cost of establishing the greenhouse and the maintenance costs (costs of seeds, fertiliser, water, pesticides, etc.). Greenhouse production is a capital-intensive technology requiring a substantial investment especially during the initial establishment period. Kassamjee (2010) found that price was a significant factor deterring farmers from buying greenhouse kit. Initial capital investment for establishing the greenhouses in the study area was at least US$ 1200, which may be hardly unaffordable by majority of small-scale women farmers whose incomes are low. This led to many farmers making their greenhouses using locally available material such as wood. Whereas use of locally available materials could have resulted in low investment and running costs, it may have compromised on efficient control of operations and performance, as has been reported by previous studies (Baille, 1999; Nordey et al., 2017).

Inadequate knowledge on greenhouse crop production

Most of the farmers also faced the challenge of inadequate knowledge on greenhouse crop production. Farmers lack information on appropriate structures, type of covering materials, agronomic practices, management of greenhouse environment, postharvest handling and marketing aspects. Discussion with farmers revealed that the greenhouses were introduced without farmers being trained on how to manage and run the greenhouses. Hence most of farmers have ended up facing limitation in selecting right materials, and other inputs for greenhouse farming. Therefore, failure of some farmers has discouraged others who are yet to adopt the technology to delay waiting to have a complete awareness of the technology which is yet to be effectively offered. Proper management and the right technical skills are important for successful greenhouse farming (FAO, 2013). Production of crops under greenhouse conditions is highly knowledge and skill intensive. Adequate knowledge enables effective use of the technology. However, farmers in the study find it difficult to get latest information and techniques on crop production under greenhouses. It has been established that extension staff farm visit frequency had a significant effect on performance of greenhouse by smallholder farmers in the region (Omoro et al., 2015). While information from suppliers is useful, it is essential that farmers have unrestricted access to unbiased technical information. Technical support for greenhouse farming and its adoption in the study region remains limited.

Unavailability of quality planting material

This related mainly to certified seeds and seedlings, and the right seeds for greenhouse crop farming. Other challenges faced by farmers related to procurement of nutrients and chemicals. Agro-dealers do not stock these locally. Most agro-dealers in the region stock supplies which are used in open field agriculture and not greenhouses. Seeds, seedlings and greenhouse inputs had to be sourced from certified nurseries in Nairobi, over 400 km away. This took quite some time, at least over one month. Lack of planting material was cited as one of the factors hindering adoption of greenhouses by smallholder farmers in Kenya (van der Spijk, 2018).

Abandoned greenhouses

These challenges led to some of the greenhouses being abandoned. Overall, 48.6% of the greenhouses had been abandoned because of the challenges. This finding corroborate the finding of Sanzua et al. (2018) who reported high failure rate of smallholder greenhouses in Kenya, ranging from 30 to 70%. Most of the abandoned greenhouses in the present study were owned by institutions (60.5%) and groups (55.6%), and lesser by individuals (38.2%) (Table 3). Number of years of

Table 3. Characteristics of functioning and abandoned greenhouses in Kisii County, Kenya.

Continuous variables		Functioning Greenhouses (n=71)*	Abandoned greenhouses (n=67)	*p*-value	*t*-value
		Mean±SE**	Mean±SE		
Years of operating the greenhouse		3.2±0.3	4.2±0.4	0.04	-2.03
Number of greenhouses owned		1.2±0.1	1.1±0.1	0.35	0.94
Categorical variables	**Category**	**%**	**%**	***p*-value**	**χ^2-value**
Gender of respondent	Male	74.6	65.7	0.25	1.33
	Female	25.4	34.3		
Ownership of greenhouse	Individual	61.8	38.2	0.05	5.87
	Group	44.4	55.6		
	Institution	39.5	60.5		
Membership to production or marketing group	Yes	21.4	15.4	0.54	0.38
	No	78.6	84.6		

*n=number of respondents per site; **SE = standard error of the mean.

operating the greenhouse significantly influenced the functionality of the greenhouses (p=0.04), with greenhouses established earlier having more likelihood of being functional. This could be due to the fact that farmers with more experience seem to have more knowledge and are better placed to cushion themselves against any adverse effects (Ozor and Nnaji, 2020). Type of greenhouse ownership also had a significant effect (p=0.05) on the functionality of the greenhouses, with greenhouses owned by individuals having higher probability of being functional (Table 3). This could be due to the fact that the risk was high for individuals who, therefore, were keen in managing their greenhouses as opposed to groups and institutions who could be engaged in wrangles due to group dynamics, thereby negatively affecting the performance of the greenhouses.

Proposed strategies to address the challenges in smallholder greenhouse farming

Pests and diseases

For pests and diseases, the main method suggested by farmers (55.5%) was use of chemicals (Table 4). This implies that pesticide use could by high in greenhouses. Whereas this study did not evaluate pesticide residues in greenhouse crops, previous studies detected pesticide residues in greenhouse crops consumed in the country (Kinyunzu 2015; Nguetti, 2019). Pesticide use in greenhouses is expected to be lower than open field cultivation, as greenhouses are constructed to grow crops in a protected growing environment (Nordey et al., 2017; van der Spijk, 2018). The fact that farmers encountered this challenge is an indication that knowledge needed in greenhouse farming is not adequate, as has been reported in this paper among the challenges experienced. Other suggested methods for dealing with pests and diseases included soil treatment using steam and water, and appropriate training on greenhouse management. Soil sterilisation using steam and water has been effective in killing soil-borne pathogens and nematode pests (Loenen et al., 2003; Luvisi et al., 2008).

Inadequate supply of water

The main strategies for addressing the challenge of inadequate supply of water included rain water harvesting and storage in tanks, collecting water from the river, and drilling boreholes (Table 5).

High cost of inputs

The main strategies for addressing high cost of inputs included acquiring inputs on credit, making savings, government subsidising inputs and getting donations from government and NGOs (Table 6).

Lack of sufficient information on greenhouse farming

The main strategies for addressing the challenge of insufficient information on greenhouse farming were appropriate training of farmers on greenhouse crop production, seeking advice from extension officers and experienced farmers (Figure 1). Knowledge on greenhouse crop production can be transferred to farmers through training of extension agents and farmers. There should also be increased extension services to

Table 4. Suggested solutions (in ranked order) for addressing pests and disease challenge in greenhouse farming in Kisii County, Kenya.

Solution	Rank (Percentage)
Spraying chemicals	55.5
Soil treatment using steam and water	12.1
Appropriate training on greenhouse management	7.4
Seek help from extension officers	5.6
No way to cope / no steps taken	3.8
Borrow from friends and relatives	2.8
Soil testing	1.9
Make savings	1.9
Plant certified sees	0.9
Contribute money to buy pesticides	0.9
Plant tolerant varieties	0.9
Crop rotation	0.9
Working in the greenhouse by self (taking time in the greenhouse)	0.9
Using new techniques to improve, e.g. using black polythene for planting	0.9
Leaving the greenhouse without crop for 2 years	0.9
Planting crops at the right time	0.9
Use insect traps	0.9
Plant onions around the greenhouse	0.9
Total	**100.0**

Table 5. Suggested solutions (in ranked order) for the challenge of inadequate water supply in greenhouse farming in Kisii County, Kenya.

Solution	Rank (Percent)
Harvest rain water and store in tanks	26.1
Collect water from the river	25.2
Drilling borehole	23.4
Buy water	4.5
Use available water supply sparingly	4.5
Piped water from municipal	4.5
Government to subsides inputs	2.7
Make savings	2.7
No way to cope / no steps taken	1.8
Fencing	0.9
Seek help from extension officers / specialists	0.9
Spraying	0.9
Shifted the greenhouse from its original location	0.9
Manual application of water using buckets	0.9
Total	**100.0**

greenhouse farmers, as it has been found that frequency of extension staff visit had a significant effect on performance of greenhouses in the region (Omoro et al., 2015).

For the challenge of lack of market, suggested solutions included carrying out market research, marketing the produce to far distance, and targeting production to coincide with the time when prices are high. For the high cost of establishing greenhouse structures, suggested strategies included government subsidy on greenhouse inputs, donation of greenhouses by development agents, acquiring inputs on credit and constructing greenhouses using cheaper locally available materials. As earlier pointed in this paper, use of locally available materials

Table 6. Suggested solutions (in ranked order) for addressing the challenge of high cost of inputs in greenhouse farming in Kisii County, Kenya.

Solution	Rank (Percentage)
Acquire inputs on credit	22.8
Make savings	17.5
Government to subsidise inputs	12.3
Donation from Government and NGOs	12.3
Use manure	7.0
Contribute money to buy pesticides	5.3
No way to cope / no steps taken	3.5
Working in the greenhouse by self (taking time in the greenhouse)	3.5
Seek help from extension officers / contact specialists, technicians	1.8
Go for cheaper inputs	1.8
Developing wooden structure	1.8
Set funds aside for greenhouse management from Institution kitty	1.8
Cooperation from group members	1.8
Improvising the natural one	1.8
Use ITK	1.8
Own manufacture of planting bags	1.8
Support from spouse business	1.8
Total	**100.0**

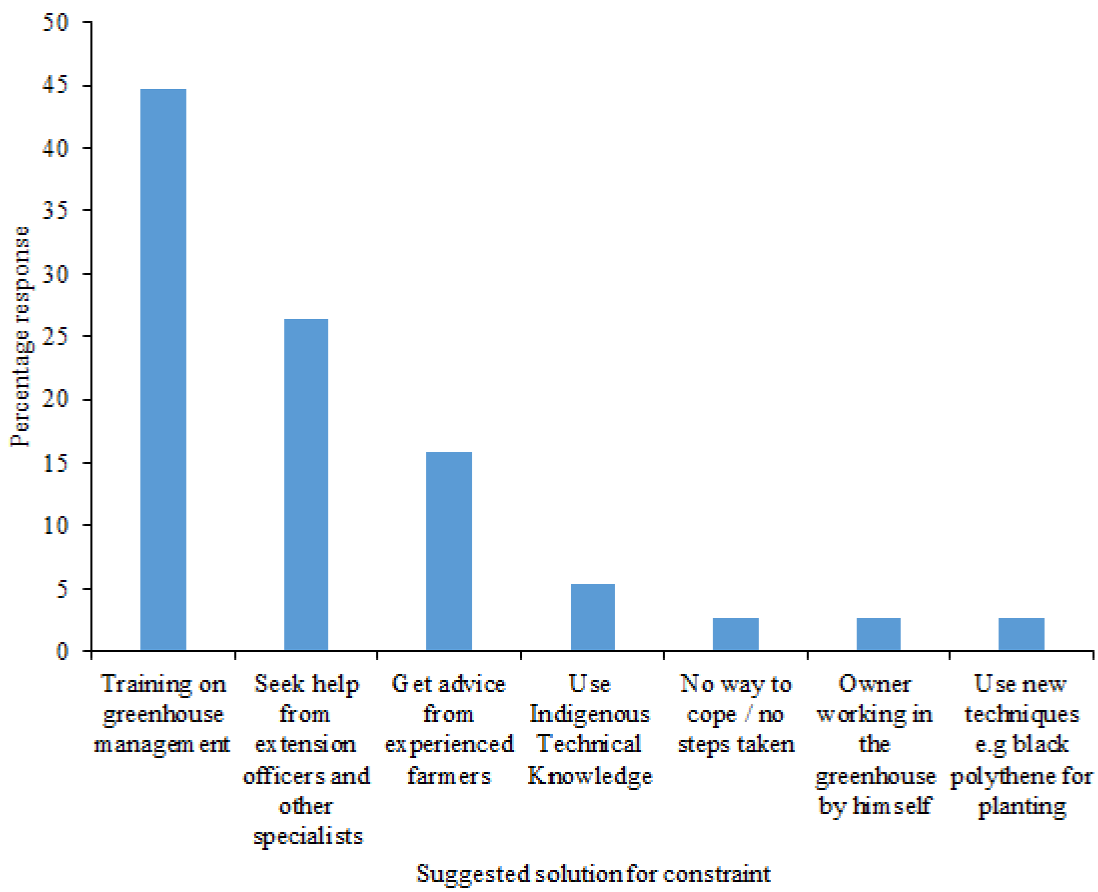

Figure 1. Suggested solutions for addressing the challenge of lack of information on greenhouse crop farming in Kisii County, Kenya.

could have resulted in low investment and running costs, but also compromised on efficiency of the greenhouses, as has been reported by previous studies (Baille, 1999; Nordey et al., 2017).

Conclusions

This study has shown that smallholder greenhouse farmers face several challenges and constraints, which prevent the farmers from realising full benefits from the technology. The main constraints include pests and diseases, inadequate supply of water, high initial costs for establishing and operating the greenhouses, and insufficient knowledge on greenhouse crop farming. Primary interventions for revitalising smallholder greenhouse farming include linking farmers to appropriate sources of funding to acquire the funds to construct and operate the greenhouses, and capacity building of farmers on greenhouse crop farming. Such trainings should ensure that the workers involved in greenhouse activities are invited, and not necessarily the owners of the greenhouses. This is important to ensure the knowledge gained is applied to improve the greenhouse production. There should be follow-up extension visits to farmers. There is also need to organise study tours for farmers to successful areas, especially large-scale horticultural farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Determination of solar energy requirements for indirect cooling combined with evaporative cooling for storage of fresh produce

Sipho Sibanda[1*] and Tilahun Seyoum Workneh[2]

[1]Agricultural Research Council, Institute for Agricultural Engineering, Pretoria, South Africa.
[2]University of KwaZulu Natal, School of Engineering, Pietermaritzburg, South Africa.

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A solar photovoltaic (SPV) system generating power to run a 53-m^3 storage for indirect air-cooling combined with evaporating cooling (IAC+EC) for providing a cool environment for storage of tomatoes under small-scale farming was evaluated. The experiment consisted of nine 330 W solar modules, twelve 230 AH Gel batteries, 145 VDC solar charge controller, 5 kW inverter, 290 W ventilation fan, 260 W water pump, psychrometric unit, and a 3.8-ton tomato storage chamber constructed and assembled on site. The psychrometric unit consisted of three-cooling pad layers and a 1760 W indirect heat exchanger. The solar modules were arranged in three series-three strings and were used in conjunction with a three string-48V bank facility. The performance evaluation of the system was conducted with full recirculation of air inside the storage chamber using solar module yield and efficiencies of inverter, battery and charge controller. Based on the experiment data the SPV system produced 2873.5 W that is 98% of the design power output at 80% probability of exceedance. The power yield of 2873.5 W was 24% higher than the power required in running the electrical appliances for IAC+EC system. Tracking the SPV system under ambient conditions with an average daily generation during the period of the experiment, the electrical power efficiency was 14.9%. The power output of modules increased with temperature of the module to 24°C and declined thereafter. The power generated by the SPV system depended on the solar irradiance availability, ambient temperature at the site and the time of the day. It was found that the SPV system could power the IAC+EC during daytime for the summer season, and the excess power stored in the battery could run the system until 22.00 h at night when temperatures were low enough for storage of tomatoes and SPV system was then switched off.

Key words: Small-scale farming, design power, theoretical power, efficiencies, actual power.

INTRODUCTION

Most of the industrial energy requirements that include the cold chain for fruit and vegetables (FV) use non-

*Corresponding author. E-mail: sibandas@arc.agric.za.

renewable energy sources for power generation resulting in greenhouse gas emissions (Charf et al., 2018). Grid electrical energy is a convenient form of energy source but is expensive and in some instances impractical especially for remote, dispersed populations with low and scattered energy demands (Cecelski, 2000). Cooling for FV is required in such areas and technologies like mechanical refrigeration, hydro-cooling, forced air-cooling and vacuum cooling exists and are potentially viable options (Prusky, 2011). However, such methods are expensive to small-scale farmers (SSF) because of high initial capital investments, high-energy input, and higher production volumes for economies of scale (Yahaya and Akande, 2018). It is therefore, necessary for SSF in remote areas to access appropriate low cost, low energy cooling technology for FV with better eco-sustainable characteristics. This study then considers adoption of an indirect air-cooling system combined with evaporative cooling (IAC+EC) which can work both in hot and dry climates and hot and humid areas through incorporation of an indirect heat exchanger (IHE).

Tolesa and Workneh (2017) investigated the effect of IAC+EC on the quality of stored tomato fruit over a 30-day period compared to ambient conditions. The tomatoes stored under IAC+EC showed higher firmness and hue angle, maintained lower concentration of sugars, had lower physiological weight loss and improved shelf-life and marketability as compared to ambient conditions. Sibanda and Workneh (2020) tested the performance of such a system and it provided storage conditions of temperature of 15.7 to 16.4°C and relative humidity of 89.6 to 93.8%. Both studies provided the efficacy of the system except the energy requirements. Incorporation of an IHE requires energy input that grid electricity could provide. However, in remote off-grid areas, renewable energy sources like solar can be utilised through use of stand-alone or hybrid solar photovoltaic (SPV) systems as they generate power with less maintenance and operational costs (Khatib et al., 2016). The integration of IAC+EC with solar energy in sub-Saharan Africa (SSA) where the average solar radiation is 4.5 to 6.5 $kWh.m^{-2}$ for 6 to 7 h a day can provide a cooling facility to SSF in areas with no access to grid electricity (Saxena et al., 2013). There is no information on energy requirements for such a system derived from actual performance data.

The sizing of stand-alone SPV systems depends on the efficiency of modules and other factors, such as ambient temperature, the quality and quantity of solar irradiance available in the location (Almarshoud, 2016). The efficiency of solar energy conversion depends on whether the module is monocrystalline, polycrystalline or thin-films type (Huang et al., 2013). Monocrystalline modules have the highest energy conversion efficiency; polycrystalline is in between, whilst thin-films are both least expensive and efficient in comparison (Bai et al., 2016). For solar arrays to produce maximum power output, they must be at an optimal tilt angle to trap maximum radiation (Tripathy et al., 2017). The optimal tilt angle depends on the season and the latitude of the area (Kaddoura et al., 2016). For higher power output, incorporation of solar trackers allows automatic adjusting of the collector tilt angle to follow the sun's change in elevation during the day (Pedro et al., 2016). In this study for cost reduction purposes, the optimum tilt angle was determined from historical data as provided by Schulze et al. (1999). The aggregate sun-oriented radiation received at a given geographical location varies depending on the length of the insolation on a specific day, the power of sunlight-based vitality, and the day or time of the year (Safa et al., 2016; Tripathy et al., 2017).

There is limited literature on sizing SPV systems for cooling; however, there are several published papers on utilisation of solar energy for water pumping applications for irrigation. There are various methods for sizing the SPV array for water pumping, some are simple, others in between while others are complex. One such simple method considers the monthly average of daily solar insolation to determine the associated peak sun hours (Hankins, 2010). Almarshoud (2016) summarized other simpler methods from literature on practical methods for SPV array sizing based on the peak sun hours. Other research work used the monthly average of daily global solar radiation and sunshine hours to size the SPV array for each month of the year, and then selected the highest values to increase the reliability in low radiation months. There are methods that are not simple but in between in sizing the SPV system required to operate a water pump like use of monthly average solar radiation on tilted surfaces of the worst month of the year (Munzer et al., 2013). Some authors have used the worst monthly average of solar radiation in addition to the maximum monthly water demand for sizing (Abidin and Yesilata, 2004). In other instances, the worst daily collectable solar energy in addition to the system efficiency has been applied (Campana et al., 2013). Complex methods include development of simple algorithms based on the monthly average of global horizontal irradiance only, while more complex approaches use dynamic programming to size the SPV array based on the average of solar energy available per day, taking into account all relevant elements of SPV pumping system (Zvonimir and Margeta, 2007; Kaldellis et al., 2007). Eltawil and Samuel (2007) sized a vapour-compression refrigeration system for storage of potatoes by estimating the battery capacity, the average load energy and the ampere-hour required per day, from the load profile. Instead of using the peak rated power of the modules at standard operating conditions they used several complex multiple regression equations to predict the SPV panel power output and its temperature in addition to energy consumption.

This paper proposes a simple and accurate approach for sizing the SPV array using the actual power requirements of the electrical accessories. When an IAC+EC system starts operating accessories like the water pump and heat exchanger will draw high current so, the SPV array must be able to provide the required current. The proposed approach will, integrate the simple approaches mentioned earlier; use the solar radiation data measured in Pietermaritzburg (PMB) over 50 years by Schulze et al. (1999) and the actual solar radiation measured in this experiment for the month of June and September. The accurate sizing of the SPV array is essential, because under sizing will make the IAC+EC system unable to power the electrical accessories resulting in failure to provide optimum storage conditions for fresh produce. On the other hand, over sizing of SPV array leads to unnecessary cost incurred in acquisition of additional modules and batteries (Almarshoud, 2016). Specifically, this study will provide actual data on the performance of SPV in powering a 3.8-ton (53 m^3) sized storage chamber for tomatoes. The main objective of this study is to design, assemble and evaluate the performance of SPV-battery based IAC+EC system.

MATERIALS AND METHODS

Experimental study

To determine the actual power requirements to operate an IAC+EC system, a case study for Pietermaritzburg, in South Africa was chosen. The IAC+EC system was used for cold storage of tomatoes over a period of 28 days in the summer month of September. The SPV system to power the IAC+EC system consisted of solar panels, solar charge controller and inverter, as well as a battery bank facility. This system was constructed and assembled on site at Ukulinga research station at the University of KwaZulu Natal, in PMB. The site is located at 30°24'S, 29°24'E at an altitude of 721 m. The SPV system and a battery bank facility provided electrical energy to the IHE, centrifugal pump and fans to facilitate airflow across the storage chamber of the IAC+EC.

Indirect air-cooling combined with evaporative cooling system set up

The IAC+EC consisted of a storage chamber, IHE, multiple charcoal cooling pads, buried water tank, a pump and two fans as shown in schematic diagram of Figure 1. The storage chamber had white double-jacket walls and a roof of 1 mm zintec (mild steel) both on the outside and inside. The floor was made of concrete mortar. The inner dimensions of the unit were 2340 mm high × 5880 mm long × 3880 mm wide giving a storage chamber volume of about 53-m^3 with a holding capacity of 3.8 tonnes of tomatoes. The cooler had a 60 mm zinc wall thickness with 58 mm polyurethane insulation in between the zintec layers. The IAC+EC system design specifications provided environmental conditions of dry bulb temperature of 14 to 20°C, relative humidity of 89 to 94% and cooling efficiency of 88 to 96% depending on the time of the day.

Solar photovoltaic system set up

Nine solar panels were connected in three series-three strings arrangement in conjunction with a with a three-string series 48 V battery bank facility (batteries of 230 AH) were used to power the electrical appliances. The characteristics and dimensions of the modules are shown in Table 1 while Figure 2 shows the solar panels and battery connections.

The solar array system was designed to provide enough power to operate electrical appliances of the IAC+EC system. These included a 1730 W Lytron indirect heat exchanger (M14-120) with 33 W fan (OW354) to facilitate airflow across, a 290 W second fan (6/P3HL/25/PA) ventilating the storage chamber and 260 W centrifugal water pump (Pedrollo SPVm 55) to reticulate water through the cooling pads. From 08.00 to 17.00 h, the SPV system powered the electrical appliances and thereafter the system was powered by battery bank facility. The hybrid SPV system was optimized by considering the number and sizes of modules as well as batteries required and balancing that with the system voltage and current. A number of combinations were considered as recommended by Goel and Sharma (2017). From these permutations, a three-series-three-string connection was chosen as it gave the highest output of 3503.8 W and did not overload the available solar charge controller.

The battery capacity was determined with reference to the electrical appliances specifications for the daily watt-hours at 50% discharge using a 48-V system and the available battery in the market, which was a 230 AH with a 90% efficiency. The number of batteries required to operate the IAC+EC system with 3.8 tons of tomatoes was determined as twelve. The total load from the electrical appliances was determined as 2343 W and the allowable battery discharge was 28116 W and such a system would produce 4196.4 W. h^{-1} if the sunshine hours are 6.7 to cool 3.8 tons of tomatoes.

The solar modules were dusted and dirt removed from the surface following which they were installed away from trees and buildings on a fixed rectangular metal manual tilt-frame and mounted facing south on an inclined angle of tilt = -15° (Sun et al., 2016; Ronoh, 2017). In order to optimize solar radiation, the tilt angle varied at ±15° to the latitude of the area. In the design four different tilts: horizontal (0°) and tilt angle -14.6006, -29.6006 and 44.6006° were considered and from calculations the highest insolation was obtained at tilt angle of -14.60°. Therefore, a tilt angle of tilt = -15° was chosen to determine the optimal power and energy output. The DC power generated from the SPV modules was transmitted to the solar charge controller prior to charging the solar batteries and thereafter the inverter converted the generated DC power to AC power. Figure 3 is a schematic layout of the solar system and shows how the rest of the components were connected. The solar charge controller (60A, 145 VDC, SANTAKUPS PC16-6015F) ensured constant voltage and current to the load from the batteries according to Deveci et al. (2015). The chosen sinewave inverter (125A, 5 kW) matched the system in terms of voltage input, AC power output, frequency and voltage regulation (Chandel et al., 2015). Twelve fully charged 230 AH batteries, which were arranged as a three-string series 48V system, were used to start up the SPV system. These batteries also temporarily stored energy generated by solar panels for overnight use.

Determination of theoretical design power and energy

Monitoring solar radiation data for PMB took place during the year 2018. However, for this study, only data for June and September was used since PMB receives the least amount of solar radiation in June while September was the month of the experiment. Data recording occurred at the research site using South African Weather Services – Agricultural Research Council (SAWS-ARC) weather station based at the research site. The solar radiation data was used to determine the actual performance of the SPV system. The solar radiation values recorded by Schulze et al. (1999) over 50 years' for PMB were extracted to obtain values for solar radiation received at 80% probability of exceedance at different tilt angles. These values were used to determine the theoretical power. The theoretical power represents the power available to run the

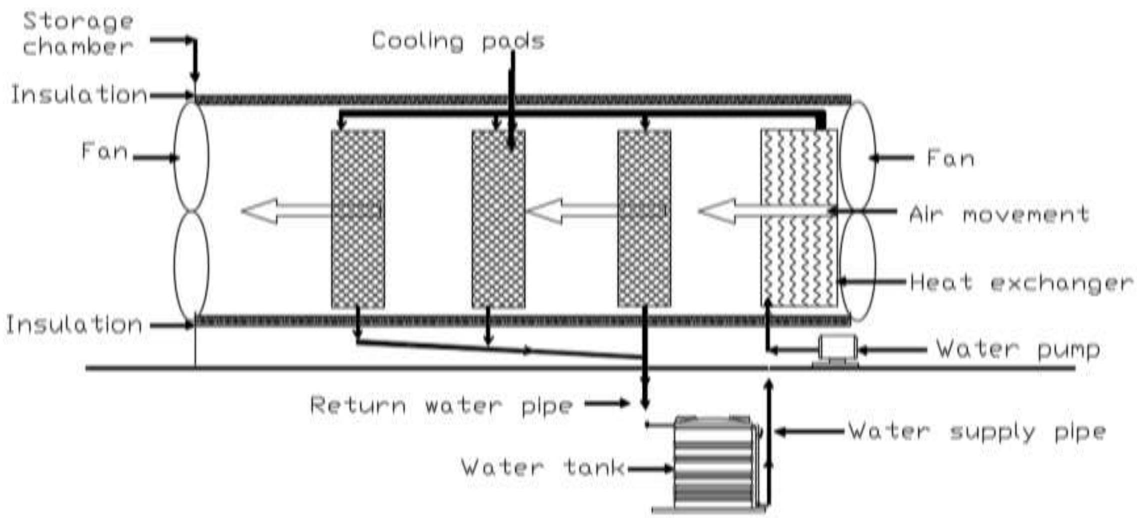

Figure 1. Schematic diagram the solar energy process flow.

Table 1. Monocrystalline solar panel (SETSOLAR) specifications under STC of insolation of1000 W. m^{-2}, the cell temperature at 25°C and air mass at 1.5.

Nominal power	350 W
Maximum power (STC) Pmax	36.6 V
Rated current I_{mpp}	8.2 A
Minimum power	330 W
Short circuit current (Isc)	8.7 A
Open circuit voltage (Voc)	44.8 V
Efficiency of panel	15-19%
Dimensions of panel	2.01 m × 1.02 m

electrical components of the IAC+EC system at the design stage. Equations 1 and 2 were used to determine the output power and energy based on the average theoretical design solar radiation for the two months at different tilt angles. The power output from Equation 1 was used to size the solar modules.

$$P_{out} = \eta_{panel} \times G \times A_{panel} \times N_{panels} \quad (1)$$

where P_{out} = average monthly power output (W), η_{panel} = overall SPV module efficiency (=0.1522); N_{panels} = number of SPV modules, A_{panel} = area of the module, G = solar radiation (W.m^{-2}).

$$E_{produced} = \frac{P_{out} \times D_1}{N_{panels} \times A_{panel}} \quad (2)$$

where $E_{produced}$ = energy produce on a day length D_1 (Wh. m^{-2}) and D_1 = average monthly day length (h).

