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Table of Content

Table of Content

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Full Length Research Paper

Interaction effect between *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici* on selected tomato (*Solanum lycopersicum* L.) genotypes

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Development of diseases in cultivated crops depends on the complex inter-relationship between host, pathogen and prevailing environmental conditions. The significant role of nematodes in the development of nematode–fungus interaction is demonstrated in many crops throughout the world. However, there is scanty research information in Ethiopia. Therefore, the main objectives of this study were to: (i) investigate the effect of *Meloidogyne incognita* (MI)-*Fusarium oxysporum* f.sp. *lycopersici* (FOL) interaction on selected tomato genotypes based on their order of inoculation and (ii) evaluate the reaction of selected tomato genotypes against the MI-FOL interaction. The greenhouse experiment was laid out in a completely randomized design (CRD) factorial with four replications. Three-week-old tomato seedlings were inoculated with MI suspension at a rate of 3000 second-stage juveniles (J_2) and 10 ml FOL suspension ($3x10^6$ conidia/ml/pot) around the root rhizosphere. Tomato growth, biomass and pathogen related data were recorded starting first week after inoculation to eight weeks of post inoculation. The result revealed that simultaneous inoculation of MI and FOL (NF) and FOL 10 days after MI (N1F2) was found significantly ($p \leq 0.05$) reducing tomato growth, biomass and pathogen related parameters compared to single pathogen or un-inoculated control. Among the three tomato genotypes tested, Assila was moderately resistant as measured by the lower number of root gall and egg mass per plant, that it could be of a good choice to manage this disease complex or interaction. Performance evaluation study at MI-FOL hot spot farmers' field should be investigated in the near future.

Key words: *Fusarium oxysporum* f.sp. *lycopersici*, *Meloidogyne incognita*, resistance, management, synergistic effect and tomato varieties

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops across the world next to potato (McGovern, 2015). The fruits of tomato are popular throughout the world, are used in all kinds of vegetables as raw salad, and as processed products such as paste and juice. Ripe tomato fruit has high nutritive value and a good source of vitamin A, B, C and minerals (MoARD, 2009). Recently, it started gaining more medicinal value because of its high content of antioxidant including carotenoids, ascorbic acid, phenolics and lycopene (Oduor, 2016). It also serves for export, making a significant contribution to the national economy of Ethiopia with most of the exports to Djibouti, Somalia, South Sudan, Middle East and European markets (Tabor and Yesuf, 2012).

Tomato production reached to more than 163.4 million tons cultivated on more than 4.6 million hectares of land worldwide (FAOSTAT, 2016). In Ethiopia, it is being grown in about 6,298.63 hectares with an annual production of 28,364.83 tones (CSA, 2017). Large-scale production of tomato is being carried out in the central rift valley (CRV) under irrigated and rain-fed conditions having unlimited potentials for expansion if certain production constraints are avoided (Wondimeneh et al., 2013). Despite its rapid spread across the different localities and agro-ecologies, the production and productivity remains low. This is attributed to several biotic and abiotic factors. Among the biotic factors, plant diseases caused by plant-parasitic nematodes (PPN) are a costly burden. There are over 4100 species of PPN currently identified collectively causing an estimated loss of $80–$118 billion per year in damage to crops. Of these species, 15% of them are the most economical species directly targeting plant roots of major agricultural crops and prevent water and nutrient uptake resulting in reduced agronomic performance, overall quality and yields (Bernard et al., 2017). In nature plants are rarely, if ever, subject to the influence of only one potential pathogen and this is especially true of soil-borne pathogens like fusarium wilt (*Fusarium oxysporum*) whereby further opportunities exist for interactions with other microorganisms occupying the same ecological niche (Back et al., 2002).

The combined effect of wilt causing fungi and PPN causes serious damage to different economically important crops worldwide (Mai and Abawi, 1987; Chen et al., 2004). Based on its worldwide distribution, extensive host range and involvement with fungi, bacteria and viruses in disease complex, root knot nematodes (RKN) rank first among the top 10 damaging genera of PPN affecting the world's food supply (Jones et al., 2013). Obtaining optimum crop quality and economic production of tomato depends on development and exploitation of an eco-friendly, sustainable, economical and alternative methods of nematode-wilt disease complex management. Being aware of the array of organisms influencing the crop and the nature of various organisms' interactions is therefore essential (Webster, 1985). This indicates that development of simple technology for efficient and reliable integrated management of soil-born plant pathogens is highly dependent on knowledge of this interaction effect. Hence, there is an urgent need to generate basic information regarding disease complex involving RKN and soil-born fungi. The main objectives of this research work were to: (i) investigate the effect of the interaction between *Meloidogyne incognita (MI)* and *Fusarium oxysporum* f.sp*. lycopersici (FOL)* on selected tomato genotypes based on their order of inoculation and (ii) evaluate the reaction of selected tomato cultivars with different degree of resistance to *M. incognita* against *M. incognita-F. oxysporum* f.sp. *lycopersici* (MI-FOL) co-infestation under greenhouse conditions.

MATERIALS AND METHODS

Fungal isolates

Monoconidial isolates of *F. oxysporum* f.sp*. lycopersici* (FOL-W, FOL-P and FOL-V) were used in this study. These isolates were collected from infected tomato plant from the Central Rift Valley (CRV), Ethiopia and were characterized according to Leslie and Summerell (2006). Diseased plant specimens (stem bases and roots) were subjected to running tap water and then rinsed with distilled water. Diseased specimens were cut into pieces (2 cm) and surface sterilized with 2% NaOCl for 2 min followed by three changes of sterile distilled water and dried in between two sterilized blotting paper. Sterilized and dried specimens were plated out on potato dextrose agar (PDA) media in sterile Petri-dishes and incubated at 25 ± 2°C for 7-9 days.

Pathogenicity test was used to confirm the identified *F. oxysporum* formae specials. Three-week-old susceptible (Marmande) tomato genotype seedlings were inoculated by standard root dip method (Srivastava et al., 2009). Four treatments: (i) Marmande + White FOL isolate, (ii) Marmande + Pink FOL isolate, (iii) Marmande + Violate FOL isolate and (iv) un-inoculated check with five replications were set under controlled greenhouse conditions. Pure culture of the aggressive fungal isolate (FOL-W) was used as a starting culture for the disease complex or interaction study (Figure 1). The test fungus was multiplied with PDA medium on 9 cm diameter Petri-dishes to get enough inoculum to help initiate the actual experiment. The three tomato genotypes, resistant (Assila), moderately resistant (Cochoro) and susceptible (Moneymaker) were inoculated with FOL conidia suspension based on the treatment requirement. Inoculum density (conidia concentration) of the pathogen was adjusted to $3x10^6$ conidia/mL/plant (Lobna et al., 2016) using a Haemocytometer and 10 mL of this solution was delivered into holes in the soil surface of pots.

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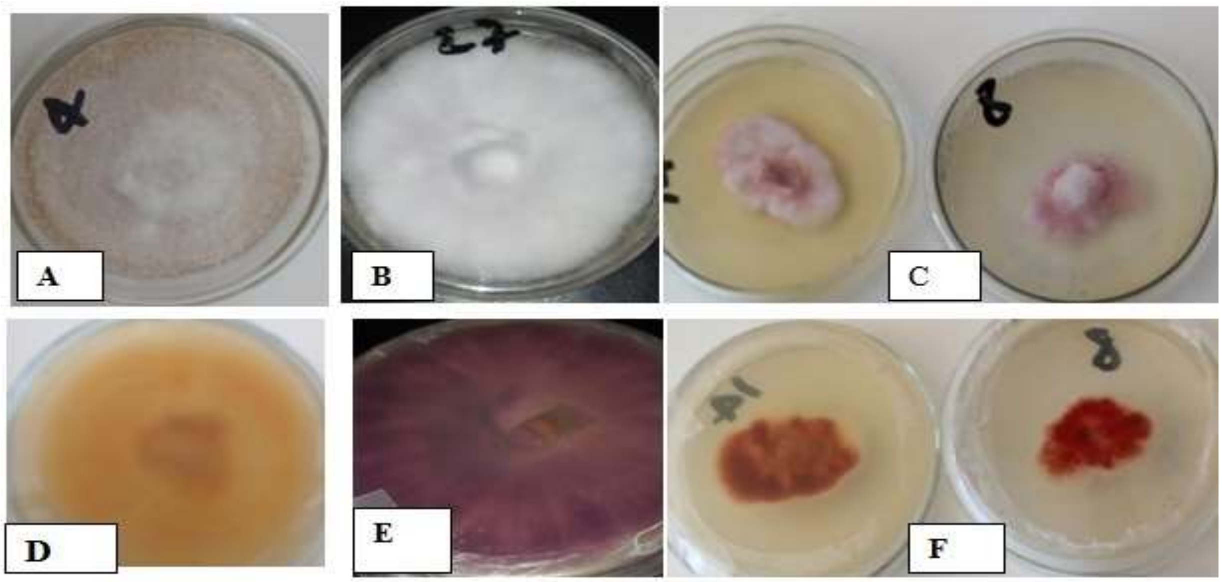

Figure 1. Morphotypic isolates of *Fusarium oxysporum*; White (A: in face, D: Back), Violet (B: in face, E: Back) and Pink (C: in face, F: back) of 9-days (A, B, D, E) and 3-days (C, F) old culture.