The theoretical power out of the SPV is the input power of the solar charge controller. Equation 3 determines this power by incorporating the efficiency of the charge controller.

$$P_{out} = \eta_{controller} \times P_{in} \quad (3)$$

where P_{out} = power output from controller (W), η_c = efficiency of the charge controller from the supplier (90%) and P_{in} = power input to the charge controller.

The output power from the charge controller is the input power to the inverter. The output power of the inverter was calculated by incorporating the efficiency of the inverter. The power output from Equation 4 should be higher than the power requirements of electrical appliances.

$$P_{out} = \eta_I \times P_{in} \quad (4)$$

where P_{out} = power output from inverter (W); η_I = efficiency of the inverter from the supplier (90%) and P_{in} = power input to the inverter.

Performance evaluation

Measurement of parameters

On the days of the experiment (1 to 30 September 2018), the solar modules supplied the energy requirements during the day from 08.00 to 17.00 h. Thereafter, the battery bank supplied energy until 22.00 h when the system was switched off until 08.00 h of the following day as ambient temperature had fallen between 18 and 20°C. Fresh produce like tomatoes can tolerate such ambient temperatures for a short time. Therefore, there was no need for further cooling overnight time as recommended by Punja et al. (2016). The solar irradiance and ambient temperature for the period of the experiment was obtained at SAWS-ARC weather station located at the Ukulinga Research Station in PMB. The values were used to determine solar irradiance power using equation 4. The other meteorological parameter data like the temperature of the solar panels was measured using an infrared thermometer with K thermocouple (Fluke 63). Thermocouples connected to data loggers measured the SPV module temperatures at hour intervals to determine the influence of module temperature on the performance of solar systems (Sun et al., 2016).

Measurement of module current and voltage at different points

For the SPV electrical system, there were four positions (Figure 4) identified to evaluate the performance of the solar array system. A

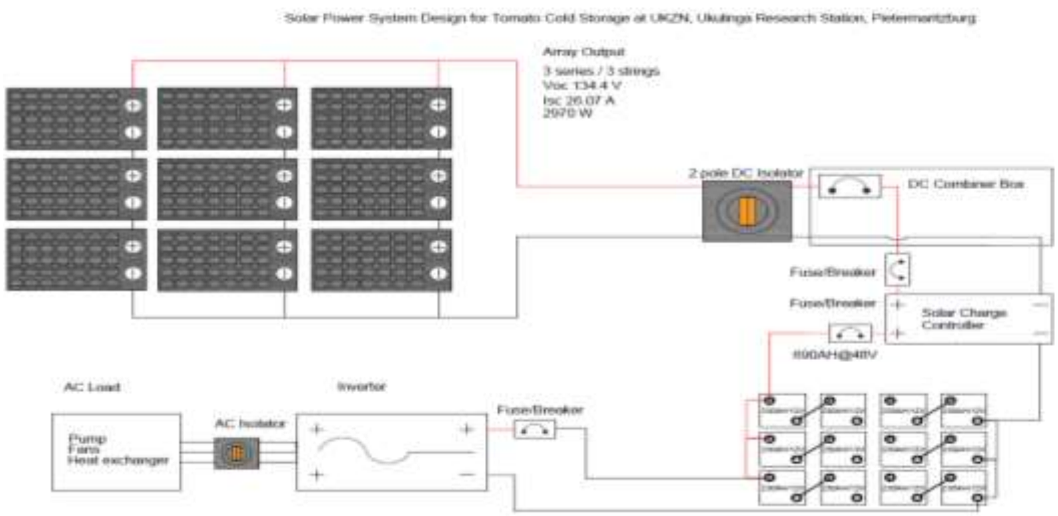

Figure 2. Solar photovoltaic system and battery bank facility for the evaporative cooling system.

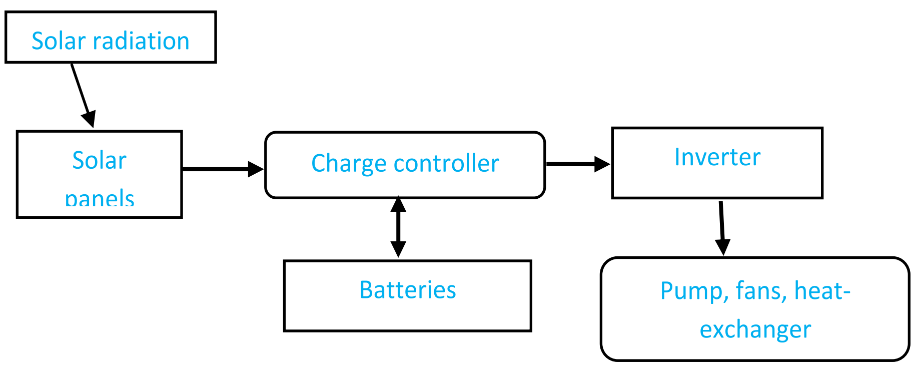

Figure 3. Schematic diagram the solar energy process flow.

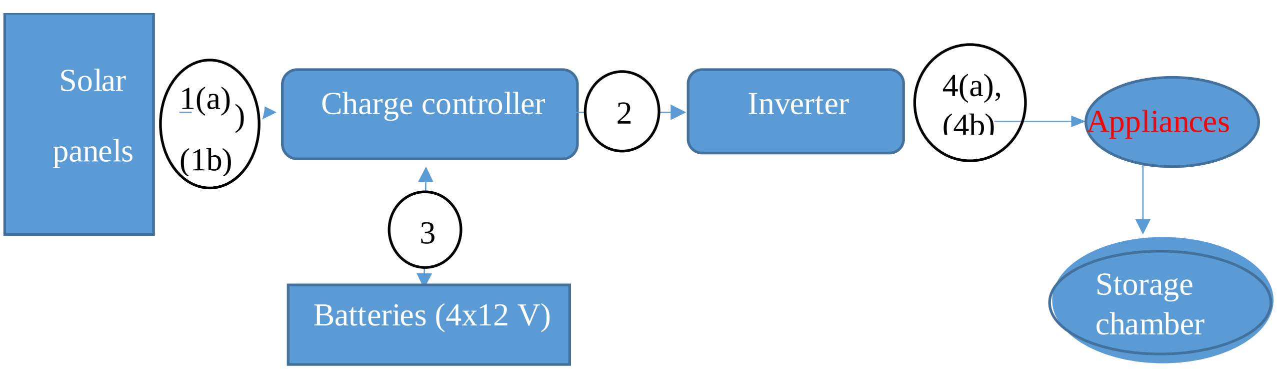

Figure 4. Schematic diagram showing points of measurements of current and voltage.

digital multi-meter (Fluke 381) measured both open circuit voltage and current under these different positions. Ohms law (Equation 5) was used to determine power:

$$Power = VI \tag{5}$$

where V = voltage (v) and I = current (A).
The test procedures to be followed were:

(The power output tests were done by measuring both the voltage and current at different points and these values were used to

Table 2. Probability of exceedance of a monthly solar radiation for June and September.

Month	CV	Tilt angle	Exceedance probability solar radiation (W.m^{-2})			Maximum power at 80% exceedance probability
			20%	50%	80%	
June	15.10	+15^{o}	565.07	548.94	522.81	1468 W
September	8.00	-15^{o}	1 199.90	1 102.71	1047.49	2941.7 W

calculate the power output using the Ohm's Law.
The test procedures followed were:

(a) Measurements at position 1 of the system (exit point of solar panels [1a] and the input side of the solar charge controller [1b]). The voltage and current measured at this point were used to calculate solar modules power output and was compared with the theoretical calculation of the power output from the solar modules;
(b) Position 2 measures both voltage and current at the exit of the charge controller and the input of the inverter.
(c) Position 3 read voltage and current to and from the batteries, and
(d) Position 4a read current and voltage between the inverter and heat exchanger, pump and fans.

Measurements at this point provide how much power the appliances draw. Position 4b reads the current and the voltage drawn by electrical appliances.

RESULTS AND DISCUSSION

Theoretical power and energy

The performance of SPV systems depends on the tilt angle and orientation of the array. In studying the effect of insolation on modules a solar tracking device helps in adjusting the position of the solar panels so that the highest possible energy output obtains compared to a fixed SPV system. This necessitates that installations of the modules be at an optimal tilt angle that maximizes the solar radiation captured by SPV panels. Solar radiation data for PMB from Schulze et al. (1999) and from SAWS-ARC weather station was collated at tilt positions +15° for June and -15° September to determine probability of exceedance which information was used to calculate theoretical power and energy. It is important to base the design of a SPV system on a higher chance that the expected solar radiation will occur or exceeded in the given period and therefore a probability of exceedance is considered. The solar radiation data at 80% probability of exceedance at different tilt angles data for PMB was considered and only data for maximum theoretical output was extracted. Table 2 summarises the data of the probability of exceedance of monthly solar radiation for June and September. From Table 2 at 20% of the time in each month there was a higher radiation received in PMB than in 50 and 80% of the time. As the exceedance probability increased, the amount of radiation received decreased. Relatively lower percentages were recorded at high irradiance levels and the converse is true. The high irradiance levels, are associated with a direct beam component, that is spread more widely with very small individual frequency percentages. For the purpose of calculation, 80% exceedance probability was used, as the values are closer to reality as is possible. From Table 2, the average optimal solar radiation received at 80% probability of exceedance in PMB in June and September were 522.81 W.m^{-2} at tilt = +15° and 1047.49 W.m^{-2} at tilt = -15°, respectively. The optimal power for the months of June and September in PMB are 1468 and 2 941.7 W, respectively and from Equation 2, this translated to optimum energy output of 628.5 and 1068.1 Wh. m^{-2} for the two months, respectively.

The theoretical power and energy were low in June because solar insolation levels were low. To generate adequate energy under such circumstances would require more solar modules and this would increase the cost of installation. The sizing of stand-alone SPV considers meeting electrical loads requirements with lowest average daily solar insolation on the array surface, which usually occur during winter months. However, the temperatures are also generally low in winter (June), and the maximum temperatures are 16 to 20°C in PMB. Under such conditions for tomatoes and many tropical and sub-tropical FV in SSA, either no cooling or minimal cooling will be required during short periods as alluded to by Punja et al. (2016). To ensure optimization of the solar insolation, a switch could be incorporated to the system coupling the electrical load (pump, fans and heat exchanger) to the SPV array system. This will allow the SPV system to switch off when the battery bank facility is fully charged. Optimising the system is important, as the costs of installation are reduced to ensure the IAC+EC systems translate to a low cost cooling technology (Chandel et al., 2015; Goel and Sharma, 2017).

The theoretical power output for the month of September is very significant for design as this month is the beginning of summer and higher solar radiation is received in subsequent months until April of the following year when temperatures begin to fall. In subsequent months, the theoretical power output is higher as the area receives more solar irradiation and this coincides with higher cooling loads as the ambient temperature is also relatively higher. This is the reason why most of the large-scale SPV systems are built in arid and semi-arid areas, where the solar insolation levels are high (Sayyah et al., 2014). However, caution has to be taken as high ambient temperature affects performance of the SPV

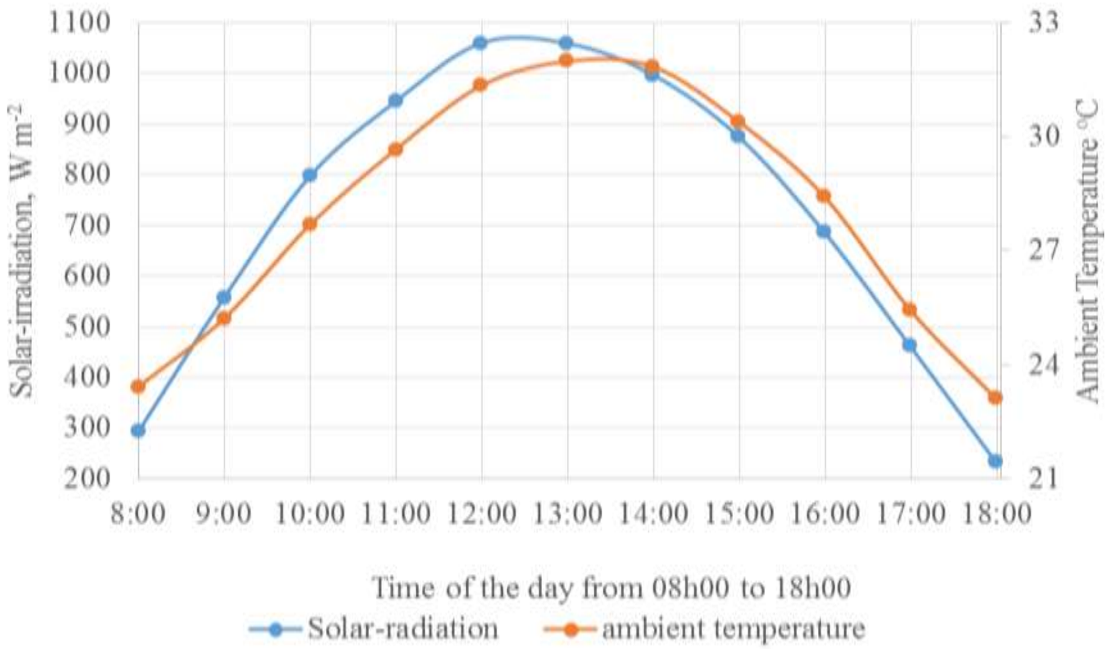

Figure 5. Variation of solar radiation and ambient temperature during the month of September.

system due to high cell temperature (Ronoh, 2017).

From Equation 3, the theoretical output power from the charge controller was:

$$P_{out} = 0.9 \times 2941.7 = 2647.6\ W$$

in September. From Equation 4, the optimum output power of the inverter is $P_{out} = 0.9 \times 2639.7 = 2382.8\ W$ in September. This means that the theoretical power available to run the electrical components at 80% probability of exceedance in September in PMB was 2382.8 W.

Variation of insolation with ambient temperature

Ambient air temperature and solar radiation outside the IAC+EC system around the SPV system was studied in the month of September 2018. It was observed that ambient temperatures and solar irradiance were low in the morning and increased from 08.00 h to between 12.00 to 14.00 h and thereafter decreased towards 18.00 h (Figure 5). Ambient temperature increased due to increasing incident solar radiation from morning until afternoon 13.00 to 14.00 h and then decreased from then onwards towards sunset as also confirmed by Madhava et al. (2017). The average insolation values rose from 293.4 W.m^{-2} at 08.00 h in the morning to 1 059.6 W.m^{-2} at mid-day. Eltawil and Samuel (2007) observed a similar trend. At any location like PMB, the length of the path the radiation takes from source to ground level varies with time of the day as the spectrum of the radiation changes through each day because of the changing absorption and scattering path length (Ronoh, 2017). Figure 5 relates to data obtained on clear days of September where the solar insolation increased from early morning to a peak at midday and then decreases to zero at night. The peak is achieved at midday as the sun is overhead and its path length is shortened. At midday, less solar radiation is scattered or absorbed by atmospheric mediums, and more radiation that is direct reaches the modules compared to any other time of the day and Olomiyesan et al. (2015) complements these results. The highest average solar irradiance received in September 2018 of 1 059.6 W.m^{-2} was slightly higher than the average value of 1 047.9 W.m^{-2} received over 50 years at 80% exceedance for the same month. This implies that the average insolation received in this month should produce enough power and energy for the designed electrical appliances.

Solar photovoltaic module power and solar irradiance power

The SPV module power (P_{module}) and solar irradiance power ($P_{irridiance}$) were studied in the month of September on clear and, sunny days selected (11 days) for the experiment. The P_{module} was obtained by measuring voltage and current between the solar modules and solar charge controller while the $P_{irridance}$ values were obtained by converting solar irradiance values in Figure 5 to power by using Equation 1. Figure 6 shows the variation of

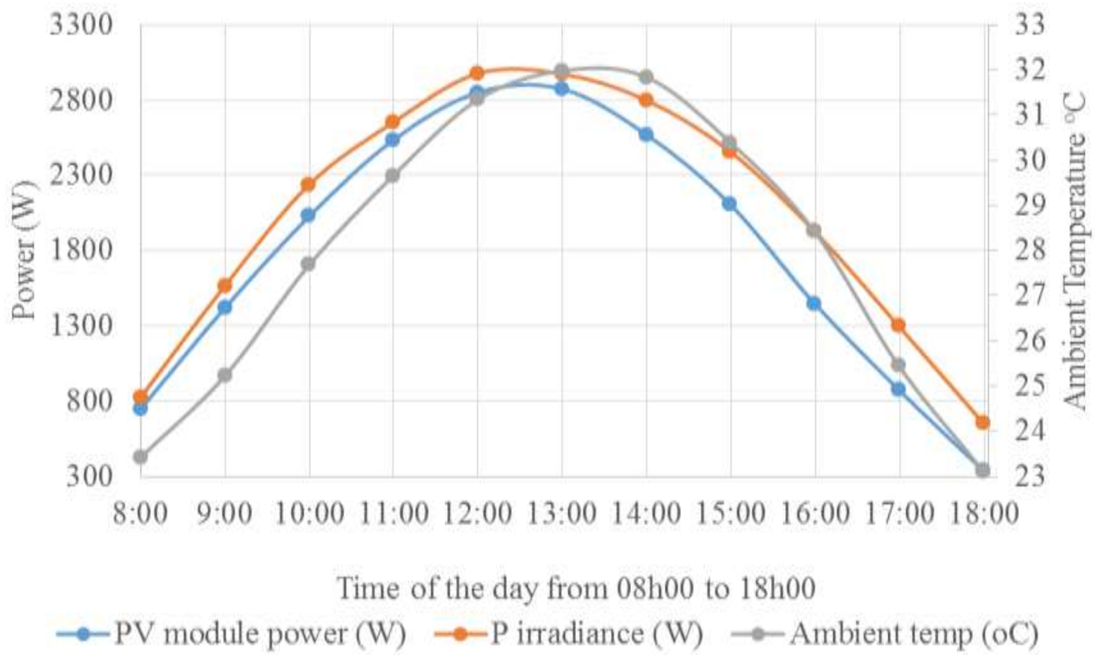

Figure 6. Variation of module power and solar radiation with time during the month of September.

P_{module} (W) and the $P_{irridance}$ (W) during the period of study from 08.00 to 18.00 h. The P_{module} increased with the $P_{irridance}$ to a peak between 12.00 and 14.00 h and decreased thereafter as the solar radiation intensity decreased. The results from the present study agree with findings of Charfi et al. (2018) in Tunisia who obtained similar trends. The $P_{irridance}$ received and P_{module} output had very similar trends with the maximum and minimum values at the same hours during the period of the experiment. This shows that the amount of electricity generated by SPV system is largely depended on the availability of the solar energy at a particular location as corroborated by Li et al. (2005) and Chaabane et al. (2019). The design power which is the maximum average solar power received in PMB over 50 years in the month of September at 80% probability of exceedance is 2941.9 W (Table 2) while the peak $P_{irridance}$ in the month of September 2018 was 2975.4 W (Figure 6). Therefore, $P_{irridance}$ was just 1% higher the design power. This shows the importance of basing the design on higher percentage of probability of exceedance.

The peak P_{module} was 2 873.5 W, which was about 3.5% lower than the peak $P_{irridance}$ of 2975.4 W during the period of the experiment. The difference between the P_{module} and the $P_{irridance}$ is attributable to the efficiency of the SPV system that peaks at 14.9% (Figure 8) which value was lower than the rated solar panel efficiency of 15 to 19%. The other contributors are environmental factors including module temperature, soiling material accumulating on the module surfaces, resistance in the wiring and connections and in some instances, modules of the same type have slight differences in electrical characteristics. Ghazi et al. (2014) mentioned that solar modules need regular cleaning as soiling is regarded as one of the significant contributors to reduction of the power output of SPV systems as it reduces the solar radiation reaching the surface of modules. When modules are soiled, the dust particles deposited on the surface absorb and scatter the incoming incident light and this might have contributed to the reduction of the P_{module} value (Sayyah et al., 2014). The peak P_{module} of 2873.5 W was 24% higher the design load for electrical appliances of 2343 W. However, it is important to note that the efficiencies of the controller and inverter could account for 24% more power generated by the modules.

Solar photovoltaic module parameters and ambient temperatures

The P_{module} output and module temperature increased with ambient temperature to about 32°C (Figure 7), which coincided with the highest ambient temperature at midday. The maximum P_{module} output occurred at 31 to 32°C ambient temperature (Figure 6) and the system was most efficient at this period (Figure 8). Thereafter both power output and ambient temperature declined after midday as shown in Figure 5. Ya'acob et al. (2014) made similar observations in Malaysia where they had the highest generated power data at midday with ambient temperature at 32.5 to 34.5°C. The P_{module} output increased as short circuit current increased with insolation due to the increase in the number of photons generating the current. Increased solar panel temperature

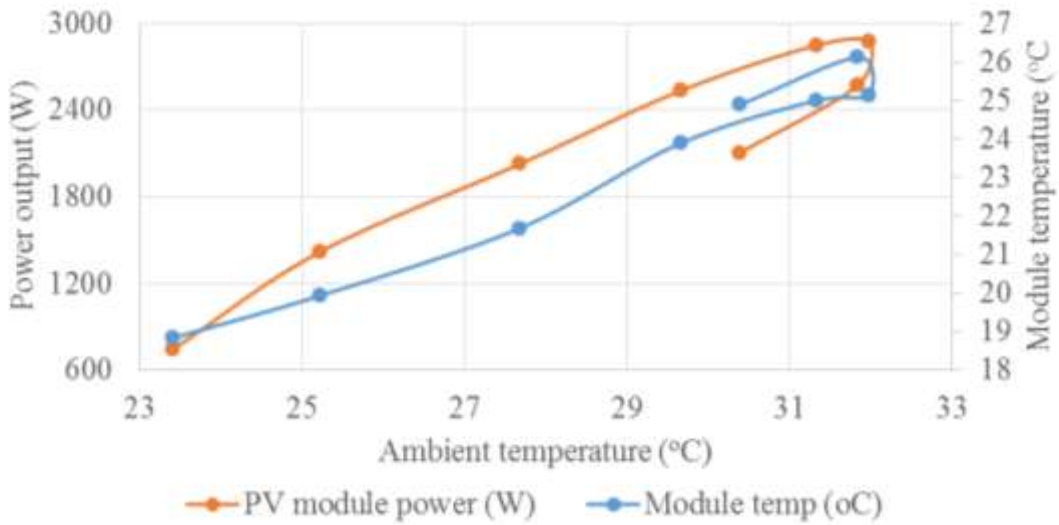

Figure 7. Variation of power output with temperature of the solar panels.

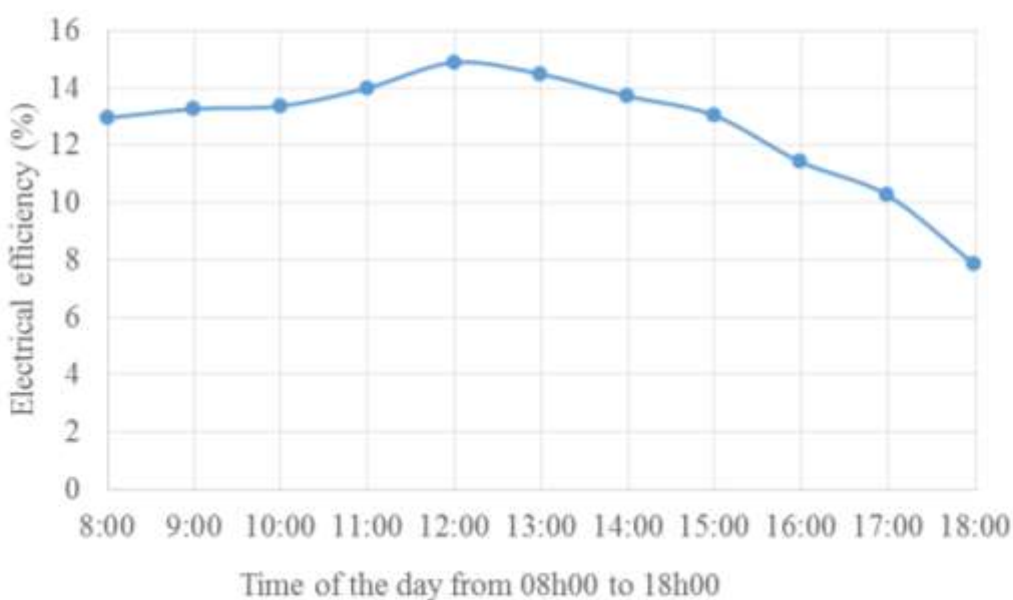

Figure 8. Variation of electrical efficiency with time of the day.

also increases the kinetic energy of the photons resulting in increased current and module output. Figure 7 shows that P_{module} increased with module temperature until 25°C and decreased thereafter.

This corroborates the work done by Bai et al. (2016) and Chaabane et al. (2019) which showed that though solar panels are designed to operate in the presence of the sun, high heat reduce panels' capacity to generate power. The increased SPV module temperature arose from high insolation heating and high ambient temperature. When the module surface temperature increases beyond a certain level, the atoms in the material vibrate resulting in a reduction in the conductance of the electron traveling through the electrical component (Olcan, 2015). Many standard grade solar panels have 0.5 to 1% less photoelectric conversion efficiency for every 1°C SPV module temperature increase above 25°C (Rawat and Kumar 2013; Bai et al., 2016).

In Figure 8, it is clearly visible that the electrical efficiency increased up to noon due to the positive effect of the solar cells temperature during this period of the day and the converse is true beyond midday. It is noted that the efficiency of the system was below the 15 to 19% given by the manufacturer, and Ya'acobs et al. (2014)

Table 3. Current and voltage at different locations of Figure 3.

Position	Current (A)	Voltage (V)	Power (W)
1a	21.82	131.7	2 873.7
1b	21.82	130.7	2 851.9
2	20.17	130.1	2 624.1
3	20.16	127.3	2 566.4
4a	18.43 *	205 *	2 403.3 **
4b	22.30	129.3	2 883.4

*The current and voltage are alternating. **A factor of 0.636 was used to convert AC power to DC power.

explain that efficiency will vary once modules are bundled in series or parallel and under different climatic characteristics from those the manufacturer tested under. According to Rawat and Kumar (2013), high incident radiation increases the surface temperature of solar cells and that in turn decreases the photoelectric conversion efficiency of cells.

Performance of the solar photovoltaic system

The peak P_{module} of 2873.5 W translated to 5146.6 $W.h^{-1}$ actual energy produced by the solar modules. This was the actual produced and stored by batteries in order to cool the 3.8 tons of tomatoes from 17.00 to 22.00 h. Therefore, to cool one ton of tomatoes, using IAC+EC system requires 1354.4 $W.h^{-1}$. This value is comparable to the value of 700 $W.h^{-1}$ for forced air evaporative cooling of tropical FV using a 0.1 HP fan mentioned by Kitinoja and Thompson (2010). The difference in power requirements can be attributable to the additional IHE and water pump incorporated in this study. The power requirements for the solar powered IAC+EC system were low when compared with hydro-cooling (immersion type) to 0 to 2°C or hydro-cooling (shower type) to 7°C where the energy required to cool 1 metric tonne of produce is 35 to 150 kWh.

Solar energy is one of the major sources of renewable energies available in SSA and SPV are currently utilised in many agricultural applications. For this study the SPV system of 9 modules (3-series 3 string) of 330 W each and a 48-V battery bank of 3-strings 230 AH batteries was able to supply the appliances with the needed electrical power and provided sufficient energy to charge the battery bank. Non-passive evaporative cooling can be viewed as a cooling technology with low initial investment and installations costs as a system of 1 to 2 MT can be constructed for US$1,300 at an energy use per MT of 0.7 kWh (Kitinoja and Thompson, 2010). However, the cost of a solar-powered 3.8 tons IAC+EC system requiring about 4.7 kW of energy input is as high as US$7500 with solar power system alone costing at least 80% of the total (Sibanda, 2019). The costs of an IAC+EC system compares well with a small-scale mechanical refrigeration system with a storage capacity of 2 tons that requires about 7 kW of electricity and has a slightly high cost of US$8,500 (Kitinoja and Thompson, 2010). Therefore, optimal sizing of SPV systems in order to supply load demand is important because of high capital investment costs of solar energy powered IAC+EC systems.

Performance evaluation of the electrical components of the design

There were four major tests to evaluate the performance and assess the electrical components of the design for the 3-string 3-series solar module system and three-string 48 V battery system. Table 3 provides the values of the current and voltage measured at difference locations in Figure 3. Equation 5 (Ohm's law) was used to determine the power input/out at the different locations.

The current and voltage measured at the exit point of the solar modules and at the entrance point of the solar charge controller were used to determine the percentage voltage drop through the SPV cables as follows:

$$V_{drop}\,(\%) = \frac{131.7 - 130.7}{130.7} \times 100\% = 0.8\%$$

This practical voltage drop as calculated provides reasonable efficiency of operation occurrence as the voltage drop is less than 3% as defined by Early et al. (2014).

Table 3 shows that the power from modules into the charge controller was 2851.9 W and that the average current and voltage supplied by the solar to the batteries were 20.16 A and 127.3 Vdc, respectively. Therefore, the battery bank facility had enough power to start up the SPV system and to operate the IAC+EC system from 17.00 to 22.00 h at evening to nighttime. The input power to the converter was 2624.1 W which was converted from DC to AC. The AC current and voltage measured at position 4 between the inverter and the load was 18.43 AAC and 205 VAC providing a DC power of 2403.3 W. Therefore, the power supplied by the invertor is enough to run the electrical appliances that include the heat exchanger, water pump and two fans.

Efficiencies of the designed system

The solar panel efficiency is calculated from the relationship between current and the voltage measured between the solar panels or batteries and the charge controller and theoretical power output of the solar panels. The current and voltage drawn by the load from the batteries through the inverter were 22.3 Adc and 129.3 Vdc giving a DC power of 2883.4 W (Table 3).

$$\eta_{solar\ panel} = \frac{P_{measured}}{G \times A \times N} \times 100\% = \frac{2883.4}{1059.6 \times 2.0502 \times 9} \times 100\% = 14.7\%$$

The efficiency of the solar panels was 14.7% as solar cells have a threshold photon energy corresponding to the particular energy band gap below which electricity conversion does not take place. Photons of longer wavelength do not generate electron-hole pairs but only dissipate their energy as heat in the cell. However, most common SPV module converts 4 to 17% of the incoming solar radiation into electricity as explained by Chow (2010). The reasons an efficiency less than 15% was obtained could be that solar modules work best when module temperatures is below 25°C. Higher ambient temperatures of about 32°C increase the module temperature and that could cause a slight increase in the electrical current as the semiconductor properties of solar cells shift, resulting in a much larger decrease in voltage as alluded to by Bai et al. (2016). Some solar panels may produce as much as 1% less electricity for every -9.44°C temperature above 25°C. The other reason why there is a variation could be that the peak annual accumulated output is calculated using the SPV module efficiency under a reference sunlight of irradiance 1 000 W.m^{-2} with a solar cell temperature of 25°C. In reality, solar radiation at a location varies with the weather conditions; season and time of day, as a result the technical information provided for STC might not occur in practice.

Conclusion

The use of SPV systems is increasing as installations costs are decreasing and the application is finding expression in remote and isolated communities and in new farming setting ups of SSF with no access to cooling facilities. Electricity supply is of great concern, as it is inadequate and in SSA, connection to the national grid for most SSF seems highly unlikely in the near future. This has turned interest to renewable energy sources like solar as a means of bridging the energy gap and providing environmentally friendly energy. In this study, a SPV system IAC+EC was evaluated based on actual performance. Furthermore, this experiment explored the possibility of integrating solar energy to power IAC+EC system targeting SSF in remote areas with no access to grid electricity.

Most of the literature does not give actual values of energy required by different cooling systems, but merely states which cooling systems are more energy intensive than others are. Energy required to operate modern cooling systems are greater than the energy required to operate IAC+EC system. The SPV systems used in the study supplied energy during the critical period of the day when temperatures were high from 08.00 to 22.00 h. To cool one ton of tomatoes using IAC+EC requires 1354.4 W. h^{-1} and the batteries had to store 5146.6 W. h^{-1} to provide energy for the 3.8-ton storage chamber to cool tomatoes from 17.00 to 22.00 h when the IAC+EC system was switched off. The efficiency of the solar panels was 14.7%. The energy to power an IAC+EC system relates to the size of the solar array system required to provide the energy and the cost of the system. The study concludes that combinations of the solar array system can power the cooling system at daytime during summer season and the excess energy can be stored in the battery to run the system for another five hours into the night. A bigger system is required to run all-night and in the near future, this will be possible, as the prices of modules continue to decline.

Therefore, where grid electricity or other commercial energy sources are unavailable and solar energy is available, IAC+EC system is a viable alternative to these more complex and costly modern day cooling systems. This shows that stand alone SPV systems have an expression in rural, dispersed and remote areas where grid electricity supply may not be readily accessible. Integrated solar and indirect evaporative cooling is an attractive alternative for SSF with no access to cooling technologies in developing countries, especially in Africa.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Field evaluation of lentil germplasms for their resistance to Ascochyta blight (*Ascochyta lentis*) under field conditions

Asrat Zewdie* and Bekele Gemachu

Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center, P. O. Box 32, Bishoftu, Ethiopia.

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Ascochyta blight caused by Ascochyta lentis is a fungal disease with a major importance in Ethiopia. It has the potential to cause appreciable reduction in yield. The present study was conducted to identify the sources of resistance in lentil to ascochyta blight in 2018 main cropping season in hot spot area in AlemTena research station. The total of 148 accessions were received from Ethiopian Biodiversity Institute were grown in augmented design without replications that only checks were replicated. The spacing was 20 cm between rows with 4m row length. The disease severity was recorded three times at different growth stage every seven to ten days intervals using (1-9) point disease ratings scale. There were high variations in resistance among the tested lines ranged from resistant to highly susceptible. It compares that about 22 were resistant, 58 were moderately resistant and other become susceptible to highly susceptible which is 56 and 11 lines, respectively. In comparison, there are promising lines to use as source of parental materials in which most of the released cultivars lacking the resistance. A wide range of variation to ascochyta blight disease reaction was observed among lentil genotypes. More resistance resources need to be identified to back up breeding programs.

Key words: Ascochyta blight, *Ascochyta lentis*, genotypes, resistance.

INTRODUCTION

Ascochyta blight, caused by *Ascochyta lentis*, is one of the most globally important foliar disease of lentil. It has been reported to be a major lentil disease in many lentil-producing countries, including Argentina, Australia, Canada, Ethiopia, India, New Zealand, Pakistan and the Russian Federation (Sheikh et al., 2010). The disease has a potential to cause appreciable reduction in yield foliar infection up to 40% yield losses (Gossen and Morall, 1984). The disease has considerable effects on both seed quality and yields (Cromey et al., 1987). *A. lentis* is specific to cultivated and wild species of lentil (Hernandez et al., 2006; Tullu et al., 2010). It is morphologically indistinct from *Ascochyta fabae* but the latter is unable to infect lentil species. *A. lentis* populations are highly variable in terms of aggressiveness on different lentil cultivars and wild accessions (Davidson et al., 2016). Movement of the host germplasm has disseminated the pathogen worldwide where it is primarily introduced to new sites through infected seed (Kaiser and Hannan, 1986; Nasir and Bretag, 1997b; Khan et al., 1983; Hawthorne et al., 2012). Despite extensive agronomic and chemical control

*Corresponding author. E-mail: asratzew@gmail.com.

studies, no efficient method has been devised to control ascochyta blight in lentil. The use of resistant varieties is an environmentally sound and a feasible option for resource poor farmers (Erskine et al., 1994). Many resistant cultivars/lines have been identified in both cultivated and wild lentil. The genetics of resistance to Ascochyta blight coming from Lens orientalis was first reported by Ahmad et al. (1997). Resistance to Ascochyta blight in lentil is mainly under the control of major genes, but minor genes also play a role (Ye et al. 2002). However, none of the varieties that have been released, for production in low potential areas, are not having good level of ascochyta blight resistance. Resistances identified so far in lentil crops against the ascochyta blights provide only incomplete protection (Ford et al., 2011). Resistance breeding in lentil crops has been slow due to the complex nature of resistance and the relatively low investment on genetics, genomics, and biotechnology of legume crops. However, continuous cultivation of relatively few resistant cultivars with narrow genetic base has likely led to episodes of resistance breakdown through selection of adapted and aggressive isolates (Davidson et al., 2016; Sambasivam et al., 2017). Breeding for resistance has been suggested as an efficient means to reduce the economic loss caused by ascochyta blight in lentil (Erskine et al., 1994; Ye, 2000). These resistances are mostly polygenic traits controlled by quantitative trait loci (QTLs) (Rubiales and Fonde Villa, 2012). Quick shifts in aggressiveness of the population of the causal agent *A. lentis* mandates developing germplasm with novel and durable resistance (Sari et al., 2018). Moreover, the efficiency of resistant in lentil cultivars in Ascochyta blight is limited by pathogenic variability in the natural populations, location-specific occurrence of races which causes resistant cultivar to lose resistance over a period of time, breakdown of resistance, which is a consequence of directional selection for better-adapted mutants, recombinants or immigrants and also by widespread and intense deployment of R genes favored by monoculture practices (Suhas et al., 2006). Also, available information on levels of resistance and on the responsible mechanisms is often incomplete. The disease appears regularly in alarming epidemic form in main season especially in early planting season. The present study was to evaluate the lentil lines for resistance to ascochyta blight.

MATERIALS AND METHODS

Field experiments were conducted during 2018 main cropping seasons at AlemTena (8°18'24.4''N, 38°57'05.3''E and 1610 m.a.s.l) and the average annual rainfall is about 728 mm and the maximum and minimum annual mean temperatures are 29.8 and 12.9°C, respectively, and the relative humidity ranges between 67 and 83% which is the sub centers of Debre Zeit Agricultural Research Center under natural infested field in hot spot area (DZARC, 2018). The number of entries was 148 lentil accession were obtained from Ethiopian Biodiversity and evaluated for their reaction to ascochyta blight disease that had grown in augmented design without replications that only checks were replicated. Ten test entries after one cultivar of susceptible check (Teshale) is sown. The spacing was 20 cm between rows with 4m row length. After germination, observation was recorded regularly for the appearance of ascochyta blight and severity. The disease severity was recorded three times at different growth stage every seven to ten days intervals using (1-9) point disease ratings scale. Evaluation of resistance has generally been performed at the seedling stage, 11 to 28 days after infection, although Gupta et al. (2012) co-assessed resistance in both the seedling and mature pod-bearing plant. Final disease assessment was made on whole plants when discrimination of disease reaction between susceptible and resistant plants was distinct (Ford et al., 1999). One observation was made from each seedling. The subjective 1 to 9 disease index used by previous researchers (Nasir and Bretag, 1997a; Ford et al., 1999; Sambasivam, 2011) was modified by specifying a size limit of small lesions and percentage leaf drop.

The scores were: 1 = no visible disease symptoms; 3 = leaf lesions only, chlorosis of affected leaves, < 10% leaf drop; 5 = leaf lesions, up to 25% leaf drop, stem flecks or lesions < 2 mm; 7 = leaf lesions, up to 50% leaf drop, stem lesions > 2 mm; 9 = leaf lesions, potential defoliation, stem girdling, potential plant death.

Test genotypes were further categorized for their reaction to AB infection on the basis of Gowen et al. (1989) scale, according to this scale; 1-<2 = Highly resistant (HR); 2<4 = resistant (R); 4-<6=moderately resistant (MR); 6<7= moderately susceptible (MS); 7-<9= susceptible (S); and 9-10=highly susceptible (HS).

RESULTS AND DISCUSSION

It was provided that 22 lines were resistant, 58 were moderately resistant, 56 were susceptible while 11 were highly susceptible to the ascochyta blight in the field (Figure 1). The 22 accessions (36144, 242603, 244614, 27879, 221720, 36044, 36048, 23754, 23971, 28747, 235015, 244603, 242604, 24175, 241132, 237987, 23898, 36124, 23970, 244628, 36159, 244635) were resistant to *Ascochyta lentils* (Table 1). None of the lines was found immune against the disease. Erskine and Bayaa (1991) found 30 accessions of lentils with strong resistance reaction. Similar finding was reported by Iqbal et al (1990). Of 152 cultivar those genotypes tested, 17 were highly resistant, 40 were resistant, 34 had an average level of resistance and the rest were susceptible which is in concurrent with (Singh et al., 1982). This supports similar findings in the Canadian study (Ahmed and Morrall, 1996) and is in broad agreement with the theory that the resistance that plants deploy against ascochyta blight is polygenic. Moreover, many valuable resistant resources have been identified by germ plasm screening in cultivated and wild lentil species (Morrall and Sheppard, 1981; Singh et al., 1982; Kapoor et al., 1990; Erskine and Bayaa,1993; Bayaa et al., 1994). Ye et al. (2001a) found that the gene conferring high resistance in "ILL 5588 is allelic to that in ILL 5684. Of the 139 entries tested, none was categorized in highly resistant, 35 were in resistant and 22 were in moderately resistant (Seid and Beniwal, 1991). There was a varied reaction to the disease among the test entries, ranging from resistant to susceptible. High disease pressure and better disease resistant selection intensity was observed in the cropping

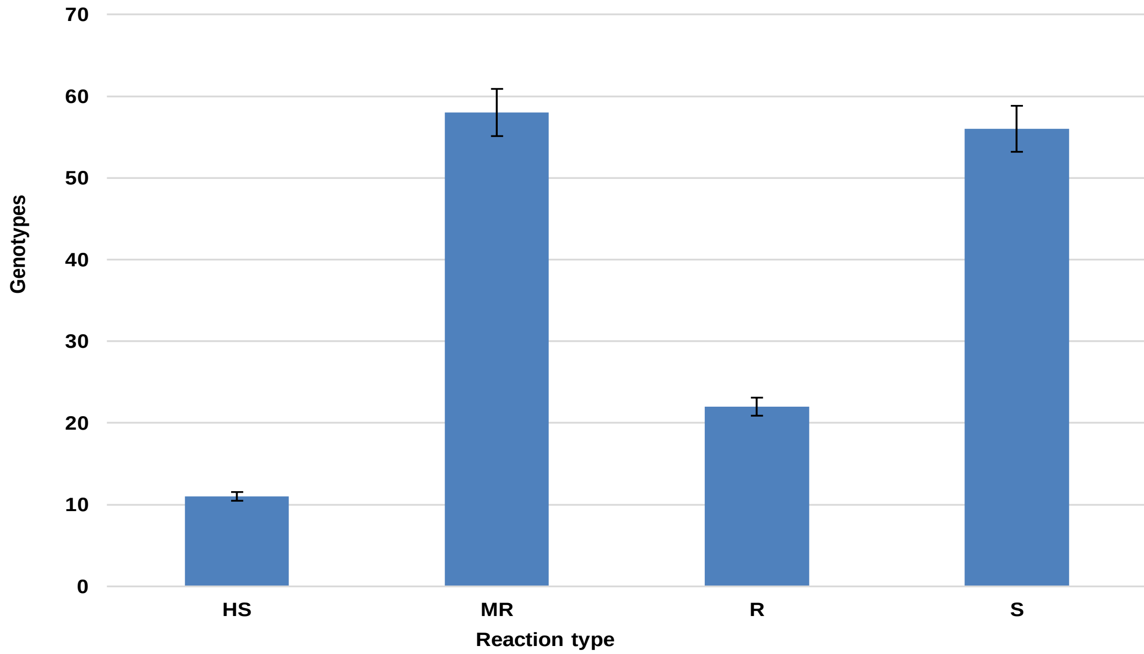

Figure 1. Reaction type of genotypes to ascochyta blight disease R= Resistant, MR= Moderately resistant, S= susceptible, HS= Highly susceptible).

Table 1. Reaction of Ascochyta blight diseases on lentil accessions.

Disease reactions	Accessions
Resistant	36144,242603,244614,27879,221720,36044, 36048, 23754, 238971,28747,235015,244603, 242604,241785, 241132, 237987,238988,36124, 238970,244628,36159,244635
Moderately resistant	36156,243434,36090,244607,244611,28747,230837,244617, 243444,204621,233973,36139,242605,243440,236456,28745, 245447,235384,237024,244615,243436,15345,243432,244629, 36007,28747,36087,220120,236889,19066,244606,243435, 244616,244623,244612,244605,244610,36089,244624,238991, 36086,36087,244631,243438,15317,237502,36013,236484,238987, 235016,36016,36092,36120,243441,223221,36027,238977,237502
Susceptible	36033,243449,243443,36003,36014,36072,237503,238987,235013,244625, 235014,36007,235011,243445,244627,36008,235382,244608,36032,36018,18007, 36058,238980,244618,244609,36007,244626,241784,236487,238974,235385,36091, 244620,24178,36012,243437,236890,243439,238990,36015,243446,244608,22918, 239184,243437,15346,243433,208758,238989,36045,28745,230015,244604, 238979,36005,244622,
Highly susceptible	22322,244627,244803,230656,28748,235012,36043,230016,243448,36046,244613

season.

The pathogens require most effort to be achieved durable resistance and so breeding effort should concentrate on quantitative resistance which is renewed regularly to stay ahead of the pathogen (Cowger and Mundt, 2002; Pariaud et al., 2009). So far, released cultivars were becoming susceptible to ascochyta blight. The rapid loss of resistance in released cultivars indicates there may be one or more major genes for resistance that have been rendered ineffective by changes in the pathogen population. Wild species have the potential to be an important source of resistance to ascochyta blight in lentil, compensating for the comparatively low intraspecific variability that is the characteristic of domesticated lentil species (Abo-elwafa et al., 1995; Tullu et al., 2010).

However, in addition to changes on specific hosts there is an apparent continuum of aggressiveness among the *A. lentis* isolates when assessing the mean reaction across the entire host set. Screening in controlled conditions with field observations indicates that isolates aggressive to cultivar have become more frequent and widespread in the *A. lentis* population, possibly as a selective response to the widespread presence of this cultivar in the farming system (Banniza and Vandenberg, 2006). The resistant lines of the germplasm evaluated could further be tested for their yield potential or these may be used as source resistant parents to transfer their resistance into commercial cultivars lacking resistance.

Detailed understanding of the genetics resistance to *A. lentis* is essential for the successful future deployment of ascochyta blight resistance in lentil lines.

The identification of highly significant differences in disease reactions between specific isolates against specific cultivars in the phenotyping experiments provides opportunity for further study into the genetic differences involved. The use of resistant varieties against this pathogen is the most practical and cost-efficient individual disease control measure for management of Ascochyta blight of lentil. Intensive cropping of single cultivars can lead to loss of resistance by selection for aggressive isolates that are already present in the naturally variable population.

Conclusion

Ascochyta blight, caused by *A. lentis*, is an important disease of lentil in Ethiopia. Due to the continuous accumulation of new pathotypes there is constant need to evaluate new varieties using different methods against virulent ascochyta blight for sustainable agricultural practices. Therefore, under present study identified new sources of ascochyta blight resistance in some genotypes. The use of resistant cultivars was the most efficient and economical for controlling the disease. A wide range of variation to disease reaction was observed among lentil accessions in field evaluation. The local germplasm has the adaptability genes and carries the advantage over exotic genetic material for use in the breeding program to develop varieties for high yield potential with wider adaptability and disease resistance against ascochyta blight pathogens. Intensive cropping of single cultivars can lead to loss of resistance by selection for aggressive isolates that are already present in the naturally variable population. The identification of highly significant differences in disease reactions between specific isolates against specific cultivars in the phenotyping experiments provides opportunity for further study into the genetic differences involved. For this reason, resistance to ascochyta blight has been considered a priority with a significant amount of research and breeding effort put into accessing and introgression sources of resistance to *A. lentis*. Large-scale screening of germplasm for resistance is required. It is necessary to evaluate the resistance several times and to test against different isolates. More resistance resources need to be identified to back up breeding programs. Moreover, use of resistant cultivars would enhance the efficacy of other disease control measures in an integrated management strategy. It is suggested that breeding programs should be used on crossing the identified resistant lines with high yield cultivars, multi-location testing and genotypes may be used directly as potential lentil varieties in area having low severity of disease. For this reason, resistance to ascochyta blight has been considered a priority with a significant amount of research and breeding e ort put into accessing and introgression sources of resistance to Ascochyta lentis. Large-scale screening of germplasm for resistance is required.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic diversity analysis and construction of selection index of selected tomato genotypes

Mahmudul Hassan[1], Basudeb Roy[2], Nurhasan[3], Kawsar Ahmed[4] and Omar Kayess[5*]

[1]Department of Agricultural Extension, Ministry of Agriculture, Chirirbandar, Dinajpur, Bangladesh.
[2]Department of Agriculture Extension, Ministry of Agriculture, Khansama, Dinajpur, Bangladesh.
[3]Department of Agriculture Extension, Ministry of Agriculture, Kaharole, Dinajpur, Bangladesh.
[4]Department of Agriculture Extension, Ministry of Agriculture, Bochagonj, Dinajpur, Bangladesh.
[5]Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

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To study the genetic variability and selection index of selected tomato genotypes, an experiment was conducted at the farmer's field of Chirirbandar Upazilla of Dinajpur District, Bangladesh. Ten tomato genotypes were evaluated in a randomized completely block design with four replications. The study results displayed high heritability plus higher genetic advance for all the traits studied. Among the traits, plant height showed high genotypic and phenotypic coefficients of variation over other traits. Yield per plant alone had 100% expected genetic gain and the relative effectiveness of the other functions was calculated in like manner. Selection indices exhibit eight traits association (yield plant-1 with plant height, canopy width, days to flowering, fruit diameter plant, fruit length, single fruit weight), and therefore the number of marketable fruit is simpler for genetic improvement thanks to the most expected genetic gain (665.23) with maximum relative efficiency (125.88%). Based on the selection index value and total rank value for selection criteria, the eight-traits combined with V6 and V10 are the promising genotypes that can be used as potential gene donor for further breeding program.

Key words: Genetic variability, selection index, tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a member of Solanaceae family (Sharma et al., 2019) and belongs to the genus Lycopersicum (Mital et al., 2016). It is generally accepted that tomato originates within the new world (The America), that is the Andean region comprising Bolivia, Chile, Colombia, Ecuador, and Peru (Blanca et al., 2012). In Bangladesh, tomato is growing with the overall production of 385 thousand metric tons (BBS, 2018). In the face of higher yield potential, the potentiality of Bangladeshi tomato genotypes is comparatively lower than other tomato grower countries (Ali et al., 2014). Due to the rapid growing population of the country as well as

*Corresponding author. E-mail: kbdkayess@yahoo.com.

their rapid change in food habits, it is desired to have a higher yield per unit area to meet the increasing demand for tomato. For the improvement program of any crop it is important to have the knowledge of the relationship between yield and yield contributing traits. To select breeding materials and to formulate breeding programs, a comparative study of various quantitative traits especially those associated with the yields is helpful for plant breeders.

The genetic variability in the existing materials is the prime source of effective selection for high yielding types. For effective selection, an assessment of the magnitude of genetic variability and its interaction with environmental variables is very useful for a plant breeder. Enough genetic variation is needed for an effective plant breeding program for the selection of better types. Careful selection and hybridization may help the plant breeder to obtain lines higher in yields with better quality. For the effective selection, genetic variability can offer an opportunity for high yielding tomato variety rich in fruit quality. To determine the most valuable genotypes along with the most suitable combination of traits selection index helps with the intention of indirectly improving yield in different plants (Sabouri et al., 2008; Rezai and Yousofi, 2008).

The individuals and progenies undergoing the selection process are classified by the selection index. The selection index is proposed by maximizing the correlation between the indexes itself and aggregating the genotypes (Smith, 1936; Hazel, 1943). For selection, construction of selection indices and the analysis of the phenotypic values of each of two or more traits to be used simultaneously would give the most appropriate weightage. That is why this research was performed to apply the selection index which combines the genotypic and phenotypic values of different yield contributing traits of tomato genotypes that could enhance the genetic improvement for yield and also to select promising genotypes.

MATERIALS AND METHODS

Experimental site

The research work was conducted at the farmer's field of Chirirbandar Upazilla of Dinajpur, Bangladesh, from October 2017 to April 2018 in the Rabi season. The experimental field was located at 25.130 N latitude and 88.230 E longitudes at an altitude of 37.5 m above the mean sea level.

Soil and climate

The experimental land was medium-high. The soil belongs to the old Himalayan Piedmont Plain of the Agro-Ecological Region (AEZ-1). The initial soil (0-15 cm depth) test revealed that the soil contained 0.10% total nitrogen, 1.06 organic matter, 24 (µg/g) available Phosphorus, 0.269 (meq/100g) available Potassium, 3.2 (µg/g) available sulphur and 0.27 (µg/g) boron. The experimental area possesses a sub-tropical climate. Usually, rainfall is heavy during the Kharif season (March – September) and scantly in Rabi season (October – February).

Experimental layout and planting materials

The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. The layout of the experiment was prepared for distributing the genotypes into every plot of each block. The individual plot size was 12 m × 1 m. Each replication contained 10 plots. Each plot contains 15 plants. The distances between the rows were 1m and plant to plant distances were 0.80 m. The distance between the block was 1m. There were 528 m^2 plots in this experiment. Each of the tomato genotypes was produced in the 2017-2018 cropping season, and the purity with germination percentage was leveled as around 98 and above 95, respectively. The experimental materials were collected from the BARI (Bangladesh Agricultural Research Institute) and local market of Chirirbandar upazilla. For this research the genotypes were designated as V1, V2, V3, V4, V5, V6, V7, V8, V9, and V10.

Production procedure and data collection

The plot selected for the experiment was opened in the first week of November 2017 and it was partitioned into the unit plots in accordance with the experimental design. Bangladesh Agriculture Research Council (2018) recommended doses of manure and fertilizers (Cow dung, Urea, Triple Super Phosphate, Murate of Potash, Zinc sulphate and Gypsum at 5000, 150, 50, 75, 25 and 5 kg ha^{-1}) were mixed with the soil. At the time of final land preparation, the whole amount of well-decomposed cow dung, Triple Super Phosphate, Muriate of Potash and half amount of urea were applied to the field. The rest half of the urea was applied in two installments (15 days after transplanting and a week before flowering) in equal quantity as ring placement method. The seed was sown on 12 October, 2017 and transplanting was done at in the afternoon of 12 November 2017 when the seedlings were 30-day old. Watering was done for seven days. Various intercultural operations like irrigation, gap filling, weeding, mulching, stacking with pant protection measures were done as per necessary. Depending on variable maturity time harvesting continued for about one month because fruits of different genotypes matured progressively at different dates and over a long time. The fruits per entry were allowed to ripe and then seeds were separated from them and then the seeds were collected from them for future use. Data on different yield and yield contributing traits were recorded on the plot and plant basis as per the experimental requirement. Data were recorded; ten (10) plants from each unit plot were selected randomly in replication and were tagged as individual plants. The traits plant height, canopy width, days to flowering, fruit length, fruit diameter, number of marketable fruits, and yield per plant were recorded in the field and the single fruit weight, shelf life, and thousand seeds weight were recorded in the laboratory after harvest.

Data analysis

The genotypic and phenotypic coefficient of variation was calculated according to Burton (1952). Broad sense heritability was estimated by the formula of Lush (1940) that was suggested by Hanson et al. (1956) and Johnson et al. (1955). The expected genetic advance for various traits under selection was estimated using the formula suggested by Lush (1940) and Johnson et al. (1955). For calculating the genotypic and phenotypic coefficient of correlation for all possible combinations the formula suggested by Hanson et al. (1956) and Johnson et al. (1955) was adopted. For

Table 1. Different genetic variability parameters of ten traits of the selected tomato.

Traits	Genotypic variance	Phenotypic variance	Genotypic coefficient of variation (%)	Phenotypic coefficient of variation (%)	Heritability (%)	Genetic advance	Genetic advance (%)
Plant height (cm)	650.10	665.12	30.11	30.17	98.53	52.90	62.01
Canopy width (cm)	130.42	138.19	21.30	22.91	97.41	24.01	47.32
Days to flowering	1.49	2.25	5.19	6.31	67.66	2.81	8.87
Fruit diameter (mm)	1.35	1.53	7.46	7.40	89.33	2.50	14.01
Fruit length (cm)	0.75	0.82	10.85	10.87	96.50	1.65	23.11
Single fruit weight (g)	26.11	26.81	14.41	14.49	93.10	10.11	28.02
Number of marketable fruits plant^{-1}	119.00	132.01	27.01	27.01	90.14	22.01	48.99
Thousand seed weight (g)	0.10	0.12	12.02	12.11	98.00	0.75	26.19
Shelf life (days)	3.71	3.86	18.00	18.01	97.14	3.49	34.00
Yield plant^{-1} (g)	10400.02	117139.31	22.00	22.01	87.11	530.66	33.02

creating selection indices R- Program version 3.2.2 was used.

RESULTS AND DISCUSSION

Genetic parameters of tomato genotypes

Significant differentiation was observed among the genotypes for all the studied traits. It exhibits the presence of greater genetic variation among the studied genotypes. At genotypic and phenotypic levels, the assessment of variance and coefficients of variation in a populace is a crucial factor for choice and efficacy of assortment of individuals for forthcoming breeding plan in crop species. Among the traits plant height (665.12), canopy width (138.19), number of marketable fruits plant^{-1} (132.01), and yield plant-1 (117139.31) displayed above 10% phenotypic variation so considered as high. On the other hand, fruit length (0.82), single fruit weight (26.81), thousand seed weight (0.12), and shelf life (3.86) exhibit a medium phenotypic coefficient of variation. Only days to flowering (2.25) and fruit diameter (1.53) showed lower phenotypic variation value. At genotypic level plant height (650.10), canopy width (130.42), number of marketable fruits plant^{-1} (119.00), and single fruit weight (26.11) showed higher genotypic variation but slightly lower than the phenotypic variation (Table 1).