Meliodogyne incognita population

Molecularly (DNA-based and Isozyme techniques) identified *M. incognita* population by Seid et al. (2017) was used as a starting pure culture. This species originated from the central rift valley area from the 2016 tomato-growing season. The root galls containing the egg masses were used to inoculate three-week-old susceptible (Moneymaker) tomato seedlings grown in sterilized soil for mass multiplication of nematode under aseptic conditions. The culture was multiplied on several pots using the same genotype to get enough inoculums. After giving sufficient time of 70-80 days to complete 2-3 generations, the plants were de-potted carefully and root system was washed free of soil. Then roots free of attached soil particles were submerged on Phloxine B (0.15 g/L) for 15 min (Holbrook et al., 1983) to clearly observe the egg-masses and facilitate counting.

Second stage juveniles (J_2) were obtained from egg masses by incubating large number of egg masses at room temperature in water. After 10 days of incubation, every two days the J2 in water were collected and kept in a refrigerator (8°C) in a 100 mL beaker and volume of water was made up to 50 mL. The nematode suspension (water containing J2) was fetched with the help of 10 mL pipette and an aliquot of one mL was transferred to counting dish for counting the juveniles under dissecting-binocular microscope. For inoculation, juvenile levels were adjusted with water so as to add equal volume of nematode suspension (3000 J_2) in each treatment and were added to pot seedlings to the 2 cm deep holes around the roots of the tomato seedlings or rhizosphere soil.

Treatments and experimental design

The experiment was conducted in greenhouse with 18 treatment combinations using a completely randomized design (CRD) factorial with four replications (Table 1). Plants were maintained in a greenhouse at the temperature of 26 ± 2°C. The experiment lasted a total of two months and at the end, each treatment was hand harvested. The following data on *M. incognita* and *F. oxysporum* f.sp. *lycopersici* and plant related parameters were collected starting from 7 days after inoculation (DAI).

Pathogen related parameters

After cutting the top parts of the plants, all the pots were turned upside down with care, to discharge the soil and the roots were made free of soil. Finally, the plant roots were gently washed with tap water to remove adhering soil particles. Then the number of root-galls per root system was counted manually aided with hand lens. Roots containing egg-masses were soaked in Phloxine B (0.15 mg/L tap water) solution for 15-20 min and then the roots were rinsed in tap water to remove residual stain. The egg-masses were stained pink to red and observed and counted (Coyne, 2007) to determine number of egg-masses per plant (EMPP). Root gall index (RGI) and egg-mass index (EMI) per plant were determined from each pot and based on 0 to 5 rating scale (Taylor and Sasser, 1978); where, 0 = no galls or egg masses; 1: 1-2 galls or egg-masses; 2: 3-10 galls or egg-masses; 3: 11-30 galls or egg-masses; 4: 31-100 galls or egg-masses and 5: over 100 galls or egg-masses.

The final nematode population density (*Pf*) was estimated from organic (root) and mineral (soil) fraction per pot. The mean number of J2 in the roots was estimated from the whole root system after extracting nematodes from a sub-sample of 5 g roots per plant based on (Hussey, 1973). Nematodes from soil samples were

Table 1. Treatment combination of three selected tomato genotypes (Assila, Cochoro and Moneymaker) with two pathogens: *Meloidogyne incognita* (MI) and *Fusarium oxysporum* f.sp. *lycopersici* (FOL).

Treatment number	Treatments
1	Assila + FOL
2	Assila + MI
3	Assila + (MI+ FOL) simultaneously
4	Assila + FOL 10 days prior to MI
5	Assila + MI 10 days prior to FOL inoculation
6	Assila (Un-inoculated check)
7	Cochoro + FOL
8	Cochoro + MI
9	Cochoro + (FOL +MI) simultaneously
10	Cochoro + FOL 10 days prior to MI
11	Cochoro+ MI 10 days prior to FOL inoculation
12	Cochoro (Un-inoculated check)
13	Moneymaker + FOL
14	Moneymaker + MI
15	Moneymaker+ (FOL + MI) simultaneously
16	Moneymaker + FOL 10 days Prior to MI
17	Moneymaker+ MI 10 days prior to FOL inoculation
18	Moneymaker (Un-inoculated Check)

extracted using a modified Baermann funnel technique. It was expressed as J_2 per 100 gram of soil. The *Pf* of *M. incognita* was counted by transferring the suspension to nematode counting dish under a stereo microscope.

Disease severity was assessed weekly (visual observation) starting one week after inoculation up to eight weeks of post inoculation, where the final estimation was recorded and rated on a scale of 0-4 (Song et al., 2004); where, 0: no infection; 1: slight infection which is about 25% of full scale (one or two leaves become yellow); 2: moderate infection (two or three leaves became yellow, 50% of leaves become yellow); 3: extensive infection (all plant leaves became yellow, 75% of leaves become wilting) and 4: complete infection (the whole plant leaves became wilting, and growth was inhibited). Area under the disease progress curve (AUDPC) was calculated according to the method of Shaner and Finney (1977) using the formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1}[0.5(Xi+1+Xi)(ti+1-ti)]$$

Plant related parameters

Plant height (PH) was measured from the soil level to the main apex of the plant eight weeks after transplanting and mean values were calculated per treatment and expressed in cm. Root length (RL) was taken after the adhering soil was gently washed away from the roots using tap water and excess water was removed after blotting with tissue paper. The root length per plant was measured from the soil level to the tip of 75% of roots end and expressed in centimeter.

The tomato plant was cut at the crown level in each pot and the fresh shoot weight (FSW) was measured (in gram) using electronic balance soon after cutting. After cutting the top parts of the tomato plants, all the pots were turned upside down with care, to discharge or dislodge the soil and the roots were made free of adhering soil. Finally, the plant roots were gently washed with tap water to remove adhering soil particles. Then, the fresh root weight (FRW) was measured (in gram) using electronic balance. The shoots were put in paper bag and brought to laboratory just after taking the fresh weight and kept in an oven at 105°C for 24 h, and allowed to come to room temperature and the dry shoot weight was measured (in gram) using electronic balance to determine its dry shoot weight (DSW).

Data analysis

Data obtained from the greenhouse experiment were subjected to analysis of variance (ANOVA) and means were separated using LSD at *P*=0.05 by GenStat software (16th edition).

RESULTS

Identification of *Fusarium oxysporum*

The length and breadth of macroconidia usually varied between (15.9-46.98 x 1.83-4.88 µm) and that of

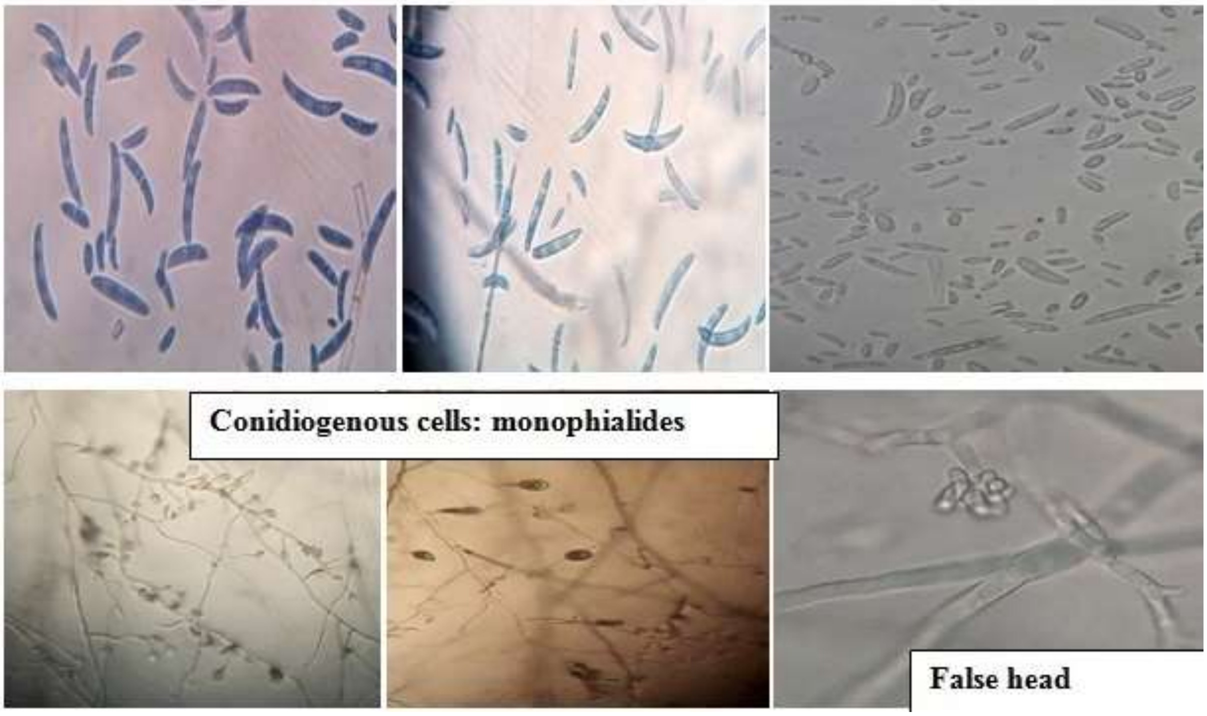

Figure 2. Macro-and micro-conidia, Conidiogenous cells and false heads of FOL (40x magnification compound microscope of 9-days-old culture).

microconidia was 6.75-13.56 x 1.93-3.4 µm under 40 x compound microscope magnification. The number of septation of macroconidia ranges from 1.5-4.3. The shape of macroconidia varied from straight, slightly curved to sickle shaped. The shape of microconidia was oval, elliptical or kidney and usually 0-septated with infrequent occurrence of a single septation (Figure 1). False head structures with short monophialides were there in the aerial mycelium while it was observed under compound microscope without disturbance of the existing mycelium (Figure 2).

Pathogenicity of *Fusarium oxysporum*

Various symptoms on aerial parts and within the stem tissues of tomato plants infected with *F. oxysporum* were noted starting at 33 DAI. Yellowing of the lower leaves at early stage of the plant and leaf necrosis and later, dropping due to the infection were the most prominent symptoms. However, there was no statistically significant difference in virulence among the isolates in mean disease severity and area under disease progress (Table 2). As expected, however, there was significant difference ($p \leq 0.05$) between the inoculated treatments and the un-inoculated check regardless of the isolates (Table 2).

Number of root gall and root gall index

Data presented (Table 3) revealed that mean number of root gall per plant (RGPP) has significantly ($p \leq 0.01$) increased in the treatment where the nematode was inoculated simultaneously with the fungus (NF) as compared to the treatment which received nematode 10 days later to fungus inoculation (F1N2) and the control (C) on Moneymaker genotype. The maximum mean number of RGPP (496.8) was recorded from treatments that received both pathogens simultaneously followed by FOL inoculated 10 days after *M. incognita,* N1F2 (453.8) and *M. incognita* alone, N (360.2) on susceptible genotype Moneymaker, although no significant variation among one another. However, the minimum number of root galls was observed on roots of Assila and Moneymaker genotypes when fungus infection preceded nematode in 10 days (F1N2).

There was a significant difference in number of RGPP

Table 2. Virulence analysis of *Fusarium oxysporum* f.sp. *lycopersici* isolates as measured by disease severity and AUDPC.

***F. oxysporum* isolates**	**Disease severity**	**AUDPC**
White	3.188[b]	71.75[b]
Pink	3.062[b]	71.75[b]
Violet	2.938[b]	64.75[b]
Control	0.000[a]	0.00[a]
LSD (5%)	0.3194	7.80
CV (%)	9.0	9.7

Means followed by the same letter (s) within the column in each parameter are not significantly different at 5% level of significance; LSD, least significant difference; CV, coefficient of variation; AUDPC, area under disease progress curve.

Table 3. Effect of *Meloidogyne incognita* and *F. oxysporum* f.sp. *lycopersici* disease complex on number of root gall and root gall index.

Treatment	**RGPP**			**RGI**		
	Genotypes			**Genotypes**		
	Assila	**Cochoro**	**Moneymaker**	**Assila**	**Cochoro**	**Moneymaker**
N	143.50(2.102[h])	298.80(2.464[cd])	360.20(2.541[abc])	4.75[b]	5.0[a]	5.0[a]
NF	193.80(2.281[fg])	228.00(2.356[def])	496.80(2.692[a])	5.0[a]	5.0[a]	5.0[a]
N1F2	216.50(2.295[efg])	323.50(2.481[bcd])	453.80(2.64[ab])	5.0[a]	5.0[a]	5.0[a]
F1N2	140.20(2.143[gh])	293.00(2.456[cde])	308.00(2.482[bcd])	5.0[a]	5.0[a]	5.0[a]
F	0.0[i]	0.0[i]	0.0[i]	0.0[c]	0.0[c]	0.0[c]
C	0.0[i]	0.0[i]	0.0[i]	0.0[c]	0.0[c]	0.0[c]
LSD (5%)		0.1669			0.1671	
LSD (1%)		0.2222			0.2225	
CV (%)		7.3			3.6	

Number in the brackets is logarithmic transformations [log (y+1)]; where, y: original value; and means followed by the same letter (s) within the row and column in each parameter are not significantly different at 5% level of significance. LSD (5%): list significant difference at 5% level of significance; LSD (1%): least significant difference at 1% level of significance; CV (%), coefficient of variation; RGPP, root gall per plant; RGI, root gall index; N, *M. incognita* alone; NF-synchronized inoculation of *M. incognita* and FOL; N1F2, *M. incognita* 10 days prior to FOL inoculation; F1N2, FOL 10 days prior to *M. incognita* inoculation; F, FOL alone; C, control.

among the selected tomato genotypes when *M. incognita* inoculated with F1N2. A statistically highly significant ($p \leq 0.01$) difference in mean number of RGPP was also found between Assila and Moneymaker tomato genotypes in all the treatments containing *M. incognita*. However, there was no statistically significant difference between Assila and Cochoro genotypes while they were inoculated with nematode and fungus concomitantly (NF). The minimum number of RGPP (140.2) was recorded on Assila genotype when inoculated with F1N2 followed by N (143.5). It is also possible to clearly notice from Table 3 that RGPP varied according to the susceptibility gradient of the cultivars used.

The interaction effect of the three selected tomato genotypes and disease complex involving *M. incognita* and FOL on the root gall index (RGI) was insignificant ($p \leq 0.05$) except when inoculated with *M. incognita* alone (N). The effect of the treatments was also insignificant in root gall index except on Assila genotype where it was infested with the nematode, *M. incognita* alone (N) as compared to other treatments, including the un-inoculated check.

Number of egg-mass and egg-mass index

The highest number of egg-mass per plant, EMPP (347.2), was recorded from the treatment inoculated with N1F2 followed by inoculation of N (297.8) on the susceptible, Moneymaker tomato genotype (Table 4). A statistically highly significant difference ($p \leq 0.01$) in the

Table 4. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici* disease complex on number of egg-mass and egg-mass index.

Treatment	EMPP			EMI		
	Genotypes			Genotypes		
	Assila	Cochoro	Moneymaker	Assila	Cochoro	Moneymaker
N	126.0(2.041^{e})	193.8(2.284^{c})	297.8(2.463^{ab})	4.50^{b}	5.0^{a}	5.0^{a}
NF	89.2(1.937^{e})	184.5(2.264^{c})	220(2.311^{bc})	4.50^{b}	5.0^{a}	5.0^{a}
N1F2	126.22.089^{de})	213.2(2.326^{bc})	347.2(2.536^{a})	4.75^{ab}	5.0^{a}	5.0^{a}
F1N2	42.0(1.554^{f})	104.0(2.014^{e})	180.2(2.245^{cd})	3.25^{c}	4.5^{b}	5.0^{a}
F	0.0^{g}	0.0^{g}	0.0^{g}	0.00^{d}	0.0^{d}	0.0^{d}
C	0.0^{g}	0.0^{g}	0.0^{g}	0.00^{d}	0.0^{d}	0.0^{d}
LSD (5%)		0.1713			0.4919	
LSD (1%)		0.2281			0.655	
CV (%)		8.3			11.1	

Number in the brackets is logarithmic transformations [log (y+1)]; where, y: original value; and means followed by the same letter (s) within the row and column in each parameter are not significantly different at 5% level of significance. LSD (5%): list significant difference at 5% level of significance; LSD (1%): least significant difference at 1% level of significance; CV (%), coefficient of variation; RGPP, root gall per plant; RGI, root gall index; N, *M. incognita* alone; NF-synchronized inoculation of *M. incognita* and FOL; N1F2, *M. incognita* 10 days prior to FOL inoculation; F1N2, FOL 10 days prior to *M. incognita* inoculation; F, FOL alone; C, control.

mean number of EMPP was observed on Moneymaker genotype when it was inoculated with N1F2 as compared to other treatments and the control except the treatment that received N. A statistically highly significant difference ($p \leq 0.01$) in mean number of egg mass was also noted among the selected tomato genotypes but of between Cochoro and Moneymaker, while they were inoculated with NF (Table 4). Less mean number of EMPP (42.0) was recorded when inoculated with F1N2, followed by inoculation of N (89.2) on the resistant genotype (Assila).

Generally, treatments receiving N1F2 and N resulted in increased and comparable number of egg mass across all the genotypes tested. There was invariably reduced number of EMPP in all the genotypes that received F1N2 (Table 4). The lowest egg mass index, EMI (3.25) was observed from the treatment inoculated with F1N2 on the resistant tomato genotype. Highly significant ($p \leq 0.01$) interaction effect of the disease complex and tomato genotypes was also noted when fungus inoculation precedes nematode by 10 days in this study (Table 4).