A higher difference between Phenotypic Coefficients of Variation (PCV) and Genotypic Coefficients of Variation (GCV) indicates a high influence of the environment on the traits whereas low difference indicates the low influence of the environment on the traits. The values of phenotypic coefficient of variation and genotypic coefficient of variation greater than 20% are regarded as high, whereas the values less than 10% are regarded to be low and values between 10 and 20% to be medium (Srivastava et al., 1998). The fruit length (10.85), single fruit weight (14.41), thousand seed weight (12.02), shelf life (18.00) and yield plant-1 (22.00) exhibit moderate GCV value but minor than PCV value. Among the traits plant height (30.17), canopy width (22.91), number of marketable fruits plant-1 (27.01) and yield plant-1 (22.01) displayed above 10% phenotypic co-efficient of variation so considered as high. In contrast, hand fruit length (10.87), single fruit weight (14.49), thousand seed weight (12.11), and shelf life (18.01) exhibit a medium phenotypic coefficient of variation. Only days to flowering (6.31) and fruit diameter (7.40) showed lower PCV value. However, slightly higher estimation of GCV value than PCV value was found in relation to days to flowering (5.19) and fruit diameter (7.46). Generally quantitative or agronomic traits are influenced by the environment (Table 3).

In this study, the GCV values were little lower than PCV, displayed that the environment had a slightly important role for the expression of these traits or more specifically these traits varied due to the genetic makeup of the genotype itself. The next difference between PCV and GCV indicates the high influence of the environment on the traits whereas low difference indicates the low influence of the environment on the traits. The genetic advance is a valuable indicator of the development that can be projected as a result of exercising

Table 2. Selection indices for yield and the relative efficiency of the selected tomato.

Index	Expected genetic gain	Relative efficiency (%)
X_{10}	531.01	100
X_1+X_{10}	582.15	109.87
X_1+X_2+X_{10}	584.03	110.78
X_1+X_2+ X_3+X_{10}	568.13	107.23
X_1+X_2+ X_3+ X_4+X_{10}	560.21	105.99
X_1+X_2+ X_3+ X_4+X_5+X_{10}	611.22	115.81
X_1+X_2+ X_3+ X_4+X_5+X_6+X_{10}	571.13	107.91
X_1+X_2+ X_3+ X_4+X_5+X_6+X_7+X_{10}	666.23	125.88
X_1+X_2+ X_3+ X_4+X_5+X_6+X_7+X_8+X_{10}	642.99	121.05
X_1+X_2+ X_3+ X_4+X_5+X_6+X_7+X_8+X_9+X_{10}	506.23	95.88

$\mathbf{X_1}$ = Plant height, $\mathbf{X_2}$ **=** Canopy width, $\mathbf{X_3}$ **=** Days to flowering, $\mathbf{X_4}$ = Fruit diameter, $\mathbf{X_5}$ = Fruit length, $\mathbf{X_6}$ = Single fruit weight, $\mathbf{X_7}$ = Number of marketable fruits plant^{-1}, $\mathbf{X_8}$ = Thousand seed weight, $\mathbf{X_9}$ = Shelf life and $\mathbf{X_{10}}$ = Yield plant^{-1}

choice on the relevant population. The estimations of heritability perform as projecting tool in expressing the trustworthiness of phenotypic value. So, high heritability helps in effective choice for a particular trait. Again, heritability values greater than 80% are considered as very high, values from 60 to 79% are moderately high, values from 40 to 59% are medium and values less than 40% are low (Singh, 2001). All the traits under study showed higher heritability (>80%) except days to flowering which showed moderately high heritability (67.66%). How much variation of a trait due to genes within a population is compared to variation due to the environment is measured by heritability. The yield plant-1 (530.66) recorded the highest GA followed by plant height (52.90 cm), canopy width (24.01 cm), number of marketable fruits plant^{-1} (22.01), and single fruit weight (10.11 g). The lowest GA was observed in days to flowering, fruit diameter, fruit length, thousand seed weight, and shelf life.

The estimates of genetic advance as percent of mean were highest for plant height, number of marketable fruits plant^{-1}, canopy width, shelf life, yield plant^{-1}, thousand seed weight and fruit length. The rest of the traits showed lowest value of genetic advance in percent mean. It showed the predominance of additive gene action for governing these traits. Therefore, these traits can be developed only over selection. High heritability per low genetic advance was detected for thousand seed weight, fruit length and fruit diameter. It recommended non-additive gene action for the expressions of these traits. The high heritability was displayed because of promising influence of environment fairly than genotype and selection for such traits might not be satisfying. Low heritability together with high genetic advance was recorded for yield plant^{-1}. It exposed that the trait is directed by additive gene effects. The low heritability was being shown by reason of high environmental effects. Therefore, selection for this trait might be effective.

Construction of Selection Indices in tomato

With the corresponding integration of more number of traits in functions, the benefit of selection indices was increased. By using different combinations of yield and yield contributing traits different selection indices were formulated and their expected genetic grain and relative efficiencies were estimated (Table 2). Higher relative efficiency pointed improvement of the traits would be more efficient with the selection index. The expected genetic gain for yield per plant (531.01) and relative efficiency (100%) was estimated (Table 2). The relative efficiency in two and three traits combinations (109.87 and 110.78%) were increased very slightly. But at four and five traits association the relative efficiency slightly decreased from the previous combination. This may be due to the addition of variation of fruit diameter and fruit length. However, relative efficiency (115.01%) of six traits combination increased sharply indicating those traits could be improved by the breeding program (Table 2). Likewise in four and five traits association a decreasing trend of relative efficiency was also found in seven, nine and ten trait combinations indicating these combinations are less effective for improvement than other traits combination. Eight traits combination showed maximum relative efficiency (125.88%) overall the combinations. Again, a breeder may be optimistic to increase the present yield status under the selection of more traits in the function and also gradual relative efficiencies should be analyzed for each multiple function. In this research work, the maximum relative efficiency over direct selection was 125.88% in eight traits association for the construction of selection index. Therefore, selection index based on eight traits association (yield per plant with plant height, canopy width, days to flowering, fruit diameter plant, fruit length, single fruit weight and the number of marketable fruit) would be more efficient for improvement as maximum expected genetic gain

Table 3. Comparison among the selection criterions of the tomato genotypes based on the selection index with their total rank.

Generation	2 traits	3 traits	4 traits	5 traits	6 traits	7 traits	8 traits	9 traits	10 traits	Rank
V_1	8	8	8	8	7	8	8	8	8	71
V_2	7	7	7	7	6	7	7	7	6	61
V_3	5	6	5	4	5	5	5	5	3	43
V_4	2	2	2	3	3	3	3	3	1	22
V_5	9	9	9	9	9	9	9	9	9	81
V_6	1	1	1	1	2	2	2	2	2	**13**
V_7	3	4	4	5	4	4	4	4	4	36
V_8	6	5	6	6	8	6	6	6	5	53
V_9	4	3	4	4	5	3	3	3	7	37
V_{10}	1	1	2	2	1	1	1	1	4	**14**

(666.23) with maximum relative efficiency (125.88%) is suggested for the improvement of yield (Table 2). By comparing among relative selection criterion of all the traits of ten tomato genotypes based on the best selection index (yield per plant with plant height, canopy width, days to flowering, fruit diameter plant, fruit length, single fruit weight and the number of marketable fruit) with their total rank value V6 and V10 are the promising parents (Table 3).

Conclusions

The traits yield plant^{-1}, plant height, and canopy widths are attributable to belonging to additive gene, which demonstrates that advance in these traits is auspicious through hybridization followed by selection per pedigree breeding. The traits thousand seed weight, fruit length and fruit diameter with high heritability and low genetic advance representing the trait is inclined by environmental effects and selection may not be useful. Besides, by comparing among relative selection criterion of all the traits and based on the best selection index with their total rank value V6 and V10 are the promising parents those can be used for future hybridization program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The use of microchondrometer to assess test weight in small samples of triticales and oats

Lauro Akio Okuyama[1]*, Carlos Roberto Riede[1] and Man Mohan Kohli[2]

[1]Instituto de Desenvolvimento Rural do Paraná – IAPAR - EMATER, Caixa Postal 10030, CEP 86047-902 Londrina, Paraná, Brazil.
[2]Camara Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas (Capeco), Av. Brasilia 840, Asunción, Paraguay.

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The test weight or hectoliter weight is an important parameter used to classify the quality of the grain. Globally, it is evaluated by using 250, 500 or 1000 ml devices. Despite its importance, there is no standard equipment to assess it in small samples from the research plots. This study aims to test a newly developed microchondrometer (15.30 ml) in the triticale and oats. A second microchondrometer (31.26 ml) was also designed to be tested in oats. The performance of the two microchondrometers, their comparison and relationship with 250 ml commercial chondrometer were analyzed using the t-test and Pearson's correlation coefficient. The results revealed no significant differences between two microchondrometers or their relationship with 250 ml chondrometer by t-test ($p > 0.05$). Based on two-year evaluations, the correlation between the 250 and 15.30 ml was highly significant ($p < 0.0001$) for triticale (0.9873), and oats (0.9557 for 15.30 ml device and 0.9448 for 31.26 ml device). The correlation between 15.30 and 31.26 ml devices was also highly significant ($p < 0.0001$) for oats (0.9399). These results suggest that in small samples, the 15.30 ml microchondrometer can be used successfully in triticale and oats to assess its test weight.

Key words: Plant breeding, grain density, genotype screening.

INTRODUCTION

The test weight, or hectoliter weight, is an important indicator of grain quality in the milling industry. It is also used to classify the physical quality of cereal grains in international trade, where a test weight over 76 kg hl^{-1} is considered minimum for high quality wheat (Protic et al., 2007). For commercial purposes, a minimum test weight of 65.0 kg hl^{-1} is required for triticales (GTA, 2018a) and 52.5 kg hl^{-1} for oats (GTA, 2018b). Test weight analysis is also conducted in other crops such as millet, pulses, fiber, fodder, oilseed, and green manures (Deivasigamani and Swaminathan, 2018). Besides genetic differences among crop varieties for test weight (Ilker et al., 2009;

*Corresponding author. E-mail: lauro2288@gmail.com

Iqbal et al., 2016; Mut et al., 2018), it is also affected by environmental factors (Isleib, 2012; Joshi et al., 2018; Silva et al., 2019; Awulachew, 2020).

Manley et al. (2009) describe two types of devices to measure test weight. The first is equipped with a funnel to provide a uniform packaging in a measuring cup of 500 ml (in South Africa and Canada) or 1100 ml (in the USA). The second is a 500 ml chondrometer used in the United Kingdom and Australia or of 1000 ml chondrometer used in Germany and France. For experimental purposes, a small chondrometer (250 ml) has been used in wheat (Stagnari et al., 2008; Durazzo et al., 2015; Botelho et al., 2018), in triticale (Messia et al., 2012; Redaelli et al., 2015; Kuzmanovic et al., 2020) and in oats (Nava et al., 2010; Buerstmayr et al., 2007; Martinez et al., 2010; Da Silva et al., 2015).

The triticale improvement program at the International Wheat and Maize Improvement Center, CIMMYT, Mexico, emphasizes to select genotypes with higher grain density and test weight (Mergoum et al., 2004). A high genetic diversity and stability for grain production and test weight in triticale were observed by Barnett et al. (2006). They believe them to be essential traits for any breeding program to develop cultivars with high yield and test weight, as well as adaptation to a wide range of production environments. Considerable genetic diversity for the test weight in several geographical areas has also been reported by Đekić et al. (2018), who suggested that it is possible to select for specific environments.

In oats, the test weight was positively correlated with the groat percentage and had the highest heritability values among the grain quality characteristics (Buerstmayr et al., 2007). They concluded that selection for better physical appearance of the grains should result in higher test weight, thereby contributing towards the development of oat cultivars that combine higher test weight and earliness. Doehlert and Mcmullen (2008) reported that test weight has a major impact on the monetary value of the oats. They revealed that approximately 78% of the price variation was attributed to the grain density and the remaining to the packaging efficiency, which is the proportion of the container occupied by the grains.

Despite its importance, no specific device has been built to measure the test weight in small samples from studies conducted in greenhouses, plant nutrition, pre-harvest sprouting and crop breeding. Decades ago, Aamodt and Torrie (1934) emphasized the need to determine test weight in small samples of wheat and developed a method by cutting a 25 cm^3 graduated cylinder at the 4 cm^3 point. Harris and Sibbitt (1941) cut a graduated cylinder at the appropriate height (4 and 16 ml) to determine the test weight. Ghaderi et al. (1971) obtained an excellent correlation (r = 0.982) between the micro-test and the standard test weight. They used a small glass jar (47ml) to assess 59 winter soft wheat cultivars and concluded that the microtest is a reliable predictor.

Similarly, Donelson et al. (2002) used a 100 cm3 graduated glass cylinder to measure the test weight in 20 and 40g of wheat samples. They reported that 40g samples had a higher statistical validity compared to the 20g samples, but the method was severely restricted by wrinkled grains. A few years ago, Stepochkina and Stepochkin (2015) tested 10 lines of common wheat and triticale with a very small cylindrical container (2.86 cm^3 in volume). They compared the device with standard 250 ml chondrometer and reported a correlation coefficient of 0.98.

Recently, Okuyama et al. (2020) compared the15.30ml microchondrometer with the 250 ml chondrometer on fifty wheat samples ranging from well-formed grains to severely shriveled and germinated grains. They also reported a highly significant correlation coefficient (r = 0.99) between the two devices. A vast majority of the studies reported in the literature have compared different volumes of graduated cylinders with the standard devices to determine the test weight in wheat. The present study was carried out to confirm the feasibility of using a 15.30 ml microchondrometer in triticale, as well as 15.30 and 31.26 ml in oats to assess their test weight.

MATERIALS AND METHODS

The 15.30 and 31.26 ml microchondrometer were built using the 250ml Dalle Molle® chondrometer as a standard reference (Figure 1). The respective specifications of the 15.30, 31.26 and 250 ml chondrometers are as follows: total height (cm): 18.60, 19,50, 39.00; total weight (g): 639.60, 342,61 and 949.32; external diameter (mm): 28.49, 28,56 and 56.18; cutting bar (g): 16.43, 21,98 and 70.55; piston volume (cm^3): 3.59, 7,64 and 61.58, respectively.

Two experiments were conducted to test the efficacy of new microchondrometers to measure the test weight in small samples of triticale (*Triticosecale* Wittmack) and oats (*Avena sativa* L.). The research was carried out at the Instituto Agronômico do Paraná (IAPAR), Londrina, Brazil, in 2016 and 2017.

In the first experiment, the efficacy of the 15.30 ml microchondrometer was evaluated using sixty-six triticale samples in the first year and forty-four samples in the second year of the study. The second experiment was conducted to test two microchondrometers (15.30 and 31.26 ml) in thirty-five and nineteen oat samples, in the first and second year, respectively. The grain samples used in the two experiments, approximately one kg each, were obtained from experimental plots and farmers' fields. Some of these samples were forced to sprout in a mist chamber for a period of 24, 30 and 48 h in order to obtain contrasting values of test weight. The test weight was determined by weighing the grains, contained inside the cylinder, on a digital scale (Marte® AS2000; 0.01 g.), and multiplying it by 6.5359, 3.1990, and 0.40, respectively for the 15.30, 31.26 and 250 ml chondrometers.

The first experiment on triticale, consisted of three replications, and compared between 15.30 and 250 ml chondrometers. The second experiment on oats (with tips of grains clipped off) was conducted, in five replications, and compared among 15.30, 31.26 and 250ml chondrometers. All comparisons were analyzed by the

Figure 1. From left to right, the 15.30 ml and the 31.26 microchondrometers and the 250 ml chondrometer, with its respective pistons and cutter bars.

Pearson's correlation coefficient and by the Student t test using Microsoft Excel 2013 software and SAS package (SAS, 2001).

RESULTS

A wide range of the test weight values, over a two-year period, were observed in the triticale grain samples under study. Measured with the 250 ml commercial chondrometer, these values ranged from 49.92 to 73.73 kg hl^{-1} in the first year, and from 58.09 to 71.93 kg hl^{-1} in the second year (Table 1). The Student t-test conducted to compare the two chondrometers (250 ml and 15.30 ml) over a two-year period did not show any significant difference ($p > 0.05$) among their performance (Table 2).

The correlation between test weights measured by the 250 and 15.30 ml chondrometers in triticale was highly significant ($p < 0.0001$) with values of 0.9907 and 0.9743 for the first and second year, respectively.

Combining triticale samples from both years, the Student t-test between the two chondrometers (250 and 15.30 ml) did not show any significant difference ($p > 0.05$) (Table 2). On combined samples, the correlation between the test weight measurements of 250 ml chondrometer and 15.30 ml microchondrometers was highly significant ($p < 0.0001$), with r values of 0.9873. In the experiment on oat samples, the test weight values measured with the commercial 250 ml chondrometer ranged from 46.82 to 60.16 kg hl^{-1} in the first year and from 42.72 to 53.28 kg hl^{-1} in the second year (Table 3).

In oats, a comparison among the three chondrometers (250, 15.30 and 31.26 ml) to measure test weights over a two-year period, did not show any significant difference ($p > 0.05$) among their performance (Table 4). The correlation between test weights measured on the 250 ml chondrometer with 31.26 ml and 15.30 ml microchondrometers in oats were highly significant ($p < 0.0001$), with values of 0.9510, 0.9525 and 0.9076, 0.9181 for the first and the second year of tests, respectively. Furthermore, the correlation between the 31.26 and 15.30 ml microchondrometers was also highly

Table 1. Mean, standard deviation (SD), sum, minimum (min) and maximum (max) values of triticale samples measured by 15.3 ml and 250 ml chondrometers.

Year	Number of samples	Chondrometer volume (ml)	Simple Statistics Test weight (kg hl^{-1})				
			Mean	SD	Sum	Min	Max
2016	66	15.3	62.61	6.19	4132	49.83	75.27
		250	61.94	6.07	4088	49.92	73.73
2017	44	15.3	65.66	4.17	2889	57.19	73.87
		250	65.23	3.83	2870	58.09	71.93
Combined	110	15.3	63.83	5.65	7021	49.83	75.27
		250	63.25	5.51	6958	49.92	73.73

Table 2. Test weight, standard deviation and significance of the t-test of triticale grains, evaluated by the 250 ml and the 15.30 ml chondrometers.

Year	Number of samples	Test weight (kg hl^{-1})		250 ml vs. 15.30 ml		
		15.30 ml	250 ml	t-test	Prob.	Signif.
2016	66	62.61	61.94	0.63	0.5286	ns
2017	44	65.66	65.23	0.50	0.6194	ns
Combined	110	63.83	63.26	0.76	0.4461	ns

ns = non-significant (p> 0.05). Comparison in each row.

Table 3. Mean, standard deviation (SD), sum, minimum (min) and maximum (max) values of oats samples measured by 15.30 ml, 31.26 ml and 250 ml chondrometers.

Year	Number of samples	Chondrometer volume (ml)	Simple statistics Test weight (kg hl^{-1})				
			Mean	SD	Sum	Min	Max
2016	35	250.00	53.41	2.538	1869	46.82	60.16
		31.26	53.14	2.530	1860	47.88	59.03
		15.30	52.52	2.657	1838	45.73	58.18
2017	19	250.00	49.21	2.287	935.1	42.72	53.28
		31.26	50.18	2.357	953.3	44.00	53.69
		15.30	49.01	2.532	931.2	42.82	53.44
Combined	54	250.00	51.94	3.163	2805	42.72	60.16
		31.26	52.10	2.834	2813	44.00	59.03
		15.30	51.29	3.094	2770	42.82	58.18

significant ($p < 0.0001$), with r-values of 0.9189 and 0.9203 for the first and second years, respectively.

Combining the oat test weight data from two years, the Student t-test among the chondrometers (250 and 15.30 ml), (250 and 31.26 ml) and (31.26 and 15.30 ml) did not show any significant difference ($p > 0.05$) (Table 4). The correlation values of 250 ml chondrometer with 31.26 and 15.30 ml microchondrometers were highly significant ($p < 0.0001$); with r values of 0.9448, 0.9557, respectively. Furthermore, the correlation between the 31.26 and

Table 4. The test weight comparison (by t-test) of the oat samples evaluated with the 15.30 ml, the 31.26 ml and the 250 ml chondrometers.

Year	Number of samples	Chondrometer		Chondrometer		Chondrometer		
		Volume (ml)	Test weight (kg hl^{-1})	Volume (ml)	Test weight (kg hl^{-1})	t-test		
						t value	Prob.	Signif
		250.00	53.41	31.26	53.14	0.45	0.6538	ns
2016	35	250.00	53.41	15.30	52.52	-1.43	0.1573	ns
		31.26	53.14	15.30	52.52	-0.99	0.3247	ns
		250.00	49.21	31.26	50.18	-1.28	<0.2102	ns
2017	19	250.00	49.21	15.30	49.01	-0.26	<0.7967	ns
		31.26	50.18	15.30	49.01	-1.47	<0.1510	ns
		250.00	51.94	31.26	52.10	-0.28	0.7807	ns
Combined	54	250.00	51.94	15.30	51.29	-1.07	0.2850	ns
		31.26	52.10	15.30	51.29	-1.42	0.1598	ns

*ns = non-significant (p> 0.05). Comparison in each row.

15.30 ml microchondrometers was also highly significant (p <0.0001), with r-values of 0.9399.

DISCUSSION

In our earlier study on wheat, it was concluded that 15.30 ml microchondrometer was able to predict the commercial test weight (measured by 250 ml chondrometer) for a wide variety of wheat grain types, forms and densities (Okuyama et al., 2020). Based on this study and considering the similarity between wheat and triticale grains, except for grain density, we decided to further confirm its versatility in triticale as well as in oat grains.

In general, the triticale grains have lower test weight than wheat. The test weight of the samples under study and measured by the commercial 250 ml chondrometer, ranged from 49.92 to 73.73 kg hl^{-1} in the first year and from 58.09 to 71.93 kg hl^{-1} in the second year (Table 1). It should be pointed out that 65 kg hl-1 is the minimum test weight value for triticale commercialization in Brazil (Brasil, 1983), and in Australia (GRDC, 2018). Although the triticale samples varied widely, from sprouted kernels to well-formed grains, no significant differences (p> 0.05) in their test weight values were observed when measured on the commercial (250ml) chondrometer or the new prototype (15.30ml) microchondrometer (Table 2). The correlation between the two devices was found to be highly significant (p <0.0001), with r-values of 0.9907 and 0.9743 for the first and second year, respectively. For combined data of two-years, the correlation between them was also highly significant (p <0.0001), with value of 0.9873.

In their study on fifty-nine wheat lines and cultivars, Ghaderi et al. (1971) concluded that the micro-test (evaluated in a 47 ml glass bottle) was a reliable predictor of the test weight (r=0.982). Supporting their results, we also confirm the versatility of the 15.30 ml microchondrometer, to be useful in all experiments producing insufficient grain volume, to determine the test weight by traditional methods. For oats, their irregular size and shape of grains influence their packing in the test weight measuring container (Forsberg and Reeves, 1992). In order to explore the usefulness of the two microchondrometers (31.26 ml and 15.30 ml) to measure the oat test weight correctly, they were compared with the 250 ml chondrometer.

When evaluated with the 250 ml commercial chondrometer, the hectoliter weight for oat samples under study ranged from 46.82 to 60.16 kg hl^{-1} in the first year and from 42.72 to 53.28 kg hl^{-1} in the second year (Table 3). For trading purposes, the standard test weight values in oats are as follows: group 1: > 50 kg hl^{-1}; group 2: from 47 to 49 kg hl^{-1}; group 3: from 41 to 46 kg hl^{-1} and; group 4: <41 kg hl^{-1} (Brasil, 1975). For international export, a minimum test weight value of 52.5 kg hl^{-1} is required (GTA, 2018b). The comparison of the 250 ml chondrometer with the 31.26 and 15.30 ml microchondrometers, as well as between the 15.30 with 31.26 ml microchondrometers revealed no significant difference in the test weight values of oat samples over a two-year period of the study (Table 4).

The correlations of the 250 ml chondrometer with 31.26

and 15.30 ml microchondrometers were highly significant (p <0.0001), with values of 0.9510, 0.9525 and 0.9076, 0.9181 for the first and second years, respectively. Similarly, the correlation between the 31.26 and 15.30 ml microchondrometers was also highly significant (p <0.0001), with values of 0.9189 and 0.9203 for the first and second years, respectively.

In a combined analysis for two years, both the 31.26 and 15.30 ml microchondrometers correlated highly (p <0.0001) with the commercial 250 ml chondrometer, with r values of 0.9448, and 0.9557, respectively. Similar relation was observed between 31.26 and 15.30 ml microchondrometers, which was also highly significant (p <0.0001), with r value of 0.9399. Although the correlation values among the devices were slightly lower in oats than those reported in triticale, they were much higher than the values in wheat reported by Aamodt and Torrie (1934). These authors obtained a correlation of 0.834 for 59 samples of winter wheat and concluded that for practical purposes the differences were very small and insignificant. Therefore, we are confident that the 15.30 and 31.26 ml microchondrometers can be used to determine the test weight in oats in a wide range of grain conditions.

The differences in the correlation values obtained between the triticale and oat samples analyzed in this study can be caused by the differences in the size and shape of grains of the two crop species. Yet, the new microchondrometers permit their evaluation successfully under wide range of conditions. We believe its versatility to be very useful in the retention or discarding the genotypes that fail the minimum quality standard demanded by the market. It is worth emphasizing that the 31.26 and 15.30 ml microchondrometers are easier to handle than those manufactured by Taylor (1965). There is no limitation with respect to their utility in severely shrunk kernels, as observed by Donelson et al. (2002) and there is no need to compress or level the samples, as suggested by Stepochkina and Stepochkin (2015).

We reemphasize that the 15.30 and 31.26 microchondrometers were not built with the intention to replace the 250 ml commercial chondrometer, but as an option to assess the test weight in very small samples. We confirm that both of the microchondrometers reported in this study represent valid options for assessing the test weight in the research projects, especially in the evaluation of individual plants and greenhouse experiments, where the sample size is a limiting factor.

Conclusion

The highly significant correlation coefficient between the 250 ml chondrometer and the 15.30 ml microchondrometer in triticales and oats, as well as with the 31.26 ml microchondrometer in oats confirms the usefulness of these microchondrometers as an excellent alternative to evaluate the test weight in small samples in these crops. Given the lack of significant differences between the 15.30 ml and the 31.26 ml microchondrometers in oats, it is possible to choose the smaller version, due to the lesser amount of grain needed to evaluate the test weight.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of kernel type on hardness and interrelationship with endosperm chemical components of Malawian local maize (*Zea mays L.*) varieties during storage

Ephantus Nguma[1], Daiki Murayama[3], Chandiona Munthali[2], Kazumitsu Onishi[2], Masahiko Mori[4], Masayuki Tani[4], Jiwan P. Palta[5], Hiroshi Koaze[4] and Daigo Aiuchi[4*]

[1]Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan.
[2]Department of Agro-Environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan.
[3]Department of Food Science, University of Wisconsin-Madison, Babcock Hall, 1605 Linden Dr, Madison, WI 53706, United States.
[4]Research Center for Global Agromedicine (GAMRC), Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan.
[5]Department of Horticulture, University of Wisconsin-Madison, 490 Moore Hall-Plant Sciences, 1575 Linden Dr, Madison, WI 53706, United States.

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The aim of this study was to establish the attributes of kernel type based on kernel hardness and its interrelationship with maize endosperm chemical components, which are essential during storage. Three local varieties and one hybrid variety commonly grown by smallholder farmers in central Malawi were used. For storage experiment, the maize samples were infested with *Prostephanus truncatus*, larger grain borer (LGB) for eight weeks. Due to the high propensity of maize to cross-pollinate, the kernels were classified into flint and dent kernels to establish the physicochemical properties of the kernel type and variety independently. Local variety (L-3) exhibited the lowest number and weight of damaged grains and the highest adult LGB cadavers. Local variety, L-1 and L-3 had significantly the highest proportion of flint kernels type. Moreover, flint kernels of local varieties showed significantly the highest hardness, and the highest content of protein, total zein, α-zein and zinc ($p < 0.05$). Furthermore, hardness was significantly and positively correlated with total zein and 14 kDa β-zein content ($p < 0.05$). Zein in the endosperm, particularly 19 and 22 kDa α-zein mainly contributed to distinct hardness of the local varieties hence may contribute to physical barrier of the kernels against storage pests.

Key words: Maize, local variety, kernel type, hardness, chemical properties, larger grain borer.