Final population density (Pf) and reproduction factor (Pf/Pi)

The maximum mean final nematode population density [72002 (J_2+eggs)] and reproduction factor (24) from 100cc soil and the entire root system was observed on Moneymaker genotype when it was inoculated with N1F2 (Table 5). In contrast, the lowest mean *M. incognita* count [18552 (J_2 + eggs)] and reproduction factor (6.18) was on the resistant genotype, Assila where the reciprocal (F1N2) treatment was applied. There was highly significant variation ($p \leq 0.01$) in nematode population density among treatments on all the genotype tested. However, this variation was not significant between treatments, which received NF and N on Assila genotype. The same is true between the treatments on genotype Moneymaker when N1F2 and N. As indicated in Table 5, the final population density of *M. incognita* in the treatment that received both pathogens concomitantly (NF) was numerically lower compared to nematode alone (N) inoculated treatment though not statistically significant.

The interaction effect of disease complex and selected tomato genotypes was noted significant except between Cochoro and Moneymaker where the genotypes were inoculated with N1F2 (Table 5). However, the highest reproduction was on susceptible cultivar when nematode preceded fungus by 10 days. The reproduction of *M. incognita* invariably increased on all genotypes used when inoculated with both pathogens in the sequence of N1F2 or N.

Plant height (PH) and root length (RL)

The result indicated that when *M. incognita* was inoculated simultaneously with the FOL (68 cm) and FOL 10 days after *M. Incognita* (71.75 cm) reduced PH to a

Table 5. Effect of *M. incognita* and *F. oxysporum* f.sp. *lycopersici* disease complex on nematode population density and reproduction factor.

Treatment	*Pf*			RF (*Pf*/*Pi*)		
	Genotypes			Genotypes		
	Assila	Cochoro	Moneymaker	Assila	Cochoro	Moneymaker
N	34016[f]	49438[c]	69802[a]	11.34[f]	16.48[c]	23.27[a]
NF	29600[fg]	43762[de]	51265[c]	9.87[fg]	14.59[de]	17.09[c]
N1F2	48025[cd]	59452[a]	72002[a]	16.01[cd]	19.82[b]	24.00[a]
F1N2	18552[h]	27522[g]	42686[e]	6.18[h]	9.17[g]	14.23[e]
F	0.00[i]	0.00[i]	0.00[i]	0.00[i]	0.00[i]	0.00[i]
C	0.00[i]	0.00[i]	0.00[i]	0.00[i]	0.00[i]	0.00[i]
LSD (5%)	4965.1			1.655		
LSD (1%)	6612.2			2.204		
CV (%)	11.5			11.5		

Means followed by the same letter (s) within the column in each parameter are not significantly different at 5% level of significance; LSD (5%): list significant difference at 5% level of significance; LSD (1%): least significant difference at 1% level of significance; CV (%), coefficient of variation; RGPP, root gall per plant; RGI, root gall index; N, *M. incognita* alone; NF-synchronized inoculation of *M. incognita* and FOL; N1F2, *M. incognita* 10 days prior to FOL inoculation; F1N2, FOL 10 days prior to *M. incognita* inoculation; F, FOL alone; C, control.

Table 6. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici* disease complex on plant height and root length.

Treatment	PH (cm)			RL (cm)		
	Genotypes			Genotypes		
	Assila	Chochoro	Moneymaker	Assila	Chochoro	Moneymaker
N	92.75[cde]	78.75[hi]	100.00[bc]	38.23[abc]	29.33[g]	33.2[c-g]
NF	87.75[efg]	68.00[j]	78.25[hi]	37.15[a-e]	31.0[efg]	39.83[ab]
N1F2	89.25[def]	71.75[ij]	85.50[e-h]	37.83[abc]	37.12[a-e]	36.38[b-e]
F1N2	90.00[cd]	81.00[gh]	89.75[def]	30.12[fg]	32.45[e-g]	29.43[g]
F	95.75[cd]	85.00[fgh]	105.75[ab]	36.42[b-e]	36.15[b-f]	31.77[d-g]
C	96.75[cd]	106.5[ab]	113.00[a]	39.5[ab]	43[a]	40.25[ab]
LSD (5%)	7.599			6.243		
LSD (1%)	10.12			8.314		
CV (%)	6			12.4		

Means followed by the same letter (s) within the column in each parameter are not significantly different at 5% level of significance; LSD (5%): list significant difference at 5% level of significance; LSD (1%): least significant difference at 1% level of significance; CV (%), coefficient of variation; RGPP, root gall per plant; RGI, root gall index; N, *M. incognita* alone; NF-synchronized inoculation of *M. incognita* and FOL; N1F2, *M. incognita* 10 days prior to FOL inoculation; F1N2, FOL 10 days prior to *M. incognita* inoculation; F, FOL alone; C, control.

significant level ($p \leq 0.05$) as compared to the control (106.5 cm) and the rest of the treatments except the treatment, which received *M. incognita* alone on Cochoro genotype (Table 6). The same is true in case of Moneymaker genotype where by the lowest mean PH (78.25 cm) was recorded from NF inoculated treatment. In contrast, the highest PH (113.0 cm) and (106.5 cm) were recorded from the control treatment on Moneymaker and Cochoro genotypes, respectively.

The result of the current study also showed statistically insignificant variation ($p \leq 0.05$) between treatments receiving the two pathogens simultaneously and fungus

Table 7. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici* disease complex on fresh shoot weight (FSW), dry shoot weight (DSW) and fresh root weight (FRW).

Treatment	FSW (g)			DSW (g)			FRW (g)		
	Genotypes			Genotypes			Genotypes		
	Assila	Cochoro	Moneymaker	Assila	Cochoro	Moneymaker	Assila	Cochoro	Moneymaker
N	113.7[ef]	108.1[ef]	140.4[cd]	21.37[a-d]	15.28[ef]	20.19[a-d]	18.58[cde]	17.9[cde]	27.67[a]
NF	108.9[ef]	84.1[h]	113.7[ef]	20.29[a-d]	8.45[g]	21.9[abc]	11.47f	15.49[def]	13.9[ef]
N1F2	110.7[ef]	102.3[fg]	103[fh]	17.1[def]	13.98[f]	17.76[c-f]	13.97[ef]	15.61[def]	13.27[ef]
F1N2	100.4[fgh]	86.2[gh]	102.6[fg]	19.36[a-e]	17.03[def]	18.55[b-e]	19.68[bcd]	17.38[cde]	24.59[ab]
F	124.3[de]	112.5[ef]	161.6[ab]	21.09[a-d]	17.89[c-f]	21.51[abc]	15.42[def]	17.31[cde]	17.25[cde]
C	150.3[bc]	137.7[cd]	168.9[a]	23.7[a]	19.91[a-d]	22.56[ab]	24.45[ab]	21.56[bc]	29.55[a]
LSD (5%**)**	17.74			4.389			5.635		
LSD (1%)	23.62			5.846			7.504		
CV (%)	10.6			16.5			21.4		

Means followed by the same letter (s) within a row and column in each parameter are not significantly different at 5% level of significance. LSD (5%), Least significant difference at 5% level of significance; LSD (1%), Least significant difference at 1% level of significance; CV (%), Coefficient of variation; FSW, fresh shoot weight; DSW, dry shoot weight; FRW, fresh root weight; N: *M. incognita* alone; NF, synchronized inoculation of *M. incognita* and FOL; N1F2: *M. incognita* inoculated 10 days prior to the FOL; F1N2, FOL inoculated 10 days prior to the *M. incognita*; F, FOL alone; C, control.

after 10 days to nematode with respect to PH on Cochoro and Moneymaker genotypes. On the other hand, PH was highly significantly ($p \leq 0.01$) affected by synchronized inoculation of the two pathogens regardless of the genotypes used, including the resistant one, Assila (Table 6). The interaction effect of *M. incognita* and FOL disease complex and the genotypes were statistically insignificant between Assila and Moneymaker except when inoculated with NF. However, this is not true in case of Assila and Cochoro genotypes.

The minimum (29.33 cm) mean root length was recorded from *M. incognita* alone inoculated treatment on Cochoro genotype, followed by nematode inoculated 10 days after FOL (29.43 cm) on the genotype Moneymaker. The maximum root length (43 cm) was from un-inoculated check on the same genotype. There was no statistically significant difference in mean root length among the three selected tomato genotypes, except in between Assila and Cochoro when inoculated with nematode alone.

Fresh shoot weight (FSW)

The minimum (84.1 g) mean FSW was counted from pots inoculated with *M. incognita* and FOL simultaneously (NF) on Cochoro genotype. This was followed by the treatment, where the *M. incognita* was inoculated 10 days after FOL (F1N2); even though, there is no significant difference between each other. The maximum (168.9 g) fresh shoot weight was observed from un-inoculated treatment on the same genotype. Significant ($p \leq 0.05$) interaction effect between treatments and genotypes were also observed where the genotypes were inoculated with NF, N and F. All the treatments inoculated with *M. incognita* and FOL significantly ($p \leq 0.05$) reduced fresh shoot weight (FSW) than treatments, which received either of the pathogens alone, and the control on the susceptible genotype (Table 7).

Dry shoot weight (DSW)

The minimum (8.45 g) DSW, from *M. incognita* and FOL simultaneously inoculated pot and maximum (23.75 g) DSW, from un-inoculated pots were recorded on Cochoro and Assila genotypes, respectively. A significant ($p \leq 0.05$) synergistic interaction effect of treatments and genotypes in terms of DSW were noted if the genotypes are infected with NF and N (Table 7).

Fresh root weight (FRW)

The lowest (11.47 g) mean FRW was recorded on simultaneously inoculated treatment with *M. incognita* and FOL (FN) on Assila genotype followed by treatment received FOL after 10 days to *M. incognita*, N1F2 (13.27 g) on Moneymaker genotype. There was significant ($p \leq 0.05$) variation among the genotypes selected when exposed to *M. incognita* with absence of FOL (Table 7). Fresh root weight in *M. incognita* alone inoculated

Table 8. Effects of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici* on disease severity and AUDPC.

Parameter	Assila	Cochoro	Moneymaker	Assila	Cochoro	Moneymaker
N	1.688[f]	1.875[ef]	2.00[def]	38.5[f]	42[ef]	46.38[cdef]
NF	2.312[bcde]	2..438[bcd]	2.00[def]	54.25[bcde]	56[bcd]	44.62def
N1F2	2.687[ab]	2.562[abc]	3.00[a]	62.12[ab]	58.62[abc]	70[a]
F1N2	2.146[cdef]	2.125[cdef]	2.5[abcd]	45.5[cdef]	49[bcdef]	56.88[abc]
F	2.125[cdef]	2.063[cdef]	2.375[bcde]	49.88[bcdef]	46.38[cdef]	53.38[bcde]
C	0.00[g]	0.00[g]	0.00[g]	0[g]	0[g]	0[g]
LSD (5%)		0.5056			13.18	
LSD (1%)		0.6734			17.55	
CV (%)		18.9			21.6	

Means followed by the same letter (s) within a row and column in each parameter are not significantly different at 5% level of significance. LSD (5%), Least significant difference at 5% level of significance; LSD (1%), Least significant difference at 1% level of significance; CV (%), Coefficient of variation; AUDPC, area under disease progress curve; N, *M. incognita* alone; NF, synchronized inoculation of *M. incognita* and FOL; N1F2, *M. incognita* inoculated 10 days prior to the FOL; F1N2, FOLinoculated10 days prior to the *M. incognita;* F, FOL alone; C, control.

treatment was comparable to the un-inoculated treatment on Cochoro and Moneymaker genotype. Significant ($p \leq 0.01$) reduction in FRW invariably across the genotypes was noted when it was inoculated two pathogens simultaneously and N1F2 and there is insignificant variation among the genotypes in this regard. The interaction effect of treatments and the genotypes was observed significant ($p \leq 0.05$) if the genotypes are inoculated with *M. incognita* alone (Table 7).

Disease severity and area under disease progress curve

The interaction effect of disease complex and genotype on mean disease severity score was insignificant ($p \leq 0.05$). The main effect of the genotypes was also found to be insignificant and here under the main effect of the treatments, only is presented. This was invariably true in case of the resistant genotype, Assila and moderately resistant genotype, Cochoro on which highest mean disease score, 2.687 and 2.562 and area under disease progress curve, 62.12 and 58.62 respective order, was recorded from treatments infected with FOL 10 days later to *M. incognita* (Table 8).

DISCUSSION

This research result showed that the number of root gall and galling index caused by *M. incognita* has increased in the presence of fusarium wilt (FOL) either concomitantly (NF) or nematode 10 days prior to the fungus (N1F2). This might be attributed to increased penetration rate of *M. incognita* juveniles (J_2) into the roots due to co-infection of both pathogens. Similar result was obtained by Al-Hazmi and Al-Nadary (2015); whereby synchronized inoculation of *M. incognita* race 2 and *Rhizoctonia solani* (N + F) increased the index of rhizoctonia root rot and the number of root galls on green beans (*Phaseolus vulgaris* L.). Minimum number of root gall in F1N2 treatment might indicate the unsuitability of root for J_2 penetration and the fungus damaged lack of support for the nematodes to establish within the root system as it. *F. oxysporum* f.sp. *lycopersici* infection and establishment in plant roots previously infected by nematodes (N1F2) was enhanced as the developing root galls associated with nematode feeding may act as a nutrient sink. Elevated major organic constituents of root exudates mainly, carbohydrates and nitrogenous compounds during the first fourteen days after nematode infection is well established fact (Van Gundy et al., 1977; Mai and Abawi, 1987). These organic constituents are considered to be major nutrient consumptions for different fusarium wilt inciting fungal species like *F. oxysporum* that co-infect the same host plant. This result also supports /depicts these established research reports as determined by synergistic interaction effect of the two pathogens on the number of root galls. This is generally in line with nematode induced physiological modification/change theory of nematode fungus interaction in the host plant tomato. Maximum number of root gall on treatments when nematode was inoculated 14 days prior to the fungus and on synchronized inoculation of both pathogens with no statistically significant difference between each other on the same experimental host plant and pathogens was also reported (Khpalwak, 2012). Statistically insignificant variation between the resistant and moderately resistant tomato genotypes in number of root gall during co-infection of the two pathogens (NF) probably indicates loss of potential genetic resistance in resistant genotype due to co-infection of these pathogens and signifies the negative impact of the disease complex on the resistant potential of tomato plant as it was measured by the number of root gall. However, increased number of RGPP along with the susceptibility gradient of tomato genotypes implied that *M. incognita* resistant genotypes would also be promising for the management of the disease complex.

Similar trends of increase in number of egg-mass and egg-mass index in N1F2 and NF treatments across all the genotypes tested were observed. The influence of FOL and time of its application on *M. incognita* egg-mass development on tomato was most pronounced with N1F2. This clearly depicts the negative impact of FOL and its time of inoculation on the development of nematode egg. Similar previous findings of Yang et al. (1976), Al-Hazmi and Al-Nadary (2015) and Kumar et al. (2017) are in line with this result. This might be also attributed to reduced food sources for nematode as the root system is affected with the fungus in F1N2 inoculated treatment. Timing of application of nematode and fungi seems to matter the relationship between the invasions of tomato by *M. incognita* as it was also supported by Back et al. (2006) who prove the relationship between the invasion of potato roots by potato cyst nematodes and the percentage of stolon affected by *Rhizoctonia solani* was strongest 6 and/or 8 weeks after planting. Lowest EMI in F1N2 treatment however is against the previous result of Pauline (2016), who reported lower EMI in combined inoculation of *F. oxysporum* and *Meloidogyne* species as compared to inoculation of *Meloidogyne* species alone and highest EMI viz. single inoculation of *Meloidogyne* species on the same experimental host plant. This might be attributed to the genetic nature of tomato genotype used in the experiment as it was depicted with significant interaction effect of Assila and the treatments.

Measurement of nematode reproduction (host efficiency) and yield or growth is vital to quantify the reaction of plants to RKN infection (Mai and Abawi, 1987). High nematode population density and reproduction factor from N1F2 treatment, which was low with the reciprocal treatment (F1N2), may be explained by the nutrient competition effect of the two pathogens co-habiting the common host plant. Sugars in root exudates from *M. incognita* infected tomato increased from to 836% over un-inoculated check (Wang and Bergeson, 1974).

This indicates huge nutritional advantage of the nematode (*M. incognita*) obtained from the host plant response during *M. incognita* infection and the entire disease cycle. However, this nutritional advantage of the nematode could be suppressed if other pathogen of the same nutritional requirement existed on the same ecological niche that is soil rhizosphere and plant rhizoplane. Similar previous report in which infection of the fungus *Verticillium albo-atrum* resulted in no enhancement effect on reproduction of neither stubby root nematode (*Trichodorus christie*) nor *M. incognita* on or in the root of the host plant (tomato) was also reported (Conroy and Green, 1974). Invariably increased nematode population on all genotypes used when inoculated with both pathogens in the sequence of N1F2 indicates the unfavorable impact of *M. incognita*-FOL interactions on nematode reproduction probably due to the colonization of giant cells with fungi and thereby disturbing their function. The probable inhibitory effects of fungi metabolites on hatching of nematode eggs are also reported by Zahid et al. (2002). The decline in nematode populations involved in a disease complex with fungi may also be explained by competition for nutrients and root space between the two organisms (Back et al., 2002).