INTRODUCTION

Globally, maize (*Zea mays* L.) is the most produced crop (FAO, 2016). Most developing countries rely on maize as the main staple food and primary source of calories (Smale et al., 2011; Suleiman et al., 2015). Maize is consumed by more than 50% of the population in sub-Saharan Africa (SSA), with an average per capita annual consumption in Malawi being 129 kg, Lesotho at 158 kg, Kenya at 76 kg, Zambia at 118 kg and Zimbabwe at 93 kg (FAO, 2013; Smale and Jayne, 2003). Smallholder farmers rely on rain-fed maize farming; hence, production of the single crop in one season, which necessitates long storage period of about eight months (Maliro and Kandiwa, 2015). Furthermore, they prefer flinty varieties commonly referred to as 'local', (Smale and Heisey, 1997) which are locally adapted and traditional maize varieties inherited from the ancestors. In addition, these local varieties are associated with superior performance under abiotic stress conditions and higher grain to flour conversion ratio, than dent, white hybrids (Gilbert et al., 1994).

Maize hardness is an important quality factor to smallholder farmers. Indeed, hard kernels are less susceptible to damage during traditional storage by pests (Smale et al., 2011; Suleiman et al., 2015). Endosperm, the main component of maize kernels, significantly contributes to hardness (Mestres and Matencio, 1993; Blandino et al., 2010) with the most abundant components being starch and protein (Fox and Manley, 2009). Protein plays a significant role in influencing mechanical strength of the endosperm, (Fox and Manley, 2009; Williams et al., 2009) owing to zein, the main maize storage protein (Shewry and Halford, 2002). α-zein, which is composed of 19 and 22 kDa has been associated with vitreous kernels whereas γ-zein, which comprised 16 and 27-kDa has been associated with the floury kernels (Chandrashekar and Mazhar, 1999; Gayral et al., 2016).

Generally, normal starch consists of about 25% amylose (Sandhu and Singh, 2007; Hu et al., 2010). Although amylose is a minor compound of maize endosperm, it plays a role in the formation of a compact starch-protein matrix (Dombrink-Kurtzman and Knutson, 1997) as shown by Gayral et al. (2015) that flint maize have a higher amylose content than dent maize as well as their corresponding vitreous and floury endosperm fractions.

In our previous study, we have shown that the Malawian local varieties, which are mainly orange in color, have superiority in nutrients, cookability, and storability than hybrid varieties (Murayama et al., 2017). The local varieties showed more resistance to *Prostephanus truncatus*, larger grain borer (LGB) during a 12-week storage experiment than hybrid varieties. Furthermore, the Malawian maize varieties and particularly flinty 'local' varieties have been contaminated with dent hybrids due to natural open-pollination over time (Smale and Heisey, 1997) and have different chemical composition 1997) and have different chemical composition (Letchworth and Lambert, 1998).

In light of the aforementioned, the extent of cross-pollination and its effect on maize hardness and endosperm chemical properties, which are crucial in determining maize resistance to storage pest damage, are not well established. In the current study, we elucidated the level of natural cross-pollination among farm-stored maize varieties by smallholder farmers in central Malawi and its effect on the interrelationship of kernel hardness, storability, and endosperm chemical components.

MATERIALS AND METHODS

Maize varieties and experimental field design

Four maize varieties were used in this study. Three local varieties namely white local variety (ACC OU 549), yellow local variety (ACC OU 546-2), and yellow local variety (ACC OU 552-4) obtained from Malawi Plant Genetic Resource Center in Chitedze Agricultural Research Station (CARS) and one hybrid variety (DKC-9089) obtained from DeKalb Genetics Corp., USA (Table 1). To obtain the maize samples, eight plots were prepared as a single factor experiment of the maize varieties without randomization. The trial experiment was conducted in an experimental field in CARS, Lilongwe, Malawi during the 2014/2015 cultivation season. Each plot size was 10 m × 15 m and maize seed spacing between hills was 25 and 75 cm between the rows. A compound fertilizer of 23% N, 21% P and 0% K with urea of 46% N were applied in the ratio 2:3 to attain a standard fertilizer of 92 kg-N/ha as recommended by the government of Malawi (Mutegi et al., 2015). The actual total rainfall in 2014/2015 season was 560.4 mm as compared to the normal expected rainfall of 859.0 mm. Daily minimum and maximum temperatures averaged between 14.0 and 38.2°C, respectively (Messina et al., 2017).

Sample preparation

The maize grains were disinfected by freezing at -30°C for three weeks and thawed at 4°C for one week. Moisture standardization was done in a hot air oven (WFO-700, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) at 40°C to achieve approximately 12.0 ± 1.0% moisture content, wet weight basis. Dried samples were kept at 4°C in an airtight container for one week.

LGB resistance

LGB resistance was evaluated as described by Tefera et al. (2011) and Abebe et al. (2009) with some modifications. Two hundred grams of each maize variety were placed in glass jars (500 mL) with steel screens at the lids (60 mesh), then 30 adult *P. truncatus* of 0 to 7 days old were introduced into each jar and kept in an incubator (MIR-254, Sanyo Co., Ltd) at 28.0 ± 1.0°C. The number of live adult LGB, adult LGB cadavers and number, and weight of damaged

*Corresponding author. E-mail: aigo@obihiro.ac.jp. Tel: +81 155 49 5934. Fax: +81 155 49 5229.

Table 1. Identities of maize varieties and their sampling information.

Sample code	Variety	Accession number	Color	Sampling point			
				Latitude	Longitude	District	Province
L-1	Local	ACC OU 549	White	S14°02'08.9”	E33°36'01.1”	Lilongwe	Central
L-2	Local	ACC OU 546-2	Yellow	S14°01'27.6”	E33°34'39.8”	Lilongwe	Central
L-3	Local	ACC OU 552-4	Yellow	S13°58'32.6”	E33°41'14.7”	Lilongwe	Central
H-1	Hybrid	DKC9089	White	-	-	-	-

grains were recorded after the fourth and eighth week of incubation. All LGB were separated and removed from the maize by use of sieves with 1.00 and 4.00 mm mesh sizes (IIDA Testing Sieve, IIDA Manufacturing Co. Ltd, Osaka, Japan). The number of damaged grains was determined by randomly selecting 100 grains from the jar and counting all grains tunneled by LGB and their weight was determined in triplicate.

Kernels classification

Classification of maize kernels into flint and dent types was done based on endosperm phenotypic characteristics (Suleiman et al., 2015). Maize flour was prepared by milling with a blender (New Power Mill PM-2005, Osaka Chemical Co., Ltd., Osaka, Japan) and sieved through a 355 µm mesh sieve for chemical analysis.

Physical properties

Kernel hardness was determined using the method described by Blandino et al. (2010). Fifteen maize grains were punctured on the upper lateral surface using a texture analyzer (TA-XT2, Stable Micro System, Godalming, UK) with a flat probe P/2 (diameter 2 mm) and 25 kg load cell. Puncture test was performed at 1 mm/s of puncture speed and kernel punctured at the lateral center side, opposite to the embryo to a depth of 2 mm. Kernel hardness was expressed as the break force calculated in Newton (N).

Chemical properties

Moisture content and crude protein were determined using the standard AOAC methods (AOAC, 2005). Total starch and amylose contents were determined using Megazyme K-TSTA and K-AMYL Assay procedures, respectively (Megazyme International, Bray, Ireland). Total zein was extracted as described by Wallace et al. (1990) and the supernatant representing the total zein fraction was dried at 80°C in Kjeldahl tubes for 4 h and analyzed for nitrogen content by the Kjeldahl method (AOAC, 2005). Zein composition analysis was done by extracting zein using a method described by Wu et al. (2012) with some modifications. Twenty-five mg of flour was transferred to a 2 mL micro-centrifuge tube, then mixed, vortexed with 2 mL of 70% ethanol with 2% 2-mercaptoethanol (v/v), and allowed to stand at room temperature overnight. The mixture was centrifuged at 13,000 rpm for 10 min; 100 µl of the supernatant was transferred into a new tube and mixed with 10 µl of 10% sodium dodecyl sulfate (SDS). The mixture was dried in a rotary vacuum (DNA Speed Vac DNA110, Savant Instruments, INC. Farmingdale, NY), and resuspended in 50 µl of water by vortexing. Further 50 µl of sample reducing buffer, composed of 4% SDS, 10% 2-mercapethanol, 10% sucrose, 0.05% Coomassie blue G-250 and 1.5 M Tris-HCl pH 6.5 was added, and vortexed. Tubes were sealed and boiled for 5 min and frozen overnight. The extracted zein was separated by SDS-PAGE (49.5% T, 6% C mixture) as described by Schägger (2006), the gel scanned at 312 nm (BioInstruments ATTO, AE-6933FXES) and quantification of the bands done using ImageJ 1.50i (National Institutes of Health, Bethesda, Maryland). Mineral contents of magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca) and zinc (Zn) were determined by wet-ashing 100 mg with concentrated sulfuric acid and hydrogen peroxide (2:1 v/v), the resulting solution was diluted 25-fold with deionized water and quantified by an inductively coupled plasma mass spectrometry (ICP; ICPS-8100, Shimadzu Co. Ltd., Japan) in triplicate.

Statistical analysis

Results were subjected to statistical analysis using SPSS for Windows (ver.17.0). A t-test and ANOVA complemented with Tukey’s multiple-range test were used to determine significant differences of LGB resistance parameters for each variety, physicochemical properties, and kernel type. Significant level was established at $p \leq 0.05$. Hardness and chemical properties were correlated using Pearson’s correlation.

RESULTS AND DISCUSSION

LGB resistance

Live and dead insects, damaged grains, and their weight are commonly used to determine maize resistance to LGB in laboratory storage experiments. As shown in Table 2, there was an exponential increase in live adult LGB from the fourth to the eighth week of infestation. In addition, adult LGB cadavers and number, and weight of damaged grains increased with storage period. In the fourth and eighth week of the storage periods, there were no significant differences of the LGB resistance parameters among the varieties (Table 2). However, in the fourth week, L-3 showed the minimum number and weight of damaged grains at 15.1 and 3.2 g, respectively. L-1 and H-1 were observed to have a higher number and weight of damaged grains than L-3. After the eighth week of infestation, L-3 steadily exhibited the minimum number and weight of damaged grains and the highest number of adult LGB cadavers. LGB is known to cause damage to maize grains by tunneling at pre-ingestion phase for food and oviposition during storage period (Li, 1988). The tendency of L-3 to show resistance to damage by LGB during storage due to the less feeding observed, indicates

Table 2. Number of live adult LGB, and adult LGB cadavers, number of damaged grains and weight of damaged grains (g) on LGB infestation after a storage period of 4 and 8 weeks.

Sample code	Live adult LBG	Adult LGB cadavers	Number of damaged grains	Weight of damaged grains (g)
			4 weeks	
L-1	28.3 ± 1.2[a]	1.7 ± 1.2[a]	20.8 ± 4.4[a]	6.1 ± 1.3[a]
L-2	27.3 ± 1.7[a]	2.7 ± 1.7[a]	19.1 ± 3.0[a]	5.6 ± 0.7[a]
L-3	28.3 ± 0.9[a]	1.7 ± 0.9[a]	15.1 ± 2.7[a]	3.2 ± 0.5[a]
H-1	27.7 ± 0.5[a]	2.3 ± 0.9[a]	20.3 ± 4.1[a]	4.7 ± 1.0[a]
			8 weeks	
L-1	281.7 ± 28.5[a]	15.0 ± 5.0[a]	51.1 ± 8.5[a]	14.2 ± 2.9[a]
L-2	357.7 ± 180.6[a]	11.3 ± 4.0[a]	54.1 ± 10.1[a]	14.7 ± 2.4[a]
L-3	473.7 ± 161.5[a]	23.0 ± 2.2[a]	51.6 ± 8.3[a]	10.6 ± 1.6[a]
H-1	403.3 ± 53.9[a]	15.3 ± 8.3[a]	67.3 ± 9.2[a]	14.3 ± 2.6[a]

Means in a column with different letter are significantly different at $p \leq 0.05$ among the varieties.

Table 3. Classification (proportion/100 grains) and hardness (N) of flint and dent type kernels.

Sample code	Kernel type	Proportion/100 grains[a]	Hardness[b]
L-1	Flint	63.9 ± 9.7[ab]*	255.9 ± 50.0[b]*
L-2		31.8 ± 11.1[c]	280.5 ± 55.2[ab]*
L-3		85.6 ± 2.2[a]*	303.5 ± 40.6[a]
H-1		46.0 ± 13.3[bc]	238.8 ± 56.4[c]*
L-1	Dent	15.1 ± 3.0[bc]	193.8 ± 59.5[bc]
L-2		45.2 ± 11.0[a]	232.8 ± 59.8[b]
L-3		12.7 ± 0.3[c]	284.4 ± 41.0[a]
H-1		29.8 ± 3.0[b]	182.7 ± 35.3[c]

Means in the same column with different letter(s) are significantly different at $p \leq 0.05$ among the varieties. Means in the same column with * are significantly higher at $p \leq 0.05$ between kernel types for each variety. Mean ± SD ([a]n = 100) and ([b]n = 15).

a higher tolerance to insect damage, probably attributed to its physicochemical properties and/or genetic ability. The tendency of H-1 to exhibit the highest number of damaged grains and lower weight damaged grains depicts susceptibility to attack by LGB over the storage period.

Classification

Cross-pollination significantly affects both physical and chemical properties of maize grains (Letchworth and Lambert, 1998). Thus, the maize varieties was classified into flint and dent type kernels based on endosperm phenotypic characteristics (Suleiman et al., 2015) to highlight the interconnection between hardness and chemical composition and their effect on kernel resistance. In every 100 g of maize grains, L-3 and L-1 had significantly the highest proportion of flint type kernels at 85.6 and 63.9%, respectively. Dent type kernel proportion of L-1, L-2, L-3 and H-1 was 15.1, 45.2, 12.7 and 29.8%, respectively (Table 3), while the remaining proportion was classified as semi-flint. Local maize varieties are generally ‘flinty’, which was in agreement with our results. However, the proportion of dent type kernel in local varieties might continue to increase in the near future due to the high degree of maize to cross-pollinate and predominance of hybrid dent varieties among smallholder farmers as well as in the market outlets (Smale and Heisey, 1997). Therefore, there is a need to conserve the ‘local’ flinty maize diversity among the smallholder farmers.

Hardness

Hardness among the varieties fluctuated significantly between 238.8 and 303.5 N for flint type kernels and 182.7 and 284.4 N for dent type kernels as shown in Table 3. Blandino et al. (2010) reported results within the

Table 4. Total starch, amylose, crude protein and total zein content of flint and dent type kernels.

Sample code	Kernel type	Total starch, % db	Amylose, % ww	Crude protein, % db	Total zein, % db
L-1	Flint	69.2 ± 3.1[ab]	21.0 ± 1.2[ab]	12.3 ± 0.1[b*]	11.1 ± 0.2[b*]
L-2		72.0 ± 2.0[a]	19.1 ± 1.2[bc]	11.8 ± 0.1[c]	10.3 ± 0.1[c]
L-3		66.3 ± 2.1[b]	22.1 ± 1.2[a]	13.5 ± 0.0[a*]	12.1 ± 0.1[a*]
H-1		69.5 ± 1.1[ab]	18.7 ± 1.9[c]	11.5 ± 0.0[d*]	9.4 ± 0.2[d]
L-1	Dent	69.9 ± 1.4[ab]	20.0 ± 2.7[ab]	12.1 ± 0.0[b]	10.5 ± 0.2[ab]
L-2		72.4 ± 2.7[a]	18.8 ± 0.3[b]	11.7 ± 0.0[c]	9.8 ± 0.4[bc]
L-3		69.2 ± 1.0[b*]	21.4 ± 1.0[a]	13.2 ± 0.0[a]	10.9 ± 0.2[a]
H-1		70.9 ± 1.1[ab*]	18.6 ± 1.4[b]	11.9 ± 0.1[b]	9.3 ± 0.3[c]

Means in a column with different letter(s) are significantly different at $p \leq 0.05$ among the varieties. Means in a column with * are significantly higher at $p \leq 0.05$ between kernel types for each variety. Mean ± SD (n = 3)

same range, from 145 to 300 N for 13 commercial maize hybrids. Local varieties had a significantly higher hardness ($p < 0.05$) compared to Hybrid DKC9089 (H-1), with L-3 showing significantly the highest hardness. For the type of kernels, flint had the significantly higher hardness as compared to dent counterparts apart from L-3, although its flint type kernels had a higher hardness than the dent type kernels. These findings suggest that flint type kernels had a high ratio of vitreous compact endosperm fraction compared with the dent type kernel counterparts. Consequently, this contributed to higher physical resistance of flint type kernels during penetration and shearing by the probe.

Chemical properties

Chemical properties evaluated for flint and dent type kernels of each variety are shown in Table 4. Total starch content among the varieties ranged from 66.3 to 72.0% and 69.2 to 72.4% for flint and dent type kernels, respectively. Essentially, dent type kernels were observed to have a higher content of total starch as compared to flint counterparts (Table 4). Dent type kernels of L-3 and L-1 had significantly the lowest starch content and significantly the highest hardness whereas H-1 had significantly higher starch content and significantly the lowest hardness. These findings are in agreement with other studies that total starch content has a negative correlation with hardness (Pineda-Hidalgo et al., 2015).

Amylose content varied from 18.6 to 22.1% (Table 4) as reported in other studies (Gayral et al., 2015). Local varieties were observed to have significantly higher amylose content than Hybrid variety H-1 ($p < 0.05$) whereas flint type kernels exhibited the higher content of amylose than dent type kernels (Table 4), which was in agreement with Juárez-García et al. (2013) that vitreous kernels have a higher content of amylose than floury kernels. Amylose gradient exists in the endosperm with a gradual decrease from vitreous to the floury fraction in flint and dent maize (Chandrashekar and Mazhar, 1999; Gayral et al., 2016), thus, the higher amylose content may be responsible for the compactness of starch granules at the periphery of the endosperm as proposed by Dombrink-Kurtzman and Knutson (1997).

The average crude protein for kernel types of each variety are shown in Table 4. Flint type kernels were observed to have higher protein content as compared to their corresponding dent type kernels for all varieties with L-1, L-3 and H-1 having significantly higher protein content ($p < 0.05$). Despite the low abundance of protein in maize endosperm, its role in constituting the physical resistance in maize kernels cannot be ignored as exhibited by L-3, due to the formation of starch-protein matrix. Indeed, the compactness of the endosperm depends on the architecture of starch (Narvaez-Gonzalez et al., 2006) which is influenced by the presence of proteins leading to the formation of a starch-protein matrix (Wallace et al., 1990).

Further studies with a key interest of zein's role in kernel hardness were conducted. Total zein accounted for an average of 87.4 and 82.7% of protein content for flint and dent type kernels, respectively. Total zein content among the varieties varied from 9.4 to 12.1% and 9.3 to 10.9% for flint and dent type kernels, respectively as shown in Table 4. Similar to protein content, flint type kernels had higher total zein content compared to dent counterparts with L-1 and L-3 having significantly higher total zein content ($p < 0.05$). It is important to note that zein synthesis takes place in the endoplasmic reticulum, assembled as protein bodies, and deposited in the endosperm, (Holding and Larkins, 2006; Shewry and Halford, 2002) while Pereira et al. (2008) established that hard-endosperm maize cultivars had abundant and well-organized protein bodies. This may augment the adhesion of protein bodies on the surface of the starch granules hence increasing the rigidity of the starch-protein matrix, which explains the significantly higher hardness of the flint type kernels. It is worth noting that

Table 5. Zein composition content of flint and dent type kernels.

Sample code	Kernel type	α-22 [a]	α-19 [a]	Total α [a]	γ-27 [a]	γ-16 [a]	Total γ [a]	β-14[a]	δ-10 [a]
L-1	Flint	3.0 ± 0.1a*	6.0 ± 0.4ab*	9.0 ± 0.5a*	0.7 ± 0.2ab	0.4 ± 0.1a	1.1 ± 0.3ab	0.5 ± 0.1b	0.5 ± 0.1a
L-2		2.5 ± 0.0b*	5.4 ± 0.0b*	7.9 ± 0.0b*	0.7 ± 0.0ab	0.5 ± 0.0a	1.1 ± 0.0ab	0.7 ± 0.0a	0.6 ± 0.0a
L-3		3.1 ± 0.1a*	6.2 ± 0.2a*	9.3 ± 0.1a*	1.0 ± 0.1a	0.3 ± 0.1a	1.3 ± 0.1a	0.7 ± 0.1a	0.7 ± 0.1a
H-1		2.1 ± 0.1c	5.4 ± 0.3b*	7.5 ± 0.2b*	0.5 ± 0.2b	0.3 ± 0.0a	0.8 ± 0.2b	0.4 ± 0.0b	0.7 ± 0.1a
L-1	Dent	2.5 ± 0.1a	4.9 ± 0.2a	7.5 ± 0.2ab	1.4 ± 0.1a*	0.5 ± 0.1a	1.9 ± 0.0a*	0.4 ± 0.1ab	0.7 ± 0.2a
L-2		2.3 ± 0.0a	4.8 ± 0.1a	7.1 ± 0.1bc	0.9 ± 0.1b*	0.4 ± 0.0ab	1.4 ± 0.1c*	0.7 ± 0.0a	0.7 ± 0.2a
L-3		2.6 ± 0.0a	5.0 ± 0.1a	7.6 ± 0.1a	1.4 ± 0.1a*	0.3 ± 0.0bc	1.7 ± 0.1b*	0.7 ± 0.0a	0.9 ± 0.1a
H-1		2.0 ± 0.2b	4.9 ± 0.0a	6.9 ± 0.2c	1.0 ± 0.0b*	0.2 ± 0.0c	1.2 ± 0.0c*	0.3 ± 0.1b	0.8 ± 0.1a

[a]All data expressed as a percentage of total zein content on dry weight basis. Means in a column with different letter(s) are significantly different at $p \leq 0.05$ among the varieties. Means in a column with * are significantly higher at $p \leq 0.05$ between kernel types for each variety. Mean ± SD (n = 3).

Table 6. Mineral composition of flint and dent type kernels.

Sample code	Kernel type	Mg	P	K	Ca	Zn
		mg/100 g, db				
L-1	Flint	125.9 ± 1.3b*	314.8 ± 4.1b*	154.9 ± 2.8a*	5.4 ± 0.1a	1.6 ± 0.1b*
L-2		95.4 ± 0.2d	233.3 ± 2.4c	134.9 ± 2.4b	6.0 ± 0.5a	1.3 ± 0.0c*
L-3		150.4 ± 2.1a*	328.4 ± 3.1a*	154.1 ± 1.9a*	6.0 ± 0.9a	1.8 ± 0.1a*
H-1		113.1 ± 3.1c	242.8 ± 4.0c	149.8 ± 3.7a	5.6 ± 0.5a	1.1 ± 0.1d
L-1	Dent	113.0 ± 0.1c	274.4 ± 2.7c	149.3 ± 1.7b	6.0 ± 1.3a	1.3 ± 0.1b
L-2		121.8 ± 1.0b*	288.6 ± 1.2b*	154.5 ± 2.7b*	5.6 ± 0.4a	1.6 ± 0.0a
L-3		106.0 ± 0.9d	255.5 ± 2.7d	126.5 ± 1.7c	6.0 ± 0.7a	0.9 ± 0.1c
H-1		146.0 ± 1.7a*	313.0 ± 4.7a*	184.3 ± 2.7a*	6.9 ± 0.3a*	1.7 ± 0.1a*

Means in a column with different letter(s) are significantly different at $p \leq 0.05$ among the varieties. Means in a column with * are significantly higher at $p \leq 0.05$ between kernel types for each variety. Mean ± SD (n = 3).

non-zein protein content such as albumins, glutelins, and globulins in dent type kernels of H-1 was the highest at around 22.0% of crude protein, which explains H-1 having significantly the lowest total zein content, yet a higher crude protein content. Furthermore, dent type kernels of the H-1 had significantly the lowest hardness.

To evaluate the composition of zein sub-classes in connection with kernel hardness, extracted zein was separated by SDS-PAGE and quantified as a percentage of total zein as shown in Table 5. Distinct bands observed in the gels were similar to previous studies of maize kernels (Wu et al., 2012; Paiva et al., 1991). α-zein was the most abundant zein class with an average of 78.7% and 75.4% of total zein content for flint and dent type kernels, respectively. Notably, 19 kDa was higher than 22 kDa for α-zein content, while 27 kDa content was higher than 16 kDa for γ-zein content. Flint type kernels had significantly higher total α-zein and 19 kDa α-zein compared to dent type kernel for each variety ($p < 0.05$), whereas dent type kernels had a significantly higher total γ-zein and 27 kDa γ-zein content ($p < 0.05$). This is in agreement with Gayral et al. (2016) who established that γ-zein was significantly higher in floury endosperm and augments our earlier findings that dent kernel had a higher content of total starch. γ-zein and the most expressed 27 kDa γ-zein (Holding and Larkins, 2006) are synthesized during early stages of kernel development and as a result of programmed cell death (PCD) (Young and Gallie, 2000) are associated with the inner core floury fraction of endosperm. In addition, more mature protein bodies, mainly composed of α-zein are located at the periphery of endosperm due to PCD (Gayral et al., 2016; Holding and Larkins, 2006) which explains the high content of α-zein in flint kernels.

The main minerals in maize kernels such as Mg, K, P, Ca and Zn are shown in Table 6. The most abundant mineral was P, ranging from 242.8 to 328.4 mg/100 g dry weight (dw) while Zn was the least ranging from 1.8 to 0.9 mg/100 g dw in both kernel types. Mineral content was similar with other studies following P, K, Mg, Ca, and

Table 7. Pearson's correlation matrix of hardness and chemical properties.

Variable	TS	AM	CP	TZ	α-22	α-19	Total α	γ-27	γ-16	Total γ	β-14
HD	-0.46	0.63	0.63	0.709*	0.69	0.63	0.69	-0.22	0.15	-0.17	0.780*
TS		-0.817*	-0.782*	-0.743*	-0.64	-.720*	-0.732*	-0.13	0.29	-0.05	-0.09
AM			0.936**	0.948**	0.879**	0.62	0.776*	0.38	0.04	0.37	0.46
CP				0.846**	0.714*	0.45	0.60	0.48	-0.16	0.42	0.54
TZ					0.972**	0.713*	0.874**	0.23	0.26	0.29	0.57
α-22						0.759*	0.915**	0.10	0.37	0.19	0.55
α-19							0.957**	-0.44	-0.02	-0.42	0.15
Total α								-0.23	0.16	-0.18	0.33
γ-27									0.05	0.967**	0.17
γ-16										0.31	0.40
Total γ											0.26

HD= hardness (N), TS = total starch (%), AM = amylose (%), CP = crude protein (%), TZ = total zein (%). *Correlation is significant at 0.05 level. **Correlation is significant at 0.01 level.

Zn descending order (Chen et al., 2016; Nuss and Tanumihardjo, 2010). Maize endosperm has a negligible <1% of minerals in the grain, while 30% of zinc is localized in the endosperm (Nuss and Tanumihardjo, 2010). The concentration of zinc at a high level may be toxic to grain storage insects by impairing catalase mechanism (Lazzari and Lazzari, 2012). Moreover, for normal growth and a healthy life, smallholder farmers require maize rich in microelements such as zinc (Ortiz-Monasterio et al., 2007) considering the high prevalence of zinc deficiency in Malawi (Siyame et al., 2013). In this study, it was observed that flint type kernels had significantly higher content of zinc, which can help improve the zinc deficiency problem as well as act as an intrinsic deterrent of LGB during storage of maize.

Correlation between hardness and chemical composition

Pearson's correlation results of hardness, chemical properties and LGB resistance parameters at the eighth week of infestation are shown in Table 7. Total zein content, the abundant protein in maize endosperm was positively correlated with hardness at $r = 0.71$ ($p < 0.05$). This finding shows that total zein is strongly associated with hardness and directly influences the physical resistance of maize kernels. It is worthwhile noting that, greater than 70% of zein is composed of α-zein (Wu et al., 2012) and actually, flint type kernels had significantly higher hardness than their dent counterparts, and therefore α-zein and particularly 19 kDa α-zein certainly plays a major role in kernel hardness as shown by our ANOVA results. Furthermore, 14 kDa β-zein was positively correlated with hardness at $r = 0.78$ ($p < 0.05$). β-zein is located in the periphery of the protein bodies (Shewry and Halford, 2002) and contains methionine and cysteine hence rich in sulfur (Wu et al., 2012). L-3 showed significantly the highest 14 kDa β-zein content, whereas H-1 had significantly the lowest content. Indeed, it can be hypothesized that, probably due to the presence of methionine and cysteine, β-zein could contain a higher disulfide bridges hence increasing the rigidity of the protein bodies at the periphery, which might impart better filling of the starch intergranular spaces, therefore higher compactness and vitreousness of the endosperm.