Invariably significant synergistic effect of the two pathogens on tomato plant growth and biomass was explained by reduction of plant height, root length, fresh shoot weight, and dry shoot weight with concomitant infection (NF). Similar previous research finding (Goswami and Agrawal, 1987; Johnson and Littrell, 1970; Kumar, 2008; Kumar et al., 2017; Lobna et al., 2016; Negron and Acosta, 1989) also indicated similar research finding. However, the interaction effect of the two pathogens on plant height has been also affected by the existing varietal variation. Assila is a genotype with determinate growth habit (bushy type that grows 60-90 cm tall) whereas Moneymaker is genotype with indeterminate growth habit, vining type tomato that grows 1.5-3 m tall. Root length was found unaffected when inoculated simultaneously with both pathogens (NF) which is similar with previous research report of Kumar et al. (2017) and against the research results of Goswami and Agrawal (1978).

The increase of fusarium wilt severity in the presence of *M. incognita* may be due to the fact that infection by this endo-parasitic nematode (*M. incognita*), whether prior to the fungal infection (N1F2) or simultaneously (NF), causes physiological and anatomical changes in the root tissues predisposing the plants to increased fungal infection (Al-Hazmi and AL-Nadary, 2015). This result is also in line with the finding of Katsantonis et al. (2003) on which invasion of the roots of cotton by *M. incognita* enhanced disease severity, as measured by the height of vascular browning in the stem, following inoculation of *F. oxysporum* f.sp. *vasinfectum.* The result also indicates the importance of timing of nematode infection and plant defense mechanisms for the establishment of an interaction as supported by increased disease symptoms when *M. incognita* are inoculated 10 days before or together with the FOL. The fungus might utilize the feeding tracts of the nematode to infect the tomato plants. Research findings of (Yang et al., 1976) indicated promoted wilt development viz. *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* interaction on cotton. Highest wilt disease incidence due to infection of the nematode before the fungus on tomato plant (Khpalwak, 2012) is also proved, experimentally.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

REFERENCES

Al-Hazmi AS, Al-Nadary SN (2015) Interaction between *Meloidogyne incognita* and *Rhizoctonia solani* on Green Beans. Saudi Journal of Biological Sciences 22:570-574.

Back M, Haydock P, Jenkinson P (2006). Interactions between the potato cyst nematode *Globodera rostochiensis* and diseases caused by *Rhizoctonia solani* AG3 in potatoes under field conditions. European Journal of Plant Pathology 114(2):215-223.

Back MA, Haydock PP, Jenkinson P (2002). Disease complexes involving plant parasitic nematodes and soil borne pathogens. Plant Pathology 51(6):683-697.

Bernard GC, Egnin M, Bonsi C (2017). The Impact of Plant-Parasitic Nematodes on Agriculture and Methods of Control. In: shah MM, Mohamood M., eds., Nematology Concepts, Characteristics and Control, InTech, London 121. https://doi.org/10.5772/intechopen.68958.

Chen ZX, Chen SY, Dichson DW (2004). Nematology Advances and Perspectives: Nematode Management and Utilization. Tsinghua University Press, Beijing.

Conroy JJ, Green RJ (1974). The Stubby Root Nematode *Trichodorus christiei* with *Verticillium*. Phytopathology 64:1118-1121.

Coyne DL (2007). Practical plant nematology: a field and laboratory guide. IITA

Central Statistical Agency (CSA) (2017). Agricultural sample survey report on area and production of major crops (private peasant holdings, Meher season). Vol I. Addis Ababa, Ethiopia.

FAOSTAT (2016). Crop production: Food and Agricultural Organization of United Nations.

Goswami BK, Agrawal DK (1978). Interrelationships between species of Fusarium and root knot nematode, *Meloidogyne incognita*, in Soybean. Nematologia Mediterranea 6(6):125-128.

Holbrook CC, Knauft DA, Dickson DW (1983). A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Disease 67(9):957-958.

Hussey RS (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Report 57:1025-1028.

Johnson AW, Littrell RH (1970). Pathogenicity of *Pythium aphanidermatum* to chrysanthemum in combined inoculations with *Belonolaimus longicaudatus* or *Meloidogyne incognita*. Journal of Nematology 2(3):255.

Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-López R, Palomares-Rius JE, Wesemael WM, Perry RN (2013). Top 10 Plant-Parasitic Nematodes In Molecular Plant Pathology. Molecular Plant Pathology 14(9):946-961.

Katsantonis D, Hillocks RJ, Gowen S (2003). Comparative effect of root-knot nematode on severity of Verticillium and Fusarium wilt in cotton. Phytoparasitica 31(2):154-162.

Khpalwak W (2012). Interaction between *Fusarium oxysporum* f.sp. *lycopersici* and *Meloidogyne incognita* in tomato. Dissertation, University of Agricultural Sciences, Dharwad.

Kumar B (2008). Studies on root Knot and wilt complex in *Coleus forskohlii* (Wild.) Briq. Caused by *Meloidogyne incognita* (Kofoid and White) Chitwood *and* Fusarium *chlamydosporum* (Frag. and Cif.) Booth. Doctoral dissertation, University of Agricultural Sciences, Dharwad**.**

Kumar N, Bhatt J, Sharma RL (2017). Interaction between *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lycpersici* on Tomato. International Journal of Current Microbiology and Applied Sciences 6:1770-1776.

Leslie JF, Summerell BA (2006). The Fusarium Laboratory Manual. Blackwell Publishing, Iowa. http://doi.org/10.1002/9780470278376

Lobna H, Hajer R, Naima MB, Najet HR (2016). Studies on disease complex incidence of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* on resistant and susceptible tomato cultivars. Journal of Agricultural Science and Food Technology 2(4):41-48.

Mai WF, Abawi GS (1987). Interactions among root-knot nematodes and Fusarium wilt fungi on host plants. Annual Review of Phytopathology 25(1):317-338.

McGovern RJ (2015). Management of tomato diseases caused by *Fusarium oxysporum*. Crop Protection 73:78-92*.*

MoARD (Mintery of Agriculture and Rural development) (2009). Improved production technology of tomatoes in Ethiopia.

Negron JA, Acosta N (1989). The *Fusarium oxysporum* f. sp. *coffeae-Meloidogyne incognita* complex in 'Bourbon'coffee. Nematropica 19(2):161-8.

Oduor TK (2016). Agro-morphological and nutritional characterization of tomato landraces (*lycopersicon* species) in Africa. MSc Thesis, University of Nairobi.

Pauline KM (2016). Host resistance and interaction between root knot nematodes and fusarium wilt of tomato. MSc Thesis, Kenya University.

Seid A, Fininsa C, Mekete TM, Decraemer W, Wesemael WM (2017). Resistance screening of breeding lines and commercial tomato cultivars for *Meloidogyne incognita* and *M. javanica* populations (Nematoda) from Ethiopia. Euphytica 213(4):97.

Shaner G, Finney RE (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67(8):1051-6.

Song W, Zhou L, Yang C, Cao X, Zhang L, Liu X (2004). Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. Crop Protection 23(3):243-247.

Srivastava R, Sharma SK, Singh JP, Sharma AK (2009). Evaluation of tomato (*Solanum lycopersicum* L.) germplasms against *Fusarium oxysporum* f. sp. *lycopersici*, causing wilt. Pantnagar Journal of Research (India).

Tabor G, Yesuf M (2012). Mapping the current knowledge of carrot cultivation in Ethiopia. Carrot Aid, Denmark, pp. 1-20.

Taylor AL, Sasser JN (1978). Biology, Identification and Control of Root-Knot Nematodes. International Nematology Project, North Carolina State University, Graphics, Raleigh 111.

Van Gundy SD, Kirkpatrick JD, Golden J (1977). The nature and role of metabolic leakage from root-knot nematode galls and infection by *Rhizoctonia solani*. Journal of Nematology 9(2):113.

Wang EL, Bergeson GB (1974). Biochemical changes in root exudate and xylem sap of tomato plants infected with *Meloidogyne incognita*. Journal of Nematology 6(4):194.

Webster JM (1985). Interaction of *Meloidogyne* with fungi on crop plants. AGRIS: International information system for the agricultural

science and technology http://agris.fao.org/agris-search/search.do?recordID=US8743744
Wondimeneh T, Sakhuja PK, Tadele T (2013). Root-knot nematode (*Meloidogyne incognita*) management using botanicals in tomato (*Lycopersicon esculentum*). Academia Journal of Agricultural Research 1(1):009-016.
Yang H, Powell NT, Barker KR (1976). Interactions of concomitant species of nematodes and *Fusarium oxysporum* f. sp. *vasinfectum* on cotton. Journal of Nematology 8(1):74.
Zahid MI, Gurr GM, Nikandrow A, Hodda M, Fulkerson WJ, Nicol HI (2002). Effects of root- and stolon-infecting fungi on root-colonizing nematodes of white clover. Plant Pathology 51:242-250.