Conclusion

Based on this study, the tendency of local varieties to have resitance to LGB, especially L-3 was due to its physicochemical properties despite of natural transgene effect of cross-pollination, further depicting the preference of local varieties among smallholder farmers in Malawi. The hardness of maize kernel was highly influenced by both the composition of starch and protein in the endosperm. In this study, it has been shown that zein profile was more associated with hardness, and therefore regulating its synthesis may increase endosperm vitreousness. Consequently, L-3 exhibited highest proportion of flint type kernels and superiority in its hardness, which was closely associated with its chemical properties. These chemical properties depend on genetic traits as observed in variety and kernel type variables, although abiotic factors may also have an influence. L-3 may be proposed as a suitable farm-stored maize variety for smallholder farmers in Malawi, and highlights the importance of conserving the local flint maize varieties. Currently, breeding programs endeavour to develop insect-resistant varieties to minimize postharvest losses due to storage pests. Plant breeders may utilize local varieties like L-3 as a genetic resource to develop naturally superior pest resistant farm-stored maize cultivars for smallholder farmers in Malawi and sub-Saharan Africa (SSA) reducing the current high postharvest losses caused by LGB during storage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Cassava leaves and azolla as crude protein supplement feed to east african short horned Zebu Heifers

Bernard K. Korir[1*], Margaret M. M. Wanyoike[2], Joseph K. N. Kuria.[2], David. M. Mwangi[3] and Edward K. Muge[4]

[1]Kenya Agricultural and Livestock Research Organization, P. O. Box 12-90138, Makindu, Kenya.
[2]Faculty of Veterinary Medicine, University of Nairobi, Kenya.
[3]Kenya Agricultural and Livestock Research Organization, Kakamega, Kenya.
[4]Department of Biochemistry, School of Medicine, University of Nairobi, Kenya.

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The study was undertaken to explore the nutritive potential of cassava leaves and Azolla as supplementary source of crude protein to cattle fed on either mixed range grass hay or Bracharia Mulato II. Two experiments were carried out, the first was the determination of the biomass yield of Azolla, its relative growth rate and doubling time while the second was a feeding trial to determine the performance of small East African Shorthorned Zebu heifers of similar age and weight. The heifers (four each) were randomly assigned to six treatments in the feeding trial that ran for 16 weeks. The doubling time for Azolla was 5 days while the relative growth rate was 0.15 g/g/day. The chemical analysis indicated that Azolla is rich in crude protein (18.8%) and minerals (3.29% calcium, 2.66% potassium and 0.15% phosphorus and other minerals in trace levels). All the supplemented groups showed more weekly weight gains ranging from 0.75 to 1.11 kg per day more than the control. Overall, the treatment with Azolla or cassava showed slightly higher but insignificant increases in weight gains. Azolla and cassava leaves can therefore be considered as potential feed for livestock.

Key words: Arid and range lands, cattle, doubling time, relative growth rate.

INTRODUCTION

Improving protein supply for ruminants is a strategy for increasing productivity in ruminants that have a high protein requirement. This strategy is important when feeding young animals following weaning, those that are pregnant as well as those in lactation. The development of new feed types from the available feed resources is one approach that may help farmers overcome the low nutritional content and seasonal availability of feed for beef cattle production. The new feeds to be introduced need to be highly adaptable to the environment and have appreciably high productivity. Some of the tropical feeds that are high in crude protein and with high growth rates

*Corresponding author. E-mail: bernardkorir@gmail.combernardkorir@gmail.com.

include Cassava leaf meal, Azolla and *Brachiaria mulato* (Antoniewicz et al., 1995, García et al., 1995; Parashuramulu et al., 2013). In a study that was carried out by Oni et al. (2010), it was found that West African Dwarf goats that had been fed with on *Panicum maximum* and supplemented dried cassava leaves led to an improvement in nitrogen retention, apparent digestibility, weight gain as well as dry matter intake. In an experiment by Marjuki et al. (2008), the inclusion of cassava leaves on a dry matter (DM) basis was up to 30%.

In the tropical areas of Africa, cassava forms part of the staple foods grown as its production is efficient and requires minimal inputs and management. The crop has proved suitable with potential to assist in curbing food scarcity in the continent of Africa (Hahn et al., 1992). Cassava roots are low in protein at about 2% crude protein (CP) and because of this, most of the times it is denigrated however it has a caloric value at 16 MJ ME/Kg DM (Oerke et al., 1994). Cassava leaves are high in CP, ranging from 16.7 to 39.9% which compares favourably with forages generally regarded as good protein sources such as lucerne with a crude protein content ranging from 16.9 to 30% (Antoniewicz et al., 1995; García et al., 1995). In Africa, scientists have recognized cassava as a crop with high potential as animal feed, just as it has been used in many countries in Europe. As a tradition in many tropical countries, cassava constitutes about 20 to 40% of the feed used by livestock (Oppong-Apane, 2013). This knowledge on the use of cassava for feeding livestock needs to be enhanced and made available to more people.

Another potential livestock feed is Azolla, commonly known as floating fern that is found in most parts of the world, both the temperate as well as tropical ecosystems (Arora and Singh, 2003). It is particularly common on farm dams and other still water bodies. Azolla has an association that is symbiotic with *Anabaena azollae,* the nitrogen fixing algae, which enables it to be highly productive. It has been claimed that Azolla is one of the fastest-growing plants on Earth (Miranda et al., 2018) with the potential to produce 347 tonnes/ha of fresh material, or an average daily yield of 818 to 955 kg/ha. In a study carried out in India on the effect of Azolla on cattle growth, there was a significant increase the daily live weight gain for the animals supplemented with Azolla as compared to the control (Chatterjee et al., 2013). This study was carried out to determine biomass yield and nutrient contents of Cassava leaves and *Azolla* as well as their effect on growth performance of cattle.

MATERIALS AND METHODS

The experiment was conducted at the KALRO Kiboko research centre in Southeastern Kenya. The centre is approximately 1,000 m above sea level and lies between latitudes 2°10' S and 2°25'S and longitudes 37°40'E and 37°55'E (Hatch et al., 1984). The average annual rainfall is 548 mm and average minimum and maximum temperature is 16.6 and 29.4°C respectively (Jimmy et al., 2017). The annual mean temperature is 23°C and the average evapotranspiration of the site is 2000 mm. Samples of *Azolla* were collected then dried on a paper towel by blotting and then 10 g each were placed in a bucket and grown for 10 days, this was repeated twice. The floating mat of *Azolla* was harvested from each bucket after the 10 days of growth. The harvested material was then dried by blotting with a paper towel before weighing with an electronic balance to determine the biomass. The time taken by the *Azolla* to double (doubling time, Dt) was determined as reported by Badayos (1989), while the relative growth rate (g/g/d) were determined as by Hechler and Dawson (1995):

$$Dt = 0.693t/\ln(wf-wo)$$

$$RGR1\text{-}2 = (\ln wf-\ln wo)/t$$

Where: t = growth duration (days); wf = final biomass (g), and wo = initial biomass (g).

The feeding trial was a completely randomized design which had 24 Small East African Zebu yearlings aged about 18 months and having a mean weight of 110 ± 18.4 kg. The animals were stall-fed for 16 weeks. Two weeks were used for adapting the yearlings to the feeds and assessing the yearlings' intake of the feeds while data were collected for fourteen weeks. The 24 heiferss were randomly assigned to the feeds while the feeds were also randomized among the six treatments. The yearlings were fed such that they received dry matter equivalent to 3% of their body weight. The performance of the heifers was monitored through their body weight gain with weekly weighing done in the morning before feeding. Supplementary feeding with Azolla or cassava leaves was done in the morning (8.00 a.m.). Supplementary materials were fed first to improve on their intake because it was observed that the yearlings preferred grass and not the test materials. One half of the amount of feeding materials (which varied between 2.55 and 4.41 kg depending on treatment) was given at 10.00 a.m. while the other half was given at mid-afternoon (2.00 p.m.) to minimize wastage. Weighed amount of feed was offered to the yearlings and intake determined by collecting and weighing the remains before the next day's feeding. Water was given *ad libitum.* A mineral block (Afya Bora® stock lick) was provided in each enclosure such that the animals had *ad libitum* access. Ticks and flies were controlled using a Pour-on acaricide (Ectopor 020 SA) while disease treatment was done whenever symptoms were observed. All the yearlings were dewormed with Albendazole 10% suspension before the start of the experiment. The treatment feeds were:

Treatment 1 were fed with mixture of range grasses (control)
Treatment 2 were fed *Brachiaria mulato II*
Treatment 3 were fed Range grass plus Cassava leaf meal
Treatment 4 were fed Range grass plus *Azolla*
Treatment 5 were fed *Brachiaria mulato II* plus Cassava leaf meal
Treatment 6 were fed *Brachiaria mulato II* plus Azolla

Samples of the experimental feeds were collected, dried and then ground through a 1.00 mm sieve harmer mill. The samples which were ground were dried at 105°C overnight in the oven to find out its DM. The Kjeldahl procedure (AOAC, 2005), was used to determine the percent crude protein (CP). The neutral detergent fibre (NDF) and the acid detergent fibre (ADF) was evaluated using the Van Soest method (Van Soest et al., 1991). Burning of samples for 8 h at 550°C in a muffle furnace was used to determine the ash content. The mineral contents were determined using Near-infrared spectroscopy (NIRS). The growth performance data of the heifers were analyzed statistically by one-way Analysis of Variance (ANOVA) (Steel and Torie, 1980). Duncan's New Multiple Range Test was used to separate means at 5% significance level. The statistical model used was $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where Y_{ij} is the weight

Table 1. Relative growth rate and doubling time of azolla.

Initial weight (Lwt) of Azolla	Duration in days	Final weight (Fwt) of Azolla	Fwt/Lwt	ln(Fwt/Lwt)	Doubling time (days)	RGR
10	10	40.0	4.00	1.39	5.00	0.139
10	10	33.8	3.38	1.22	5.69	0.122
10	10	40.2	4.02	1.39	4.98	0.139
10	10	57.4	5.74	1.75	3.97	0.175
10	10	40.6	4.06	1.40	4.95	0.140
10	10	42.6	4.26	1.45	4.78	0.145
10	10	54.0	5.40	1.69	4.11	0.169
Average		44.1			4.78	0.147

Table 2. Nutritive content of the experimental feeds (the mineral contents were determined using NIRS).

Chemical composition	DM	%CP	%NDF	%ADF	%P	%Ca	%K	%Mg	%TDN
Brachiaria mulato II	93.6	14.1	59.1	31.7	0.510	0.930	2.21	0.320	60.1
Azolla	93.2	18.8	52.1	44.2	0.150	3.29	2.66	0.450	43.6
Mixed Range Grass	93.7	5.54	58.3	44.7	0.460	0.81	2.18	0.280	50.1
Cassava leaves	93.5	29.0	40.0	29.2	0.610	2.17	2.84	0.470	68.0

gain over time (dependent variable), μ is the overall weight gain mean, α_i is effect of treatment i, and ε_{ij} is a random error.

RESULTS AND DISCUSSION

The doubling time for Azolla was about 5 days while the relative growth rate was 0.15 (Table 1). The findings were different from those of Peters et al. (1980) in which the relative growth rates were found to be 0.35 to 0.39. In another study, Talley and Rains (1980), found the relative growth rate of *Azolla filiculoides,* to be between 0.245 and 0.277 g/g day. In these studies, the doubling of biomass of Azolla took about 2 days. The prevailing conditions of the environment and the growth media and the species of Azolla could be the cause of differences in the growth rates. In another study, Peters (1990) found that Azolla grows well in water that is of shallow depth and of little disturbance. Nutrients' availability to *Azolla* is mainly dependent on water and not the soils (Kushari and Watanabe, 1992).

The nutritive value of the feed materials used in the feeding experiment are shown in Table 2. The range grasses and Azolla had more ADF than Bracharia and Cassava leaves. Cassava leaves had higher levels of phosphorous than the supplement Azolla. Similarly, there were higher levels of potassium in cassava as compared to Azolla. Azolla however had more calcium than cassava leaves. Cassava leaves also had more total digestible nutrients (TDN) as compared to Azolla. Between the two grasses, Bracharia had more TDN than the range grass hay. Bracharia also had more phosphates than Azolla. Azolla had more calcium, potassium and magnesium as compared to Bracharia.

The weekly increase in the weights of the heifers are shown in Figure 1. The performance of the heifers across the six treatments was statistically insignificant ($P > 0.05$). The mean effects of the treatments on the heifers showed that supplementing with Azolla had the highest positive effect followed by cassava and Bracharia supplemented with Azolla. Those fed with Bracharia also had a higher weight gain than those fed on range grasses alone (Figures 1 and 2).

The values for the chemical composition of cassava foliage were within the range of values reported by Alli Ballogun (1995) and Oni et al. (2011). This confirms its potential as a source of supplemental protein to grass characterized by low nutrient levels in assisting the rumen microbes. Therefore, in the rumen, the amount of ammonia generated would be high and promote an efficient digestion process (Orskov, 1995). Norton (2003) observed that optimal rumen microbial activity requires levels of ammonia that can only be provided by feeds whose crude protein content is higher than 8%.

The experimental diets (Table 3), except the range grasses, had a higher crude protein content than 8% which suggested that nutritionally they could be of better quality. It has been shown that intake of protein has an important effect on the performance of ruminants due to elevated levels of available nitrogen that is fermentable and needed by the bacteria in the rumen.

For the rumen to function normally in ruminants, enough insoluble fibre material, which is linked to digestion of cellulose and rumination, is required. Tropical feed intake

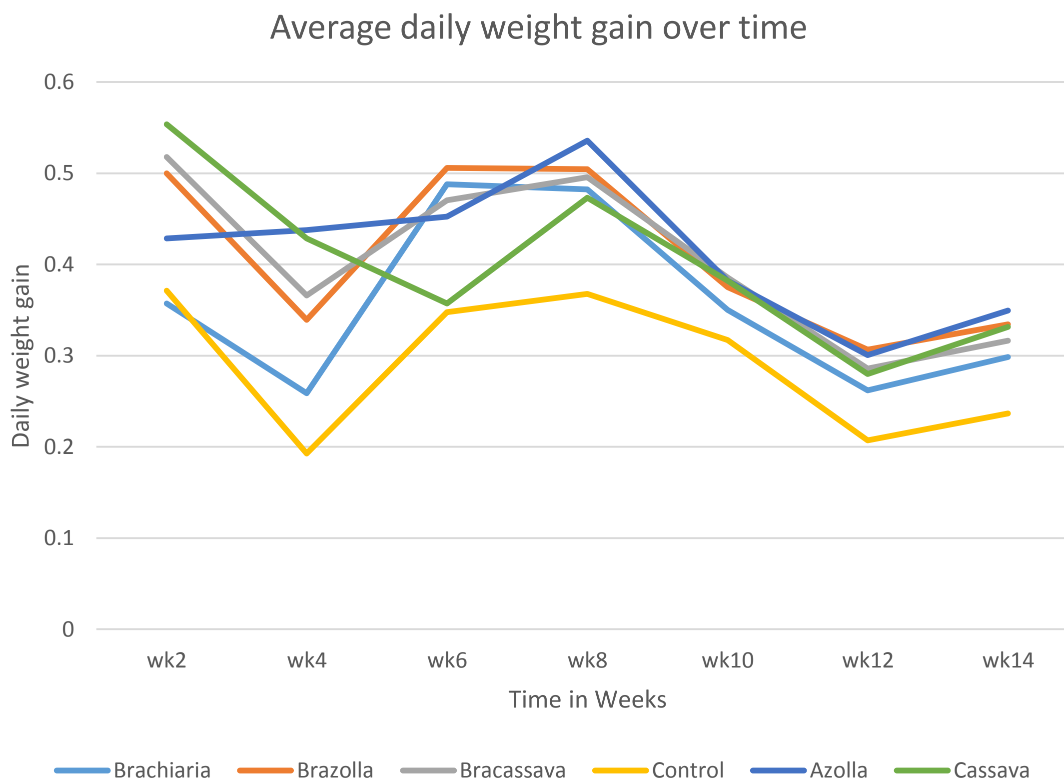

Figure 1. Performance of the zebu heifers fed on bracharia and mixed range grass basal diets supplemented with azolla and cassava leaves.

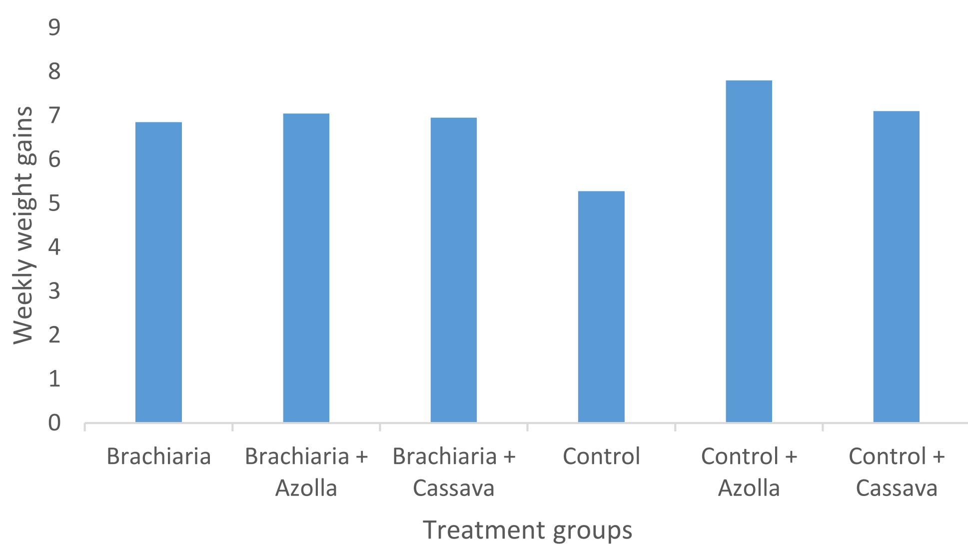

Figure 2. Mean weekly weight gains of the zebu heifers fed on brachiaria or mixed range grass basal diets supplemented with azolla and cassava leaves.

can be limited if the NDF content is above 600 to 650 g/Kg DM (Van Soest et al., 1991), of which the experimental diets were below this range. Improved digestibility of feeds as well as intake and better animal performance (Klopfenstein et al., 2001) is enhanced by moderate fibre levels which in turn facilitates the microbial

Table 3. Percentage composition of the six treatment diets fed to the heifers.

Treatment	Type of feeds used	Composition	% Dry matter intake
1	Range grasses	100%	2.9
2	*Brachiaria mulato II*	100%	3.3
3	Range grass plus Cassava leaf meal	74:26	3.25
4	Range grass plus *Azolla*	64:36	3.2
5	*Brachiaria mulato II* plus Cassava leaf meal	94:6	3.5
6	*Brachiaria mulato II* plus Azolla	91:9	3.4

organisms to colonize the ingesta in the rumen. The NDF, ADF and ADL and their concentrations in the diets affects the intakes of cellulose, hemicellulose and diet digestibility (Harper and McNeill, 2015). The fermentation rate and amount of feed consumed is reduced by too much NDF although very low levels of fibre can result in very rapid fermentation in the rumen which result in acidosis. It was observed that the groups of heifers supplemented either with Azolla or cassava leaves tended to finish their allocated feeds. The observed high total DM intake among some of the treatments corroborates earlier findings that increasing level of cassava leaf supplementation improves intake of both dry matter and nitrogen in goats and cattle fed a grass diet and supplemented with ammoniated rice straw and rice straw with para grass, respectively (Do et al., 2002; Sath et al., 2013). The low DM intake observed in heifers fed solely on range grass hay could be due to the low level of crude protein in the range grass compared to the other supplemented treatments as low protein content diets have been determined to cause reduced intake by animals (Ifut, 1988).

According to Pillai et al. (2002), there was an increase in milk yield by between 15 and 20% when commercial feeds were mixed with 1.50 to 2 kg of Azolla. About 15 to 20% of Azolla can replace similar quantity of commercial feeds such as oil cake, without any change in milk yield (Pillai et al., 2002). Also, it was realized that feeding Azolla leads to an improvement in the quality of milk as well as longevity and the health of animals. The nutritive value of Azolla compares favourably with other high valued protein sources. As indicated in Table 2, above, it has crude protein of up to 26.3%. This makes Azolla a good candidate for feeding animals. As reported by Sculthorpe (1967), large quantities of *Azolla* have been harvested in various parts of Africa and Asia and used to feed cattle and pigs.

Conclusion

The supplementation of range grasses with Azolla and cassava leaves improved feed intake, digestibility, and weight gain in the heifers. This opens an opportunity for smallholder farmers rearing cattle as an alternative supplement for their animals. Azolla which has a potential to doubles its weight every 3 or so days should be further explored as an alternative source of protein especially in areas where there are surface water bodies such as dams and rice paddies. *Bracharia mulato II* should also be promoted as a good substitute to range grasses especially due to its high CP value.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of nitrogen rate and intra-row spacing on yield and yield components of maize at Bako, Western Ethiopia

Begizew Golla[1*], Adugnaw Mintesnot[2] and Merkeb Getachew[2]

[1]Bako National Maize Research Center, Ethiopian Institute of Agricultural Research, P. O. Box 2003, Addis Ababa, Ethiopia.
[2]Department of Horticulture, Jimma University, P. O. Box 307, Jimma, Ethiopia.

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Maize is highly responsive to nitrogen fertilizer rates and plant spacing. Continuous efforts have been carried out to improve the yield potential of maize by modifying their architecture through breeding methods with aim of increasing maize yield through increasing number of plants ha^{-1}. Hybrid BH-546 is the recently released variety for this purpose but its optimum nitrogen rate and spacing has not been determined yet. Thus, a field experiment was conducted at Bako research farm in the year 2017 to determine the optimum rate of nitrogen fertilization and intra-row spacing. The experiment was laid out in a randomized complete block design in factorial arrangement with three replications. Three intra-row spacing *viz*., 75 cm × 40 cm, 75 cm × 30 cm and 75 cm × 20 cm accommodating 33,333, 44,444 and 66,666 plants ha^{-1}, respectively, with six nitrogen levels *viz*., 0, 23, 46, 69, 92 and 115 kg ha^{-1}, were assigned to the experimental plot by factorial combinations. Based on the results, the maximum grain yield (10,208 kgha^{-1}) was obtained when the hybrid was sown at the closest intra-row spacing (20 cm) with application of the highest rate of nitrogen (115 kg ha^{-1}). This result showed 8.9% yield advantages compared to the standard check. However, statistically similar grain yield (9887 kgha^{-1}) was also obtained under application of 92 kg N ha^{-1} in the same intra-spacing (20 cm). Moreover, since this experiment was conducted for one season and in one location, the comprehensive recommendation could be drawn by investigating more locations over years for this maize hybrid.

Key words: BH 546, plant density, yield, yield components.

INTRODUCTION

Though maize adapts in wide ranges of agro-climate condition and plays critical role in food security (CSA, 2017), the production and productivity of maize is highly influenced, among several factors, by nitrogen rate and plant density (Gözübenli, 2010; Shrestha, 2013). The importance of nitrogen for plant growth and productivity is increasingly being recognized, and in many cases it is considered as the most growth and yield limiting factor. The yield potential of maize is highly influenced by the amount of intercepted solar radiation, water, and nitrogen supply (Birch et al*.,* 2003). The photosynthetic efficiency of leaves depends on nitrogen concentration in leaves

*Corresponding author. E-mail: begizew06@gmail.com.

(Birch and Vos, 2000; Paponov et al*.,* 2005). N-fertilization provides sufficient nutritional requirements of maize plants and hence promotes its grain production (Khan et al*.,* 2012).

Besides plant nutrients, plant spacing significantly affects the crop yield as maize does not have tillering capacity to adjust to variation in plant stand. Plant reduction per unit area prevents maximum use of production parameters, while excessive density can increase the competition and decrease the yield (Farnia et al*.,* 2015). Thus, yield increment was observed with increasing plant density up to optimum for a maize genotype grown under a set of particular environmental and management conditions (Tollenaar, 1992; Gözübenli, 2010; Sharifai et al*.,* 2012).

However, there is no single recommendation for all conditions because optimum density varies depending on resource availability and the tolerance of a hybrid to intra-specific competition (Tollenaar and Wu, 1999; Sangoi et al., 2001). Some modern maize hybrids withstand stresses better than the earlier cultivars, and are grown at higher plant populations to maximize the interception of solar radiation (Tollenaar, 1991). The newly released hybrid, BH-546 is one of such cultivars which could be managed at higher densities to exploit its yield potential as it possesses semi erected leaf nature and narrow leaf size (BNMRS, 2014)*.* However, this opportunity of leaf architecture for increasing yield by increasing plant density is not yet determined at study area. Thus, to achieve potential economic yield of BH-546 variety modification of plant spacing with appropriate N-fertilizer rate could be determined. Keeping this in mind this study was designed with the objective of determining the optimum intra-row spacing and N- fertilizer level for BH-546 maize variety at Bako agro ecological conditions.

MATERIALS AND METHODS

Description of experimental site

The experiment was conducted at the research site of Ethiopia Institute of Agricultural Research, Bako, located at 9° 06' N and 37° 09' E, with altitude of 1650 m.a.s.l, in western Ethiopia, in the year 2017. The site represents a mid-altitude sub-humid agro ecological zone. The total annual rainfall during 2017 season was 1598.0 mm. In this season, there was a rain throughout the year except January and December, but sufficient amount of rainfall for crop growth was recorded from April to September, with maximum precipitation (291.3 mm) in August and minimum precipitation (146.5 mm) in May, within this interval. The area had also mean minimum, mean maximum and average annual temperatures of 12.8, 29.0 and 20.9°C respectively; and humidity ranged from 46 to 57% in the year of 2017 (Bako Agricultural Research Center, Ethiopia, Metrological Data, 2017). The soils of the area are dominantly nitosol.

Experimental materials (maize variety and fertilizers)

Hybrid maize variety BH-546 was used for the study. BH-546 is intermediate maturing variety released by Bako National Maize Research Center (BNMRC) in 2013. It gives 8500-9500 and 5500-7000 kg ha^{-1} grain yield on-station and on-farm experiments, respectively (BNMRC, 2014). This hybrid has narrow and erected leaf architecture which makes it unique compared to other hybrids. Nitrogen fertilizer in the form of urea (46% N) and phosphorous fertilizer in the form of Triple Superphosphate (46% P_2O_5) were used for the experiment.

Treatments and experimental design

The treatments consisted of six rates of nitrogen (0, 23, 46, 69, 92 and 115 kg N ha^{-1}), fixed inter row spacing of 75 cm, and three intra-row spacing of 40 cm, 30 cm, and 20 cm with corresponding plant population of 33,333, 44,444 and 66,666 plants ha^{-1}, respectively. The treatment set up that contain 75 × 30 cm spacing with 92 kg N ha^{-1} was used as a standard check, whereas 0 kg N ha^{-1} was used as negative control. The experiment was laid out in randomized complete block design (RCBD) in factorial arrangement with three replications. The gross plot size was 4.8 m × 3.0 m (14.4 m^2) with row length of 4.8 m, and net plot size 4.8 m × 1.5 m (7.2 m^2) was used for harvesting. The treatments were randomly assigned to the experimental unit within a block (replication). The blocks were separated by 2 m wide space.

Soil sampling and analysis

A representative soil samples were taken from 0 to 30 cm depth in a diagonal pattern among each 5 m interval before planting by vertical insertion of an auger. The sample soil was analyzed at soil laboratory for physical and chemical properties of soil using standard laboratory procedures and the result is summarized in Table 1. The experimental field was used for maize cultivation for the last five consecutive cropping seasons. Due to this reason the laboratory result of soil analysis indicated that lower amount of organic carbon, organic matter and total nitrogen as well as reduction in pH value (Table 1).

Experimental procedures

Land preparation was done by plowing three times using tractor plough during March to May 2017. Planting was done in June 2017. Full dose of phosphate fertilizer in the form of Triple Superphosphate (TSP) at the national recommended rate of 69 kg P_2O_5 ha^{-1} was applied uniformly to all plots at the time of sowing. Half dose of N fertilizer as per the treatments was applied at sowing time and the remaining half dose of N fertilizer was applied 4 weeks after sowing. Weeding and other crop management practices were applied uniformly to all plots as per the common experience of the farm. Finally, maize plants in the central net plot area were harvested at harvest maturity stage (December 06/2017) for the analysis.

Crop data collection and measurement

Data were collected from ten randomly selected samples per plot for parameters of number of ears per plant, ear length (cm), number of kernel rows ear^{-1} and number of kernels ear^{-1}. Thousand grain weights (g) were determined from 1000 randomly taken kernels from each plot and weighed using sensitive balance. All maize plants from the net plot area were mowed at the ground level, at harvesting maturity and weighed after sun drying to a constant weight to obtain above ground dry biomass. The total numbers of ears in the net plot were harvested and the field weight was

Table 1. The results of soil physical and chemical properties before planting at Bako Research Center during 2017.

Characteristic of soil	Chemical characteristic of soil						Texture		
	pH (1:2.5H_2O) suspension	OC (%)	OM (%)	TN (%)	AP (ppm)	CEC (cmol 100 g^{-1} soil)	Clay (%)	Silt (%)	Sand (%)
Value	5.01	0.88	1.51	0.05	13.33	17.17	43	8	49
Test method	Potentiometer	Walkley-Black	Walkley-Black	From %OC	Bray II	Ammonium acetate method	Hydrometer		
Rating	Strongly acid	Low	Low	Low	Medium	Moderate	-		
Textural class	-	-	-	-	-	-	Sandy Clay		

OC: Organic carbon, OM: organic matter, TN: total nitrogen, CEC: cation exchange capacity, AP: available phosphorus, pH: the negative logarithm of the hydrogen ion activity, ppm: parts per million.

measured using electronic balance after removing the husk. Grains were shelled from center of some ears of each plot and their moisture content was immediately measured using moisture tester. The measured values were adjusted to the standard moisture content of 12.5% (Biru*,* 1976)*,* and then multiplied by field weight and shelling percentage /0.8/ to determine the adjusted yield of the plot on hectare basis using the following formulas:

$$\text{Correction factor} = \frac{\text{100-Actual moisture content}}{\text{100-12.5}}$$

Grain Yield kg plot^{-1} = (100-Actual Moisture Content/87.5)×Field weight (kg)×Shelling percentage

Grain Yield kg ha^{-1} = Yield (kg/plot)×10000/Plot size (m^2)

Harvest index was then calculated as

$$\text{Harvest Index} = \frac{\text{Grain yield}}{\text{Above Ground Dry Biomass}} \times 100$$

Statistical analysis

Analyses of variances for the data recorded were conducted using the SAS version 9.3. Least significant difference (LSD) test (5%) was used for mean separation if the analysis of variance indicated the presence of significant treatment differences. Correlation analysis was made to examine the association among yield and yield related parameters.

RESULTS AND DISCUSSION

Yield and yield components of maize

The analysis of variance showed that the main effect of nitrogen fertilizer application rate and intra-row spacing highly significantly (P<0.01) influenced the ear length, number of kernel rows per ear, number of kernels per ear, thousand grains weight and harvest index, but the interaction effect of the two factors was not significant. Likewise, number of ears per plant was highly significantly (P<0.01) influenced by intra-row spacing. However, the interaction effect of N rate and intra-row spacing were not significantly influenced by these parameters. Similarly, the main effect of N rates was not significantly influenced by number of ears per plant. The statistical analysis also revealed that both the main effects (rate of nitrogen and intra-row spacing) and their interaction highly significantly (P<0.01) influenced grain yield of maize. Similarly, above ground dry biomass was highly significantly (P<0.01) influenced by the main effects of nitrogen fertilizer rates and intra-row spacing, and the interaction of the two factors was also significantly (P < 0.05) influenced this parameter (Tables 2, 3 and 4).

Number of ears per plant

Increase in plant spacing resulted to significant increment in number of ears per plant (NEPP) and the highest NEPP (1.50) was produced in the widest intra-row spacing (40 cm), while the lowest NEPP (1.08) was recorded at the narrowest intra-row spacing (20 cm) (Table 2). The reduction of ear number from each plant in response to narrow plant spacing can be attributed to more intra-specific competition for growth limiting factors such as nutrients, water, light and aeration. In such conditions plants may utilize most of such limiting growth factors for vertical growth instead of horizontal growth and consequently plants can be able to develop only one or few functional ears (ear with grain), even though some plants initially emerge more than one ear shoots. The present results are in line with the findings of Muniswamy et al*.* (2007), Karasu (2012) and Abuzar et al. (2011).

Ear length

The ear length was increased with increase in

Table 2. Effect of different nitrogen rates (NR) and intra-row spacing (IRS) on yield components and harvest index of maize during 2017/2018 cropping season.

Treatment	No. of ear plant^{-1}	Ear length (cm)	No. of kernel rows ear^{-1}	No. of kernels ear^{-1}	Thousand grain weight (g)	Harvest index (%)
NR (kg ha^{-1})						
0	1.23^{c}	15.50^{d}	14.67^{c}	535.87^{d}	303.56^{c}	32.98^{c}
23	1.27^{bc}	15.80^{d}	14.81^{bc}	548.73^{d}	311.56^{c}	33.03^{c}
46	1.33^{ab}	16.80^{c}	15.00^{abc}	564.02^{c}	317.00^{bc}	34.75^{b}
69	1.31^{abc}	17.37^{b}	15.21^{ab}	595.13^{b}	322.22^{bc}	38.26^{a}
92	1.34^{ab}	18.26^{a}	15.32^{a}	608.78^{ab}	340.89^{ab}	39.02^{a}
115	1.38^{a}	18.62^{a}	15.44^{a}	621.63^{a}	353.56^{a}	38.94^{a}
Significance	ns	**	**	**	**	**
LSD (0.05)	0.10	0.37	0.45	13.94	25.63	1.63
IRS (cm)						
20	1.08^{c}	16.50^{c}	14.76^{b}	563.69^{c}	303.28^{b}	33.99^{b}
30	1.36^{b}	16.95^{b}	15.13^{a}	581.77^{b}	329.00^{a}	36.88^{a}
40	1.50^{a}	17.72^{a}	15.34^{a}	591.63^{a}	342.11^{a}	37.62^{a}
Significance	**	**	**	**	**	**
LSD (0.05)	0.07	0.26	0.32	9.86	18.12	1.15
CV	8.27	2.26	3.10	2.51	8.24	4.69

Means followed by the same letter within column are not significantly different (P<0.05).

nitrogen rates. The longest ear 18.62 cm was recorded from treatments of 115 kg N ha^{-1} application, but it was statistically at par to ear length (18.26) which was obtained under 92 kg N ha^{-1} application. The shortest ear length (15.5 cm) was obtained at control (treatment without N application). However application of 23 kg N ha^{-1} had not brought any significant different compared to the control (Table 2). The increase in ear length in response nitrogen rates might be due to better availability of nutrients in the soil system so that the maize plant expressed fully its yield potential and produce longest ear under high rate of N. These results are in agreement with those of Sharifi and Taghizadeh (2009) and Sharifi and Namvar (2016).

The results also revealed that different intra-row spacing significantly affected the ear length of maize crop. The highest ear length (17.72 cm) was produced at wider intra-row spacing of 40 cm, while the lowest ear length (16.50 cm) was obtained at the narrowest intra-row spacing (20 cm) (Table 2). The reduction of ear length in the narrow plant spacing might be due to crowded stress and series competition among plants and among different organs within a plant for growth limiting factors (water, nutrients, air and light). These results are in line with the previous findings (Azam et al., 2017; Zamir et al., 2011).

Number of kernel rows per ear (NKRPE)

The maximum NKRPE (15.44) was recorded at an application of N in rate of 115 kg N ha^{-1}, but statistically similar row numbers per ear (15.32, 15.21 and 15.00) were also obtained under application of 92, 69 and 46 kg N ha^{-1}, respectively. The lowest NKRPE (14.67) was recorded from the control treatment, but it was statistically similar to 14.81 and 15.00 which was obtained at application of 23 and 46 kg N ha^{-1} (Table 2). This increment in NKRPE in response to nitrogen rates might be attributed to better availability of nutrient in the soil so that plants are enabled to grow vigorously and produce fully viable big ear which can carry several number of kernel rows on it. These results are in agreement with those of Aghdam et al. (2014) who reported that the use of urea fertilizer increased corn growth stages and development, which has increased the number of grain rows per ear of corn.

Main effect of intra-row spacing has also significant effect on the NKRPE. The maximum NKRPE (15.34) was noted when maize hybrid was sown at 40 cm plant spacing, but it was statistically at par with 15.13 which was obtained under 30 cm intra-row spacing. The lowest NKRPE (14.76) was recorded from narrowest intra-row spacing (20 cm) (Table 2). This could be probably crowded stress and intense competition for growth resources under narrow spacing. These results coincide with the finding of Azam et al. (2017) and Abuzar et al. (2011) who stated that number of grain rows per ear decreased as the plant population increased.

Number of kernels per ear (NKPE)

The mean comparison of the result indicated that the maximum mean NKPE (621.63) was recorded from the application of the maximum rate of N fertilizer (115 kg N ha^{-1}); however, this was statistically at par with NKPE of 608.78 produced under the rate of 92 kg N ha^{-1} application. The minimum NKPE (535.87) was produced under no N application, which was statistically similar with 548.75 produced under application of 23 kg N ha^{-1} (Table 2). This increment in NKPE in response to N application rates might be attributed to increased N availability in the soil which is required by plant in large quantity directly for growth purpose and indirectly to make available other nutrients essential for seed setting and seed development such as phosphorus and potassium (Brady, 1984). These results are in agreement with those of many other workers (Sharifi and Taghizadeh, 2009; Khan et al., 2012; Moosavi, 2012).

The main effect of intra-row spacing was also significant (P<0.01) and the highest NKPE (591.63) was recorded in the widest intra-row spacing (40 cm) followed by 30 cm (581.77). The lowest NKPE (563.69) was harvested from the narrowest intra-row spacing (20 cm) (Table 2). The decrease in the number of kernels per ear at narrow intra-row spacing could be attributed to plant competition at crowded plant stands leading to less availability of nutrients for grain formation and development. The result was in agreement to Maddonni and Otegui (2006), Azam et al*.* (2017), and Gozubenli et al*.* (2004) who reported that the grain yield of a single maize plant is reduced by proximity to its neighbors.

Thousand grain weight (TGW)

Nitrogen rates significantly increased TGW. The maximum TGW (353.56 g) was recorded in plots that were supplied 115 kg N ha^{-1} which is statistically similar with grain weight of 340.89 g produced under application of 92 kg N ha^{-1}. The minimum TGW (303.56 g) was recorded from control. The plots of those that received 23, 46 and 69 kg N ha^{-1} had not shown any significance difference in TGW compared to control (Table 2). However, numerically there was increasing trend in TGW with increasing N rate. The possible reason for increase in TGW with increased in N application rate could be due to positive effect of N on the growth of maize, increasing leaf area index and duration extending grain-filling period that allowed accumulating more photosynthetic assimilates in the grains. These results coincide with the finding of most researchers (Moosavi, 2012; Hafez and Abdelaal, 2015; Anwar et al*.*, 2017).

The intra-row spacing also had significant (P<0.01) effect on TGW. The maximum TGW (342.11 g) was recorded in a treatment where maize seed was sown in plant spacing of 40 cm, but it was statistically at par with 329.00 g which was obtained at 30 cm intra-row spacing. The lowest TGW (303.28 g) was produced at the narrowest plant spacing (20 cm) (Table 2). Reduction in TGW in response proximity of stands could be due to competition for essential nutrients and other growth limiting resources, whereas increasing intra-row spacing provides better opportunity for crop to utilize and assimilate available resources with less competition; so this might be the reason for producing higher TGW under widest plant spacing. These results agree with finding of Zamir et al*.* (2011), Khan et al*.* (2017) and Azam et al*.* (2017).

Harvest index

The harvest index was increased with increase in N rates and attained the maximum value (39.02%) at rate of 92 kg N ha^{-1} application, but it was statistically similar to harvest indices of (38.26 and 38.94%) obtained under application of 69 and 115 kg N ha^{-1}, respectively. The lowest HI (32.98%) was recorded at a plot with no N application; however, this was statistically similar with HI (33.03%) which was obtained under application of 23 kg N ha^{-1} (Table 2). In these results, we observed that application of insufficient N rate (23 kg N ha^{-1}) and or further increasing N rates beyond the optimum (more than 92 kg N ha^{-1}) did not bring any significant change in harvest index. The increase in HI due to increase in N application rate may be associated with a pivotal role of N in many physiological and biochemical processes during growth and development period that enable the crop to allocate much dry mater into grain which has direct relationship to HI. On the other hand, further increase in N application rates might have enhanced more vegetative growth that resulted to reduction in the ratio of grain to total biological yield (HI). These results coincide with the finding of Aghdam et al*.* (2014), Sharifi and Namvar (2016) and Anwar et al*.* (2017).

Likewise, HI was increased with increase in intra-row spacing and the maximum HI (37.62%) was recorded at the widest intra-row spacing (40 cm). But it was not significantly different from HI (36.88%) obtained under 30 cm intra-row spacing. The lowest HI (33.99%) was obtained at 20 cm intra-row spacing (Table 2). This reduction in HI in response to closeness among plant stands might be attributed due to high competition for light so that plant rapidly increase in plant height and allocate much of the dry mater into stalk that resulted to reduction in the ratio of grain to total dry matter. These results are in agreement with Arif et al*.* (2010), Nik et al*.* (2011) and Akhtar et al*.* (2015).

Grain yield

The ultimate goal of crop production is increasing economic yield. Maximum grain yield (10208 kg ha^{-1}) was achieved at the intra-row spacing of 20 cm with the application of 115 kg N ha^{-1}, but it was statistically similar

Table 3. Effect of different nitrogen rates and intra-row spacing on grain yield of maize (kg ha^{-1}) during 2017/2018 cropping season.

IRS (cm)	NR (kg ha^{-1})					
	0	**23**	**46**	**69**	**92**	**115**
20	6359	6392^{h}	7586^{ef}	8710^{d}	9887^{ab}	10208^{a}
30	6619^{h}	6711^{h}	7525^{fg}	8794^{cd}	9374^{bc}	9544^{b}
40	6520	6591^{h}	6906^{gh}	7712^{ef}	8234de	8203de
LSD (0.05) N × IRS			648			
CV (%)			4.27			

Means followed by the same letter within column are not significantly different (P<0.05).

Table 4. Effect of different nitrogen levels and intra-row spacing on above ground dry biomass of maize (kg ha^{-1}) during 2017/2018 cropping season.

Intra-row spacing (cm)	N rate (kg ha^{-1})					
	0	**23**	**46**	**69**	**92**	**115**
20	21773^{ef}	21843^{ef}	23250^{cd}	23843^{c}	25833^{b}	26806^{a}
30	19597^{h}	19972^{gh}	21194^{f}	22421de	23843^{c}	23972^{c}
40	18139^{j}	18250^{hj}	19106^{hi}	19750^{h}	20889^{fg}	21065^{f}
LSD (0.05) N × IRS				957		
CV (%)				2.47		

Means followed by the same letter within column are not significantly different (P<0.05).

with (9887 kg ha^{-1}) that was produced under the rate of 92 kg N ha^{-1} application for the same intra-row spacing (20 cm). The minimum grain yield (6359 kg ha^{-1}) was obtained under 0 kg N ha^{-1} at 20 cm intra-row spacing, but statistically similar grain yield were obtained under application of 0 and 23 kg N ha^{-1} at all intra-row spacing including 46 kg N ha^{-1} application in case of 40 cm intra-row spacing (Table 3). Compared to the standard control of the intra-row spacing 30 cm (44444 plants ha^{-1}) with the application of 92 kg N.ha^{-1}, the mean grain yield was increased by 8.90% when the maize hybrid was sown at intra-row spacing of 20 cm with application 115 kg N ha^{-1}.

The increase in maize grain yield under decreased spacing might be due to efficient utilization of available resources (nutrient water and light). Higher grains yield at higher nitrogen levels might be due to the lower competition for nutrient and positive effect of N on plant growth, leaf area expansion and thus increased solar radiation use efficiency that ultimately increases in grain yield. These results are in line with that of Gözübenli (2010) and Shrestha (2013).

Above ground dry biomass (AGDBM)

Application of 115 kg N ha^{-1} to maize hybrid planted at intra-row spacing of 20 cm gave the highest AGDBM yield (26806 kg ha^{-1}) followed by (25833 kg ha^{-1}) which was obtained under application of 92 kg N ha^{-1} in the same intra-row spacing. The lowest AGDBM yield (18139 kg ha^{-1}) was obtained at the widest intra-row spacing (40 cm) without N fertilizer application, but this was statistically similar with 18250 kg ha^{-1} which was harvested under 40 cm intra-row spacing with application of 23 kg N ha^{-1}. Application of 115 kg N ha^{-1} to maize hybrid planted at intra-row spacing of 20 cm gave 12.4% more AGDBM yield compared to standard check (Table 4).

The increase in AGDBM yield with the increase in nitrogen rates at closest intra-row spacing could be due to the fact that an increment in the N level increased its availability in the soil, so that the nutrient requirement of the dense stands can be optimized. On the other hand, plant grown on close spacing efficiently utilize available nutrients that resulted to increased height of each individual plant, increased leaf area index and helping to capture more light that will enable the plant utilize photosynthate more efficiently, ultimately leading to accumulation of high amount dry matter. The present results are in line with that of Moraditochaee et al. (2012) and Imran et al. (2015) who obtained maximum biological yield under maximum plant density with application of maximum N- rate and the minimum biological yield under lower plant density with no N-application.

Correlation analysis among AGDBM, yield and yield components of maize

The correlation study indicate that above ground dry

Table 5. Correlation coefficient among growth and yield parameters of maize during 2017/2018 cropping season.

Correlation	NEPP	EL	NKRPE	NKPE	TGW	GY	AGDBM	HI
NEPP	1	0.52**	0.40**	0.43**	0.60**	-0.001^{ns}	-0.46**	0.54**
EL		1	0.54**	0.89**	0.64**	0.71**	0.31*	0.83**
NKRPE			1	0.55**	0.39**	0.33*	0.01^{ns}	0.54**
NKPE				1	0.57**	0.71**	0.35**	0.78**
TGW					1	0.40**	0.004^{ns}	0.66**
GY						1	0.81**	0.72**
AGDBM							1	0.18^{ns}
HI								1

**Highly significant, *Significant, ns: non-significant.

biomass was highly and positively correlated with NKPE (r=0.35) and GY (r=0.81). It was also correlated significantly (r=0.31) with EL. But NEPP was highly and negatively correlated (r=-0.46) with this parameter (Table 5). This indicated the effect of intra-row spacing (that is, when the intra-row spacing increased the AGDBM decreased, while the NEPP increased). AGDBM was not significantly correlated with other parameters. Similarly, grain yield was highly and positively correlated with EL (r=0.71), NKPE (r=0.71), TGW (r=0.40) AGDBM (r=0.81) and HI (r=0.72). It was also positively and significantly correlated with NKRPE (Table 5). In general, HI and all of the yield components were positively and significantly correlated with GY except that of NEPP. This indicates that the GY was positively affected by most of the parameters as it is the end result of many complex morphological and physiological processes. The main case of negative correlation between GY and NEPP is similar to the case mentioned for AGDBM.

This correlation indicates that maize production was highly influenced by the yield component parameters. These parameters can be enhanced by optimizing nitrogen fertilizer rate and plant spacing which ultimately increased GY of maize.

Conclusion

Maize shows high positive responses to plant density and nitrogen. So, modification of these parameters significantly influence yield and yield components of maize. Based on the results obtained in this experiment, maize hybrid BH-546 produced the highest grain yield when the hybrid was sown at 20 cm intra-row spacing with application of either 115 kg N ha^{-1} or 92 kg N ha^{-1}.

Furthermore, the experiment indicated an additive trend of grain yield with increasing N rate and decreasing intra-row spacing. So further modification of N rates upward and intra-row spacing downward might further increase the grain yield.

More importantly, the grain yield was highly significant and positively correlated with ear length, number of kernels per ear, thousand grain weight, above ground dry biomass and harvest index.

The recommended intra-row spacing (30 cm) with 92 kg N ha^{-1} is insufficient for hybrid BH-546 maize cultivation. So closer intra-row spacing (20 cm) with 92 or 115 kg N ha^{-1} is suitable for the higher yield of hybrid maize BH-546 during main seasons at Bako. However, these results were based on single growing season; thus their confirmation should be done by further similar experiments across locations and across years.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Gene action analysis studies for agronomic traits in cassava (*Manihot esculenta* Crantz) genotypes developed using line by tester mating design

Kumba Yannah Karim[1,3], Prince Emmanuel Norman[1,3]*, Daniel Dzidzienyo[3], Beatrice Ifie[3], Peter Kulakow[2], Ismail Rabbi[2], Lucky Omoigui[2] and Elizabeth Parkes[2]

[1]Sierra Leone Agricultural Research Institute, Tower Hill, Freetown PMB 1313, Sierra Leone.
[2]International Institute of Tropical Agriculture, Ibadan PMB 5320, Nigeria.
[3]West Africa Centre for Crop Improvement, College of Basic and Applied Sciences, University of Ghana, P. O. Box LG 30, Legon, Accra, Greater Accra, Ghana.

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Despite the significance of cassava as food, feed and industrial root crop, little is known regarding the gene action determining root dry matter content (RDMC), fresh root yield, and tolerance to cassava mosaic disease (CMD), cassava green mite (CGM), and cassava mealy bug (CMB). Thus, a study was conducted to determine the general and specific combining abilities for disease, pest, RDMC, root yield and related traits by crossing 10 parents in a 6 × 4 line by tester design. The F_1 progenies and their parents were assessed in-field in a randomized complete block design (RCBD) with three replicates. Findings implied sufficient genetic variability for all traits studied. Family TMEB419×IBA030305 had the highest RDMC of 35.47%, whilst family TMEB7×IBA0000203 had the least RDMC (23.87%). Genotypes IBA020588, IBA916132 and TMEB419 were the best parents for improvement of harvest index (HI) and RDMC due to its high positive and significant GCA effects. Genotype IBA000203 contributed the highest to increased plant height, whereas TMEB1, TMEB47 and ZAR010116 had significant negative GCA effects. ZAR010116 was the best tester for HI. Families TMEB778×ZAR010116 (34.23) and IBA020588×ZAR010116 (32.78) were the best performing families for mean RDMC, with parent ZAR010116, exhibiting the highest GCA effect for RDMC. Families TMEB419×ZAR000156, IBA916132×ZAR000156 and IBA020588×IBA000156 had low mean CMD scores of 1.1, 1.2 and 1.2, respectively. The preponderance of non-additive gene actions indicated that selection of superior plants should be postponed to later generation.

Key words: Cassava, genetic improvement, agronomic traits, combining ability, heritability, progeny performance.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important staple food crop consumed by over 800 million people

*Corresponding author. E-mail: pnorman@wacci.ug.edu.gh.

worldwide (Esuma et al., 2019). Cassava has varying food, feed and industrial applications that support many livelihoods around the world. In Sierra Leone, cassava is the most consumed root crop. Cassava fresh storage roots are enrich in starch and possess small amounts of calcium (16 mg/100 g), phosphorus (27 mg/100 g), vitamin C (20.6 mg/100 g), minute quantities of protein and other nutrients (USDA, 2016). Moreover, the leaves of cassava are consumed in the country as vegetables since they contain protein such as lysine, but lack the amino acid methionine and possibly tryptophan (FAO, 2010). Other cassava products utilized in the country include cassava pellets for animal feed, cassava starch for sweeteners, thickeners and textile paper industry (Chipeta et al., 2013).

Despite its enormous significance, increased cassava productivity is fraught with a number of biotic and abiotic factors (Kintché et al., 2017). Biotic constraints such as cassava green mite can cause about 15 and 73% yield losses in resistant and susceptible genotypes of cassava, respectively (Bellotti, 2002). Cassava mealy bug damage caused about 88% yield loss in susceptible genotype of cassava (Bellotti et al., 1987). Host plant resistance is strongly advocated for the control of pests and diseases than the continual utilization of pesticides due to its adverse environmental effects on the ecosystem and unsustainability for low-income small-scale farmers (Bellotti, 2002). Environmental variability is also known to contribute to low yields of crops.

In traditional farming systems, cassava is cultivated by stem cuttings and in multiple cropping with other crops, which makes pest control difficult with consequent low yields and wide gap between potential and realized yields. In cassava breeding programmes, botanical seeds are generated through sexual recombination in the first stage (Ceballos et al., 2016). This is done to break the conventional clonal propagation of highly heterozygous cassava genotypes through crossing leading to botanical seed production and increased genetic variation (Grüneberg et al., 2009). Each plant grown from botanical seed is a potential new variety studied during the selection cycle (Ceballos et al., 2016). The efficiency of selection depends on the breeders' ability to identify useful variability created in existing and new improved populations. A robust breeding strategy that utilizes locally adapted cultivar(s) with wide adoptability in crosses for their genetic improvement for desired traits of interests may help the breeder in identifying the fewer useful crosses needed (Witcombe and Virk, 2001). Such few but "smart or clever" crosses, involving parents with desired complimentary traits is an established concept utilized in the generation of useful genetic diversity in cassava (Manu-Aduening et al., 2013).

The line × tester mating design is one of such breeding strategies that simultaneously produces both full-sibs and half-sibs. The design provides specific combining ability (SCA) of each cross, and general combining ability (GCA) of the lines and testers, with both the lines and testers exhibiting different sets of genotypes (Farhan et al., 2012). The concept of combining ability was first introduced by Sprague and Tatum (1942). Combining ability is important in plant breeding because it provides information on the nature and magnitude of gene action and for the selection of parents (Solongi et al., 2019). It comprises two types including the GCA and SCA. The GCA is the mean performance of a parent in a series of crosses, whereas SCA denotes cross combinations that perform relatively better or worse than expected mean performance of lines involved. A significant line × tester interaction provides evidence that the differential ranking of experimental lines depends on the particular tester used (Packer, 2007). This necessitates use of appropriate tester for evaluation of new germplasm lines (Ali et al., 2011). The testers used in a breeding programme may either be genetically narrow or broad-based, related or unrelated to the lines being evaluated or may have high or low frequency of favourable alleles and high or low yielding (Ali et al., 2011). The combining ability between the line and the tester determines the performance of the progenies (Fasahat et al., 2016). Thus, lines with good performances are advanced to the next breeding stage, whereas those with poor combining abilities are discarded.

There is increasing demand on cassava productivity for food, feed and industrial uses that necessitates the continued improvement of cassava for high yield, dry matter content, and food and market quality traits. Improvement of cassava for desired traits require understanding of mode of gene action controlling these traits. In Sierra Leone, no genetic studies have been done on the mode of gene action controlling the expression of pests, diseases, dry matter content, yield and related attributes. Such information would facilitate the efficient selection of superior genotypes and designing efficient cassava improvement programme that incorporate economic traits that enhance sustainable cassava production and productivity of cassava producers in Sierra Leone. The objectives of this study were to (i) determine the general and specific combining abilities and broad sense heritability estimates for cassava mealy bug, cassava green mite, cassava mosaic disease, root dry matter content, fresh root yield and related attributes for selection of elite genotypes; and (ii) determine the phenotypic associations among agronomic traits in cassava.

MATERIALS AND METHODS

Site description

The crossing trial was conducted in early May, 2015 at the Ubiaja (8°29'N, 76°57'E, 64 m altitude) crop site of the International Institute of Tropical Agriculture (IITA), Nigeria. The mean annual rainfall at Ubiaja was 1741.2 mm, relative humidity of 86.0 and mean minimum and maximum temperature of 22.5 and 29.1°C,

Table 1. Description of parent genotypes used in the line × tester mating design

Clones	Parent	Source	Characteristics
IBA020588	Line	Ibadan	White
IBA916132	Line	Ibadan	White
TMEB1	Line	Ibadan	White
TMEB419	Line	Ibadan	White
TMEB7	Line	Ibadan	White
TMEB778	Line	Ibadan	White
IBA000203	Tester	Ibadan	White
IBA030305	Tester	Ibadan	White
ZAR000156	Tester	Zaria	White
ZAR010116	Tester	Zaria	White

respectively. The seedling nursery trial was done at the Njala Agricultural Research Centre (NARC) experimental site, Njala (8°06′N latitude and 12°06′W longitude and elevation of 50 m above sea level) southern Sierra Leone during the 2016/2017 cropping season. The mean annual rainfall at Njala was 2525 mm; mean monthly maximum air temperature range from 23 to 29°C; and relative humidity ranged from 80 to 100%.

Plant materials and experimental design

Ten genetically diverse parents comprising six lines (males) and four testers (females) from the genetic gain trial cassava breeding programme at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were used (Table 1). The parents were crossed in a 6×4 line × tester mating design to produce 24 F_1 families. Controlled pollinations were performed following the standard procedures described by Kawano (1980). The F_1 seeds and parents were planted in-field in early May in a randomized complete block design with three replications. Each plot consisted of a single row with seeds planted at a spacing of 0.3 m × 1 m, between and within rows, respectively. Each row comprised 20 seeds. The recommended timely weeding was done.

Data collection

Data collected during the trial included diseases and pests: cassava mosaic disease (CMD), cassava bacterial blight (CBB) and cassava green mite (CGM) at six months after planting (MAP) using a scale of 1-5, where: 1 = no symptoms and 5 = very severe mosaic symptoms (Banito et al., 2007). Data collected at harvest (11 MAP) included: plant height (PHT), harvest index (HI), number of storage root (NSR) and fresh storage root weight (FSRW) (kg plant^{-1}) and root dry matter content (RDMC). For PHT, 10 plants were measured per plot using a meter rule as the distance from the ground to the shoot tip. Storage roots plant^{-1} were counted and weighed to obtain number of storage root (NSR) and fresh storage root weight (FSRW) (kg plant^{-1}), respectively.