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Effective policies to mitigate food waste in Qatar

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This paper highlighted food waste as one of the biggest threats to food security that put pressure on the natural resources and limit the ecological capacity of land of Qatar to continue providing renewable resources. Climate change, desertification of farmland, water shortages, soil degradation and arable land per capita decline are the main characteristics of the state of Qatar. This arid and semi-arid environment resulted in difficulties to produce food locally. Qatar used to import 90% of its food from neighboring countries before the blockade in 2017. Qatar is passing an important era of total shift from food security to self-sufficiency. In a very short time, Qatar managed to register almost full sufficiency in perishable foods and produced abundant amount of food. This shed light in the importance of sustainable production and consumption to avoid environmental disasters such as food waste that directly affect the sustainability of arable land and ground water. A panel of academics, administrators, civil society and charities came together to discuss the issue of sustainability regarding food waste, in order to formulate policies and strategies to mitigate food waste and produce compost to be used in agriculture and hence achieve food self-sufficiency. These policies will help managers and policy makers to make correct decisions to preserve the environment.

Key words: Food waste, sustainable development, policies, food security.

INTRODUCTION

Recently, food waste research is gaining more attention globally because of its direct relation to the Sustainable Development Goals such as environment and resources sustainability, food security, resource management and the higher economic and environmental costs related to food waste and loss. The growing concern of the sustainable practice of converting waste to valuable resources such as energy, that registers an ever-increasing demand for food production, is also one of the factors that increased the food waste research (Thyberg and Tonjes, 2016). Moreover, in September 2015, the United Nations General Assembly adopted seventeen goals for sustainable development as part of Plan 2030. Specifically, the 12th goal aims at *"ensuring sustainable production and consumption patterns*" The third sub goal (Goal 12.3) is to decrease the per capita share of global retail and consumer food waste and to reduce food loss, including post-harvest loss, along the production and supply chains by 2030 (United Nation 2015). According to the Food and Agricultural Organization (FAO), when all the population in a country are able to access safe, nutritious and sufficient food at all time with affordable prices, the country is considered as a food secure country. Unfortunately, the ability of attaining food

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security is threatened by the issue of food wastage (FAO, 2015a). As observed by Aktas et al. (2017), food wastage poses a great threat to the social, environmental, and economic pillars of the Sustainable Development Goals (SDGs). For instance, there is the issue of monetary value lost across the entire production and supply chain and inability to improve on the problem of malnutrition by not feeding those who cannot afford the food. Measuring food loss and waste along food chains can give decision-makers a better understanding of where and why food is wasted. These data are also the basis for prioritizing the mitigation and monitoring strategies to make progress in waste management (Flanagan et al., 2018).

Qatar has rich non-renewable (gas and oil) and renewable resources, which have been subjected to misuse and environmental pressures. A major reason is the recent population explosion resulting from the FIFA World Cup Qatar 2022 preparations, which have involved every aspect of the society. The ensuing expansion in construction, manufacturing, agriculture, and mining has put greater pressure on the country's natural wealth. The carbon dioxide levels, the air pollution, and the incidence of pollution-related diseases have increased (Richer, 2014).

The State of Qatar has responded to the sustainable development goals by formulating Qatar National Vision 2030, which calls for sectoral coordination to achieve responsible consumption and increase recycling. In order to avoid the issue of previous National Development Strategy 2016–2019 that had poor sectorial coordination implementation, the second National Development Strategy 2018–2022 has established a clear and comprehensive mechanism to enhance coordination. The second strategy included a review of the performance of the institutions in response to the first strategy. One of the advantages of National Development Strategy 2018–2022 has been its promotion of innovative policies and support for institutional reflection on the food security program (Planning and Statistics Authority Report, 2018).

Environment and food security are the most prominent sustainable development goals because they overlap and comprise all the pillars of sustainability, which are economic, social, and environmental. One of the greatest threats to food security and environmental sustainability is food waste. It has a direct effect on several interrelated aspects of human life. For example, economically, food waste leads to increased demand for food, and thus, higher prices. This leads to social cost, when prices are high; fewer individuals can purchase good quality food. It also has adverse health effects such as malnutrition-related diseases. From environmental aspect, food waste can affect the environment negatively and produce environmental pollution, that is, the decomposition of organic waste in landfills leads to higher levels of methane, which is more harmful than carbon dioxide because it accumulates in the atmosphere for a longer time. Seven percent of the greenhouse gas emissions are from food waste (Buzby and Hyman, 2012). The management of Landfills requires a lot of financial and human resources. Globally, the carbon footprint resulting from food waste greenhouse gas release is estimated at 3.3 billion tons of CO_2 and 750 dollars economic loss (FAO, 2015b).

From a religious perspective, food waste can create a sense of guilt from extravagance and often-unintentional waste, especially during the month of Ramadan when food waste is not accepted and is becoming significant ethical dilemmas. According to the Food and Agriculture Organization of the United Nations (FAO), the number of undernourished people is increasing daily and estimated to be approximately 925 million in 2015 (FAO, 2015a).

Municipal waste management and food waste are complex issues that need interdisciplinary approaches to manage and if handled carefully, it will safe considerable amount of money, feed the hungered and reduce pressure on natural resources. It can also be very beneficial and has multiple economic and ecological benefits such as, creating new employment and business opportunities. In addition, compost of food waste can be use in agriculture to improve food security. Environmental gain from waste management include, the bio-energy produced from waste and reduction of greenhouse gas emissions. Less gas emissions helps improve health and reduce health costs related to pollution (Elagroudy et al., 2016).

There is an urgent need of effective waste reduction strategies to avert environmental disasters. Therefore, the goal of this study is to highlight the importance of the appropriate management of food waste and to provide effective policies and strategies that help the government and non-government agencies to manage food waste successfully.

The State of Qatar: Strategies for the blockade and food security

Climate change, desertification of farmland, water shortages, soil degradation and arable land per capita declined are the main characteristics of the state of Qatar, arid and semi-arid environments. This results in difficulties to produce food locally (Qatar National Food Security Program, 2012). In previous years, before 2017, that is, the date of blockade, Qatar use to import 90% of its food from neighboring countries. Nevertheless, half of the Landfill components is from food waste. Given these circumstances of lower food production, wasting food, regardless of the amount, is unjustified.

The State of Qatar has established a widely recognized food security strategy. Especially since the blockade, the country allocated significant amount of financial resources to implement the strategy, which has become a major catalyst for achieving almost 100% self-sufficiency in a short time. Seventy million Qatari riyals

Table 1. Increases in agricultural land / hectares and production/ tons since the blockade.

Agricultural sources	Increase in agricultural land from 2017 2018	Increase in production from 2017 to 2018
Cold frame greenhouses	14 ha	1,686 tons
Grow houses	20 ha	4,000 tons
Exposed land	-	3,000 tons
Qatari farms that have been rehabilitated	105 subsidized farms for Qataris	59% increase in Qatari consumer products versus only 36% increase in imported products
Major strategic projects adopted by the Ministry of Municipality and Environment	1 Million square meters per project	Vegetable production

Source: Qatar News Agency (2018), Qatar's Achievements in Food Security.

Table 2. Non-vegetable agricultural products.

Agricultural product	Increase in production from 2017 to 2018
Dairy and its derivatives	346 tons
Fish	10% increase in fish farming, 2,000 tons per year of floating cages are produced annually with a capacity of 1,000 tons per year of shrimp farming project
Poultry	An increase of 29 tons per day, the GDP rose to 98%
Table eggs	An 8-ton increase, which led to a 50% drop in the price of imports, reflecting the adjustment of local market prices
Livestock: economic animals	Increase in farm animals by 1.6 million

Source: Qatar News Agency (2018), Qatar's Achievemnts in Food Security.

were allocated for each of the succeeding five years projects in support of agriculture. Agricultural production (vegetables, dates, red meat, poultry, eggs, fish, and green fodder) doubled to 400% in just one year, which is 2017 to 2018. The implementation of this strategy included the establishment of research centers to enhance agricultural production. Three centers were established to develop research on fish and animal production. This includes: (1) The settlement of Ras Matbukh, the home of the aquaculture system, which is dedicated to fish farming by floating cages and shrimps farming project (Ministry of Municipality and Environment, 2018); (2) Al Sheehaniya Health Center, which was established for animal production, dedicated to the protection of wildlife biodiversity, especially the houbara bustard, which is at a risk of extinction; (3) The Mazroa'a Center, an agricultural extension center, providing outreach services to farmers. Given that agricultural production is new to the State of Qatar, there is an urgent need to help farmers to adopt efficient techniques to increase production while maintaining the soil. It is also worth noting that the Ministry of Municipality and Environment introduced large-scale strategic vegetable production at one million square meters per project. The amount of land allocated indicates that these are indeed very large projects. The most prominent projects in Qatar's agricultural expansion are listed in Tables 1 and 2 (Qatar News Agency, 2018).

The blockade is an economic shock that forces a country to develop a short-term strategy to cope with the new situation. However, there is a need to consider the long-term negative environmental effects that accompany the short-term strategies especially since those environmental negative impacts are irreversible. The long-term negative environmental effects include the expansion of the areas allocated to agriculture. This places stress on the natural reserves and threatens the country's biodiversity. Qatar, which has the world's largest environmental footprint, will face many challenges, including land degradation, air pollution and increased waste from the agricultural and industrial expansion. This can result in a higher incidence of diseases related to low air quality, especially if the factories are dependent on fossil fuel energy.