The RDMC was determined by selecting two representative roots from the bulk roots per plant, peeling, washing, slicing and oven-drying 100 g per sample at 65 to 70°C till a constant weight is obtained at about 72 h (Fukuda et al., 2010). The fresh storage roots were washed and shredded into pieces. The DMC was calculated as:

$$\text{RDMC}(\%) = \frac{\text{FRM}}{\text{DRM}} \times 100$$

where RDMC = root dry matter content expressed as a percentage; DRM = dry root mass (kg), and FRM = fresh root mass (kg).

Data analysis

The data collected for all traits of the 24 families were analyzed using the General Linear Model procedure (PROC GLM) of SAS version 9.4 (SAS, 2013). Mean squares were calculated from type III sum of squares. Genotypes were partitioned into replication, lines, tester, line × tester and crosses. Further genetic analyses were carried out for traits among the progeny according to line × tester analysis methods as suggested by Kempthorne (1957) to partition the mean square due to crosses, lines (GCA_f), tester (GCA_m) and line × tester interactions (SCA_{fm}). The contributions of the traits to the total variability of the 24 families were analyzed according to Jollife (2002), using principal component analysis (PCA) in SAS version 9.4 (SAS, 2013). Pearson's phenotypic correlations between the 24 family means for each trait were also performed using SAS version 9.4 (SAS, 2013). The linear model for line × tester design used was:

$$Y_{ijk} = \mu + g_i + g_j + S_{ij} + r_k + e_{ijkl} \quad (1)$$

where Y_{ijk} = observed value of the cross i×j in the kth replication; μ = population mean effect; g_i = GCA effect of ith tester; g_j = GCA effect of jth line; S_{ij} = SCA effect of the cross i×j; r_k = effect of the kth block; e_{ijkl} = experimental error due to (ijk)th individual.

The variances for general and specific combining ability were tested against their respective error variances, derived from the analysis of variance of the different traits as follows:

Covariance of half-sib of line (Cov. H.S. line) = $\frac{M_l - M_{l\times t}}{rt}$ (2)

Covariance of half-sib of tester (Cov. H.S. tester) = $\frac{M_t - M_{l\times t}}{rl}$ (3)

Covariance of full-sib (Cov. F.S.) = $\frac{(M_l - M_e) + (M_t - M_e) + (M_{l\times t} - M_e)}{3r} + \frac{6r\text{Cov.H.S.} - r(l+t)\text{Cov.H.S.}}{3r}$ (4)

Average Covariance of half-sib (Cov. H.S. average) = $\frac{1}{r(2lt-l-t)}\left[\frac{(l-1)(M_l) + (t-1)(M_t)}{l+t-2} - M_{l\times t}\right]$ (5)

With the assumption of no epistasis, variances due to GCA (σ^2_{gca}) and variance due to SCA (σ^2_{sca}) are estimated as follows:

$$\sigma^2_{gca} = \text{Cov. H. S.} = \left(\frac{1+F}{4}\right) \sigma^2_A \quad (6)$$

$$\sigma^2_{sca} = \text{Cov. H. S.} = \left(\frac{1+F}{4}\right)^2 \sigma^2_D \quad (7)$$

Additive and dominance genetic variances (σ^2_A and σ^2_D) were calculated by taking inbreeding coefficient (F) equal to one; that is, F = 1 because both lines and testers were inbred.

Significance test for general combining ability and specific combining ability effects were performed using t-test.

Broad sense heritability on mean entry basis was estimated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage as described by Robinson et al. (1949).

$$H^2_b = \frac{\sigma^2_g}{\left(\sigma^2_g + \frac{\sigma^2_e}{r}\right)}$$

where σ^2_g, σ^2_e and r represent the genetic variance, environmental variance, and number of replications, respectively. The broad sense heritability was classified based on the scale described by Robinson et al. (1949): low (0 - 30%); moderate (30 - 60%); and high >60%.

RESULTS

Analysis of variance for the line × tester design crosses

Analysis of variance showed that mean squares due to crosses were significant for traits such as PHT, NSR, CBB, FSRW and HI and highly significant for CGM (Table 1). Mean squares values for line were highly significant for CGM and significant for PHT, NSR, CBB, FSRW, HI and RDMC. The mean squares values for testers were significant for PHT, CMD, CBB and HI. The line × tester interactions were highly significant for CGM and significant for CBB, FSRW and HI. The differences among the replications were not significant for any of the traits.

The broad sense heritability estimates were high for all traits ranging from 75.0 to 90.8% (Table 2).

Mean performances of progenies of 24 families

Progenies of family TMEB778×IBA000203 (208.6 cm) had the highest mean PHT, whereas family TMEB1×ZAR010116 (141.5 cm) had the lowest (Table 3). The mean NSR ranged from 1.73 (TMEB1×IBA000203) to 4.80 (IBA916132×IBA000203). The heaviest mean performance for FSRW was observed in family TMEB7×ZAR010116 (2.23 kg), whilst family TMEB778×ZAR000156 (0.90 kg) had the lightest weight. The mean performance for HI ranged from 0.29 (TMEB1×IBA0000203) to 0.44 (TMEB778×ZAR000156). Family TMEB7×IBA0000203 exhibited the least mean performance for RDMC (23.87%), whereas TMEB419×IBA030305 had the highest (35.47%).

The least mean score of 1.10 for CMD was recorded by TMEB419×ZAR010156 (family of parents tolerant to CMD) and the highest of 1.77 by IBA020588×IBA030305 (family of a cross between parents susceptible to CMD). The highest mean score for CGM of 2.53 was recorded by TMEB778×IBA000156 and the least score of 1.60 was recorded by TMEB7×ZAR010116. The highest mean score of 2.97 for CBB was recorded by IBA916132×IBA000203, whereas TMEB419×IBA030305 had the least score of 1.67 (Table 3).

General combining ability

The GCA for lines and testers are shown in Table 4. The line GCA effects for PHT was negative and significant for TMEB1 (-16.98 cm) and TMEB7 (-11.01cm) and positive and significant for TMEB778 (10.29 cm) and TMEB419 (9.88 cm), at (p<0.05). With respect to NSR, line IBA916132 showed positive and significant GCA effects (0.51), whereas line TMEB1 showed significant and negative GCA effect (-0.77). Significant and positive GCA effects were observed in IBA020588 (0.14) and TMEB778 (0.24); and TMEB778 significant and negative GCA effects in TMEB7 (-0.29) for CMD severity at 6 MAP. TMEB419 and TMEB7 showed significant and negative GCA effects of -0.26 for CBB severity at 6 MAP. Estimates of GCA effects for FSRW showed that TMEB7 exhibited positive and significant GCA effects of 0.46 kg while TMEB778 exhibited negative and significant GCA effects of -0.34 kg. The GCA effects for HI showed that IBA020588, IBA916132, TMEB419 and TMEB778 exhibited positive and significant GCA effects of 0.02, 0.03, 0.04 and 0.02, respectively. IBA020588, IBA916132 and TMEB419 exhibited significant (p<0.05) and positive GCA effects of 1.06, 1.17 and 2.32, respectively, for RDMC.

Tester IBA000203 had a significant and positive GCA effect for PHT (12.23) and significant and negative GCA effects (-1.36) for RDMC (Table 3). IBA030305 exhibited a significant and negative GCA effect for CBB severity (-0.13) and a significant and positive GCA effects (0.17) for FSRW. ZAR000156 had significant and negative GCA effects of -0.16 and -0.12 for CMD severity and CBB severity, respectively. Genotype ZAR010116 had significant and negative GCA effects of -16.23 for PHT and significant and positive GCA effects for CBB severity (0.23), HI and RDMC.

Specific combining ability

Families IBA020588×ZAR000156 and TMEB7×ZAR000156 recorded a negative and significant

Table 2. Mean squares of agronomic traits of progenies of 24 families of cassava generated using line × tester design.

Source	DF	PHT (cm)	NSR	CMD	CGM	CBB	FSRW (kg)	HI	RDMC (%)
REP	2	676.7^{ns}	0.8^{ns}	0.18^{ns}	0.005^{ns}	0.013^{ns}	0.07^{ns}	0.0009^{ns}	2.12^{ns}
Crosses	23	991.0*	1.29*	0.08^{ns}	0.24**	0.5*	0.48*	0.0049*	1.18^{ns}
Line	5	1544.9*	2.24*	0.035^{ns}	0.43**	0.83*	0.85*	0.009*	2.05*
Tester	3	2534.1*	0.19^{ns}	0.21*	0.036^{ns}	0.51*	0.33^{ns}	0.0045*	0.16^{ns}
Line*Tester	15	497.7^{ns}	1.19^{ns}	0.08^{ns}	0.22**	0.38*	0.39*	0.0036*	1.096^{ns}
Error	46	302.8	0.39	0.071	0.0413	0.15	0.14	0.0016	0.721
Mean	-	179.8	3.8	1.46	1.95	2.19	1.71	0.373	29.32
SE	-	14.06	0.5	0.2	0.3	0.6	0.29	0.03	1.89
CV (%)	-	9.7	16.39	18.3	10.4	17.5	21.7	10.56	16.1
H_b^2	-	90.8	76.5	75.0	76.6	79.8	78.7	80.3	76.4

ns, * and ** = non-significant, significant at p<0.05 and significant at p<0.01, respectively; PHT=Plant height, NSR=number of storage root, CMD=cassava mosaic disease, CGM=cassava green mite, CBB=cassava bacterial blight, FSRW=fresh storage root weight, HI=harvest index and RDMC=root dry matter content, CV=coefficient of variation, SE=standard error, $H_b^2 =$ broad sense heritability on mean entry basis.

Table 3. Performances of progenies of 24 families at the seedling stage.

Pedigree	PHT	NSR	CMD	CGM	CBB	FSRW	HI	RDMC
IBA020588 × IBA000203	203.80	3.53	1.63	2.50	2.57	1.07	0.42	26.80
IBA916132×IBA000203	180.00	4.80	1.67	2.03	2.97	1.80	0.43	32.13
TMEB1×IBA000203	177.27	1.73	1.37	1.73	1.93	1.80	0.29	28.73
TMEB419×IBA000203	189.50	4.43	1.63	1.73	1.87	1.87	0.36	29.03
TMEB7×IBA000203	192.77	3.17	1.40	1.67	1.77	2.13	0.33	23.87
TMEB778× IBA000203	208.60	4.37	1.20	1.77	2.20	1.87	0.33	27.23
IBA020588×IBA030305	187.63	3.70	1.77	2.10	2.03	1.80	0.38	30.67
IBA916132 ×IBA030305	197.70	4.57	1.63	1.87	2.00	2.27	0.41	29.50
TMEB1×IBA030305	152.83	2.97	1.60	1.67	1.97	2.00	0.33	28.70
TMEB419× IBA030305	191.83	3.40	1.40	1.87	1.67	1.87	0.37	35.47
TMEB7×IBA030305	163.93	4.70	1.47	1.70	1.93	2.17	0.33	24.43
TMEB778×IBA030305	191.67	3.33	1.43	2.40	2.73	1.20	0.39	25.57
IBA020588 ×ZAR000156	167.67	3.67	1.20	1.87	2.20	1.47	0.39	31.37
IBA916132× ZAR000156	187.00	4.20	1.20	1.87	1.93	1.97	0.35	30.70
TMEB1×ZAR000156	179.50	3.93	1.37	2.17	2.13	1.40	0.38	31.60
TMEB419×ZAR000156	205.77	3.90	1.10	1.97	1.70	1.83	0.35	31.20
TMEB7×ZAR000156	154.87	3.70	1.33	1.67	2.07	2.13	0.33	26.83
TMEB778×ZAR000156	200.67	3.83	1.60	2.53	2.37	0.90	0.44	24.05
IBA020588×ZAR010116	176.03	3.87	1.60	1.90	2.10	1.97	0.39	32.73
IBA916132×ZAR010116	169.47	3.70	1.47	1.77	2.00	1.17	0.42	29.67
TMEB1×ZAR010116	141.50	3.50	1.50	1.93	2.73	1.60	0.40	27.70
TMEB419×ZAR010116	171.47	3.87	1.60	2.50	2.47	1.07	0.42	30.90
TMEB7×ZAR010116	163.43	3.90	1.40	1.60	1.93	2.23	0.34	28.85
TMEB778 ×ZAR010116	159.27	4.60	1.47	2.07	3.27	1.50	0.41	34.23
Mean	179.76	3.81	1.45	1.95	2.19	1.69	0.37	2.30
SE	14.06	0.5	0.2	0.3	0.6	0.29	0.03	1.89

PHT=Plant height (cm), NSR=number of storage root, CMD=cassava mosaic disease, CGM=cassava green mite, CBB=cassava bacterial blight, FSRW=fresh storage root weight (kg), HI=harvest index and RDMC=root dry matter content (%), SE=standard error.

SCA effects for PHT (Table 5). Positive and significant SCA effects were observed for NSR in families IBA916132×IBA000203, TMEB419×IBA000203, TMEB7×IBA030305 and TMEB1×ZAR000156; whereas

Table 4. General combining ability effects of agronomic traits of cassava.

Parent	PHT	NSR	CMD	CGM	CBB	FSRW	HI	RDMC
Line								
IBA020588	4.03	-0.12	0.09	0.14*	0.04	-0.14	0.02*	1.06*
IBA916132	3.78	0.51*	0.03	-0.07	0.04	0.09	0.03*	1.17*
TMEB1	-16.98*	-0.77**	0.01	-0.08	0.00	-0.01	-0.03*	-0.15*
TMEB419	9.88*	0.09	-0.03	0.06	-0.26*	-0.05	0.04*	2.32*
TMEB7	-11.01*	0.06	-0.06	-0.29*	-0.26*	0.46*	-0.04*	-3.59*
TMEB778	10.29*	0.23	-0.03	0.24	0.45*	-0.34*	0.02*	-1.22*
SE	4.59	0.16	0.07	0.05	0.10	0.10	0.01	0.06
Tester								
IBA000203	12.23*	-0.13	0.02	-0.05	0.03	0.04	-0.01	-1.36*
IBA030305	1.18	-0.03	0.09	-0.02	-0.13*	0.17*	-0.01	-0.27
ZAR000156	2.82	0.07	-0.16*	0.06	-0.12*	-0.09	-0.001	0.27
ZAR010116	-16.23*	0.10	0.05	0.01	0.23*	-0.12	0.02*	1.46*
SE	3.55	0.13	0.05	0.04	0.08	0.08	0.01	0.47

* and ** = significant and highly significant, respectively, PHT=Plant height (cm), NSR=number of storage root, CMD=cassava mosaic disease, CGM=cassava green mite, CBB=cassava bacterial blight, FSRW=fresh storage root weight, HI=harvest index RDMC=root dry matter content, SE=standard error.

Table 5. Specific combining ability effects of agronomic traits of cassava.

Pedigree	PHT	NSR	CMD	CGM	CBB	FSRW	HI	RDMC
IBA020588×IBA000203	7.78	-0.02	0.06	0.46**	0.31*	-0.55*	0.04*	-2.23*
IBA916132×IBA000203	-15.77*	0.62*	0.15	0.20	0.71**	-0.04	0.04*	3.00*
TMEB1×IBA000203	2.26	-1.17**	-0.12	-0.09	-0.29	0.06	-0.05*	0.91
TMEB419×IBA000203	-12.37*	0.68*	0.18	-0.24*	-0.09	0.16	0.00	-1.25
TMEB7×IBA000203	11.78	-0.57*	-0.02	0.06	-0.19	-0.08	0.01	-0.51
TMEB778×IBA000203	6.32	0.47*	-0.25*	-0.38*	-0.47*	0.46*	-0.05*	0.49
IBA020588×IBA030305	2.67	0.04	0.13	0.03	-0.06	0.05	-0.01	0.55
IBA916132×IBA030305	12.98*	0.28	0.05	0.00	-0.09	0.29	0.01	-0.73
TMEB1×IBA030305	-11.12	-0.04	0.05	-0.19*	-0.09	0.13	-0.02	-0.21
TMEB419×IBA030305	1.02	-0.47	-0.12	-0.13	-0.13	0.04	0.02	4.09*
TMEB7×IBA030305	-5.99	0.86**	-0.02	0.06	0.14	-0.17	0.04*	-1.03
TMEB778 ×IBA030305	0.44	-0.67*	-0.08	0.23*	0.23	-0.34*	0.01	-2.27*
IBA020588×ZAR000156	-18.94*	-0.09	-0.19	-0.28*	0.10	-0.01	-0.03	0.70
IBA916132×ZAR000156	0.64	-0.18	-0.13	-0.08	-0.17	0.26	-0.05*	-0.07
TMEB1×ZAR000156	13.90*	0.83**	0.07	0.23	0.06	-0.21	0.03	2.15*
TMEB419×ZAR000156	13.30*	-0.07	-0.17	-0.11	-0.10	0.27	-0.02	-0.72
TMEB7×ZAR000156	-16.70*	-0.23	0.09	-0.05	0.26	0.06	0.02	0.83
TMEB778×ZAR000156	7.80	-0.27	0.33*	0.28*	-0.15	-0.37*	0.05*	-4.33*
IBA020588×ZAR010116	8.48	0.08	0.004	-0.20	-0.35*	0.51*	-0.03	0.88
IBA916132×ZAR010116	2.15	-0.72**	-0.07	-0.13	-0.45*	-0.51*	-0.01	-2.29*
TMEB1×ZAR010116	-5.05	0.37	-0.004	0.05	0.31*	0.02	0.03	-2.94*
TMEB419×ZAR010116	-1.95	-0.13	0.12	0.48**	0.31*	-0.47*	0.02	-2.21*
TMEB7×ZAR010116	10.91	-0.07	-0.05	-0.07	-0.22	0.19	-0.01	1.65
TMEB778×ZAR010116	-14.55*	0.47*	-0.004	-0.13	0.40*	0.26	-0.04*	4.66*
SE	7.94	0.28	0.12	0.09	0.17	0.17	0.0179	1.04

* and ** = significant and highly significant, respectively, PHT=Plant height (cm), NSR=number of storage root, CMD=cassava mosaic disease, CGM=cassava green mite, CBB=cassava bacterial blight, FSRW=fresh storage root weight, HI=harvest index, RDMC=root dry matter content, SE=standard error.

Table 6. Phenotypic correlation coefficients of agronomic traits evaluated in 24 families of cassava at the seedling stage.

Trait	PHT	NSR	CMD	CGM	CBB	FSRW	HI	RDMC
PHT	1							
NSR	0.10	1						
CMD	-0.08	0.11	1					
CGM	0.34	0.06	0.33	1				
CBB	-0.23	0.26	0.29	0.54*	1			
SRW	-0.13	0.12	-0.16	-0.82**	-0.50*	1		
HI	0.08	0.39*	0.47*	0.70**	0.61*	-0.67*	1	
RDMC	-0.10	0.17	-0.04	-0.02	0.08	0.04	0.21	1

* and ** = significant and highly significant, respectively, PHT=Plant height (cm), NSR= number of storage root, CMD=cassava mosaic disease, CGM=cassava green mite, CBB=cassava bacterial blight, FSRW=fresh storage root weight, HI=harvest index, RDMC=root dry matter content.

families TMEB1×IBA000203, TMEB7×IBA000203, TMEB778×IBA030305 and IBA916132×ZAR010116 had negative and significant values. However, the highest and positive SCA effects for NSR were observed in families TMEB7×IBA030305, TMEB1×ZAR000156 and IBA916132×ZAR010116. Family TMEB778×IBA000203 showed significant and negative SCA effects for CMD, while TMEB778×ZAR000156 showed a significant and positive SCA effect. Families with negative and significant SCA for CGM were TMEB419×IBA000203, TMEB778×IBA000203, TMEB1×IBA030305 and IBA020588×ZAR000156.

With respect to CBB, both positive and negative SCA effects were recorded with significant difference at ($p<0.01$) and ($p<0.05$), with the highest positive SCA effect recorded for family IBA916132×IBA000203. Three families (TMEB778×IBA000203, IBA020588×ZAR010116 and IBA916132×ZAR010116) showed significant and negative SCA effect for CBB. Families IBA020588×IBA000203, TMEB778×IBA030305, TMEB778×IBA000203, IBA020588×ZAR010116 and TMEB419×ZAR010116 recorded negative SCA effects for FSRW; while TMEB778×IBA000203 and IBA020588×ZAR010116 were significant and positive. Significant and positive SCA effect for HI was observed in IBA020588×IBA000203, IBA916132×IBA000203, TMEB7×IBA030305 and TMEB778×ZAR000156. The RDMC showed significant ($p<0.05$) positive SCA for families IBA916132×IBA000203, TMEB419×IBA030305, TMEB1×ZAR000156 and TMEB778×ZAR010116 (Table 4).

Phenotypic correlation coefficients of agronomic traits

The phenotypic correlation coefficients vary for the various agronomic trait associations studied (Table 6). Cassava bacterial blight (CBB) was positively correlated with CGM; whereas FSRW was significant and negatively correlated with CGM and CBB. Harvest index (HI) was significantly and positively correlated with NSR, CMD, CGM, and CBB and negatively correlated with FSRW.

DISCUSSION

Combining ability effects of agronomic traits

Traits of testers, lines and families that exhibited significant GCA and SCA mean square values indicate the preponderance of significant variations in the breeding populations, possibly attributable to additive and non-additive gene effects, respectively. Kamau et al. (2010) and Parkes (2011) also found significant effects for root number per plant and fresh root weight per plant. However, findings of the current study disagree with the non-significant GCA values reported for average root number and fresh root weight by DaSilva (2008). The variance is partly attributable to the different populations developed and environments in which they were assessed. In this study, the GCAs of CMD for all lines and GCAs of number of storage roots and CGM for all testers were non-significant.

Genotypes with significant negative GCA values for CMD, CGM, CMB and CBB indicate that they possibly possess desirable alleles for resistance to CMD, CGM, CMB and CBB that are needed in the development of new varieties resistant to the studied pests and diseases. Thus, lines and testers possessing these attributes are useful parental genotypes for cassava population improvement aimed at generating resistant genotypes to the biotic constraints (Owolade et al., 2008).

Genotypes with significant positive GCA values for plant height, root number per plant, fresh root weight per plant, harvest index and dry matter content were considered superior genotypes that contributed most to variability in the traits, while those with significant negative values were undesirable since they performed below average. Since none of the lines and testers used in this study possessed the overall best general combiner attributes for all the traits that qualify it for the improvement of all the traits in a breeding program, indicate the relevance of recombination to incorporate desired traits from parents with complementary desired alleles of traits of interests.

The distribution of family (pedigree of F1 progenies) for the studied traits relative to GCA values of parental combinations showed that most of the SCA effects were obtained from different GCA values rather than from best general combiners indicating that the inheritance of these traits involved both allelic and non-allelic interactions. These findings concurred with those reported by Saleem (2008). For instance, family TMEB419 × IBA030305 (high × low), TMEB7 × IBA030305 (low × low), TMEB778 × ZAR000156 (low × intermediate) combinations for dry matter content, IBA020588×ZAR000156 (high × intermediate), TMEB778 × IBA030305 was obtained from (high × low), TMEB7×IBA030305 (low × low) combinations for CGM. Based on the performances of hybrids depicted by the SCA values, best progenies might not always be obtained from crosses among parents with highest desirable GCA effects. These findings concurred with the suggestion by other researchers that best progenies are not always obtained from parental crosses with the highest desired GCA effects (DaSilva, 2008; Owolade et al., 2008; Mtunda, 2009; Kamau et al., 2010).

Gene action and significance of general and specific combining abilities

Plant height had lower general combining ability relative to specific combining ability indicating that the SCA was more important in predicting progeny performance for expression of this trait. However, the remaining traits exhibited higher GCA relative to SCA except for CGM, indicating the relevance of GCA in predicting progeny performance for the expression of the traits. Findings partly agree with DaSilva (2008) and Parkes (2011) who reported that SCA was more important for prediction of progeny performances of cassava. Since GCA and SCA effects were relevant for traits studied, findings agree with the suggestion that GCA and SCA gene action effects should be part of breeding schemes targeted at selection of superior genotypes (Arunga et al., 2010). Since cassava is a highly heterozygous crop cultivated using vegetative propagules such as stem cuttings, identification and selection of superior clones with desired traits can be perpetuated intact through the various breeding stages.

Phenotypic correlation and heritability estimates of agronomic traits

The positive correlation between fresh storage root weight and root number per plant implies that indirect selection for storage root weight is achievable to certain level by selecting for root number and that decrease in root storage number contributes to decreasing yields in cassava. This result is in concurrence with those noted by DaSilva (2008), Akinwale et al. (2009, 2010) and Kamau et al. (2010). Similarly, positive relationships between other economic traits were: dry matter content and root number per plant, dry matter content and fresh storage root weight, dry matter content and harvest index, and between root number per plant and harvest index.

The negative correlations between CMD and fresh root weight, CGM and fresh root weight, and between CBB and fresh root weight, indicate that severe attacks of these diseases contribute to low storage root yields in cassava. Findings agree with Bellotti (2002), who noted the impact of CGM on cassava root yield. Yield reduction by any biotic factor can be decreased through incorporation of host plant resistance in cassava breeding programme. Moreover, a significant negative association between harvest index and fresh root weight also indicates that higher fresh root yields are achieved by decreasing harvest index. The results are consistent with Akinwale et al. (2009) who opined that traits that are significantly and positively related with fresh storage root yield are important in the formulation of an efficient cassava breeding programme aimed at improving fresh root yield, while negatively correlated ones may reduce the rate of improvement for some traits under selection in a breeding programme.

The high heritability in the measured traits of the studied plants indicates the preponderance of larger genetic effects contributing to the total phenotypic variance and that the alleles of these traits could be passed intact to subsequent generations of the cassava root crop. Our findings are consistent with the high broad sense heritability values reported for these traits in cassava (DaSilva, 2008; Akinwale et al., 2010; Parkes, 2011). Findings corroborate with the suggestion by DaSilva (2008) that broad-sense heritability predominates in cassava hybrids. Thus, the high broad sense heritability in the studied progenies implies that a large proportion of the heritable variation could be exploited by plant breeders (Akinwale et al., 2010).

Conclusion

Genotypes with good GCA and cross combinations with desirable SCA for the traits studied were identified. Parental genotypes TMEB419, IBA020588, BA916132 and ZAR010116 had good general combining ability for dry matter content, whereas TMEB7 and IBA030305 exhibited good general combining ability for storage root weight. Among the testcrosses, TMEB778×ZAR010116 and TMEB419×IBA030305 were good specific combiners for dry matter content and could be used for heterosis breeding programmes in cassava.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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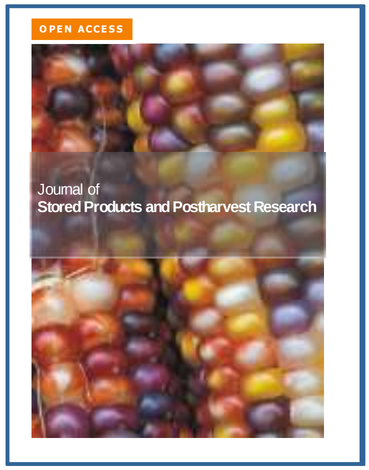

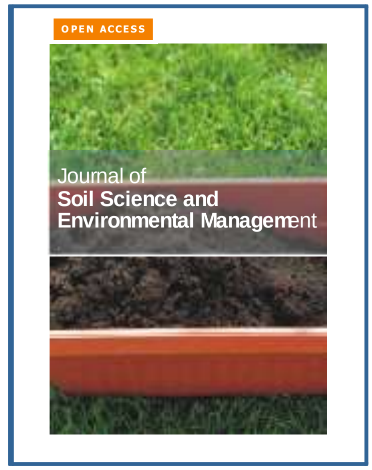

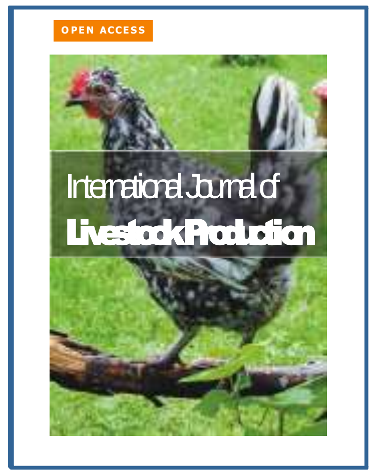

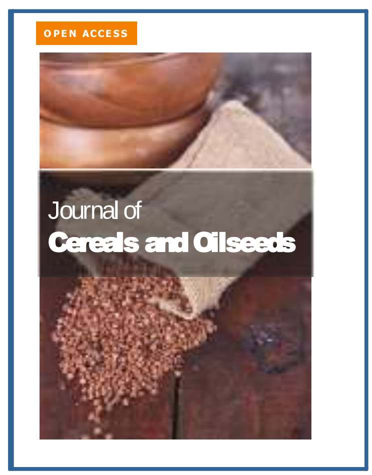

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