Caution is required for the policy of increasing food production. Sustainable production and environmental and natural resource management is very vital to be addressed at this stage. Because of the blockade, agricultural expansion has also led to water waste in a country that relies on water treatment, thereby increasing the burden on the financial resources.

The State of Qatar has addressed these challenges

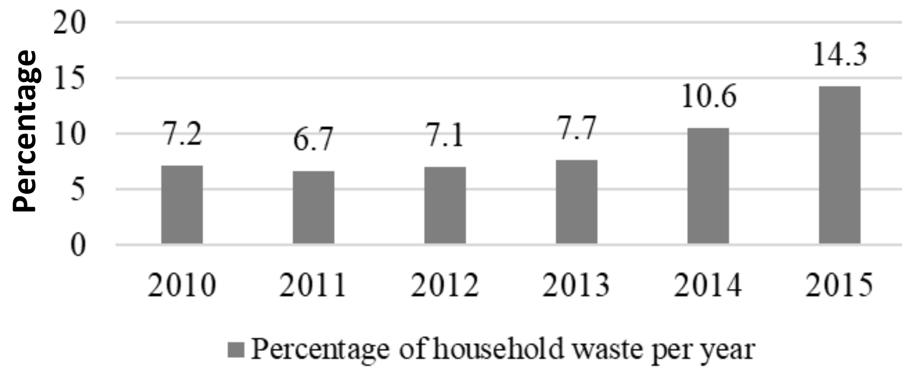

Figure 1. Percentage of household waste per year compared to total waste.
Source: Planning and Statistics Authority (2017). Qatar's Voluntary National Review, 2017.

through the development of sustainable environmental management policies. For example, the government has shown an interest in sustainable food production techniques to reduce water consumption and environmental waste. There are also policies to encourage multidisciplinary studies, such as assessments of the sustainable productivity of the renewable natural resources to ensure long-term sustainable development (General Secretariat for Development Planning, 2008).

Greater attention should be paid to the problem of food waste because of the expansion of the agricultural sector, e.g., vegetable and fruit production. The acceleration in food production leads to an increase in supply and, thus, a reduction in prices, this in turn results in increased food waste. FAO noted that the estimated average food wastage by inhabitants in Qatar is around 250 kg per day (Adema, 2016). Approximately 20 million kilograms of food in Qatar are either destroyed or discarded before reaching the end-consumer (Adema, 2016). The increase in food waste in Qatar has been associated with the population growth. Figure 1 illustrates the increase in food waste from 2010 to 2015.

The social impact of food waste on Qatar society

One of the social impacts associated with the intensified food wastage in Qatar is the increase in food prices which, eventually exacerbating the issue of food security resulting in the problem of malnutrition. This is primarily because when the food prices go up, some of the population will be unable to afford quality foods implying that they will not be in a position to meet their dietary needs.

In their research study, Baiga et al. (2018) underscored the fact that the Gulf Cooperation Countries (GCC) takes the lead of the global top food wasters. An example case scenario is the case of Ramadan when considerable amount of food is wasted. In the Qatari context, the issue of food wastage was cited as a major problem in the country (Adema, 2016). In 2012, the total food consumption and wastage estimation stood at 1.4 million metric tons (Adema, 2016). However, it is also imperative to acknowledge the fact that Qatar is one of the GCC countries that have experienced a rapid and monumental economic growth over the recent decades after the oil discovery. It follows that the per capita income has increased and hence money is not a deterrent factor when it comes to the quality and quantity of food that the population demand (Adema, 2016).

Based on the existing literature, the recklessness in food consumption is a common trend in Qatar. When it comes to traditions and customs that revolve around the food industry, Qataris are known for their generosity. The tradition of hospitality is largely acknowledged and practiced in the Qatari context. The tradition has continued to take a center stage in the country. Edelstein (2011) who notes that the culture of generosity is largely felt across Qatari supports the sentiments. This is just like in the traditional times when the host of a party such as weddings or any other form of communal dining was expected to demonstrate unfailing generosity and hospitality. As a way of extending and celebrating the particular family or traditional feast, visitors engage in informal and warmth filled conversations. This act of hospitality and generosity is extended beyond the home settings and into the restaurants and other eateries. For instance, and according to Sillitoe and Misnad (2014), it is highly welcoming to dine with the Qataris. The above-cited authors noted that a Qatari will always insist that the particular visitor eat or take the meal or drink respectively to the last piece or drop. This demonstrates their undying generosity. Nevertheless, some of the Qataris will always insist to settle the pending meal or drink bills in a restaurant. Qataris are also encouraged to share a meal or drink with anyone who sits closer to them. Similar to other nations and cultures, family life does have an influence on the food consumption pattern in Qatar. As observed by Al-Thani et al. (2017), personal preferences coupled with the individual family resources will play a pivotal role concerning the choosing foods and consumption patterns. The Qatari society is highly multi-diverse owing to the surging number of expatriate community. The more the available resources, the more choices that a family has with regard to food and consumption pattern.

It is partially on this basis that the problem of food waste has been rampant in the Qatari context. There is a clear relationship between a family's economic status and its social position. Education levels and family incomes influence food behaviors and consumption patterns. With oil recovery, there has been intensified economic growth translating into increased per capita income. In the midst of such dynamics, people are able to purchase more than they can consume. For instance, those from the well-to do families have a choice of buying fast foods at the expense of cooking their own food staffs. However, it is also important to mention that the issue of healthy eating comes into consideration when choosing the consumption pattern. For instance, the diet is often rich in meat protein and carbohydrates rather than fruits and vegetables. As such, and regardless of the economic status, some of the Qataris continue to embrace traditional foods as opposed to junk foods (Al-Thani et al., 2017).

In addition, the phenomenon of the dumping of leftover food is widespread in the Arab countries, especially during the month of Ramadan. It should be noted that observances, e.g., Ramadan, affect the dietary habits and traditions of Qatari families. Despite the large number of Ramadan-related food projects that aim to help the needy, the phenomenon of food waste remains a feature of Ramadan (Al-Thani et al., 2017).

A study showed some interesting results by reviewing a sum of empirical studies conducted in Europe. It revealed that the feel of guilt that household have from wasting food, is only generated by financial loss and has no relation with environmental protection or social implication. They also mentioned that elder people have higher tendency towards reducing food waste however, household with more children tends to waste more food (Schanes et al., 2018).

From a health perspective, food waste has complex effects on health such as increase mortality, chronic health conditions, health deterioration, behavioral problems, and poor mental health. Food waste directly harms the environment. Human health and well-being are affected by air and water pollution, and poor air and water quality contributes to chronic health conditions, such as asthma, bronchitis, and other lung diseases. It also negatively affects well-being. The symptoms include headaches, aches, pain, and chronic fatigue. These symptoms can be related to inflammatory responses to air and water pollution and they could contribute to autoimmune diseases, such as type 1 diabetes, lupus, and multiple sclerosis (Bos-Brouwers et al., 2014). Therefore, better Understanding of the extent of food wastage is very important for changing attitudes and behaviors towards food waste and formulates sustainable policies accordingly.

MATERIALS AND METHODS

Although food waste is not an easy problem and has significant social, economic and environmental negative impacts, government and policy makers still cannot magnify this extent. FAO in 2012 estimated the food loss and waste in United States reached approximately 936 billion dollars, which is larger than the Netherland GDP at that time (FAO 2018). El-Agroudy et al. (2016) mentioned that half of the world's population lack proper access to waste management services. The main waste-disposal method is open dumping in most developing countries, with unlimited negative consequences.

Food waste is one of the most significant challenges facing the Arab world. For example, in Kingdom of Saudi Arabia, the estimated annual food waste generated was around 7.7 million tons with an average of 0.71 kg per capita per day (Mu'azu et al., 2019 cited in their publication). Moreover, Abiad and Meho (2018) found that food waste in the Arab world was 210 kg per capita. The Food and Environment Protection Project implemented by Georgetown University has found that 90% of the waste in Qatar is food waste (Aktas et al., 2017). In 2016, the Ministry of Municipality and Environment indicated that 31% of the waste was organic. Abdelaal (2017) said that, "*there is a great discrepancy between the figures published in the news on the Internet and official blog articles and data regarding the quantities of waste generated annually in Qatar*". The annual environmental statistics report, published by the Planning and Statistics Authority in Qatar in 2015, indicated that 613,226 tons of solid household waste was treated at the local solid waste management center, and another 482,640 tons of domestic solid waste was treated in Mesaieed outside the local solid waste management center (Abdelaal, 2017). However, rapid population growth remains the biggest challenge to exaggerate the food waste reduction. Because of the contradictory data, the present study created a mechanism for academics and administrators to study food waste collaboratively. Consequently, a closed panel discussion gathered policy makers and academics to discuss food wastage and suggest policies and strategies accordingly.

Closed panel discussion

As was previously mentioned, Qatar achieved self-sufficiency in perishable food within a short period. The abundance of locally produced food has led to lower prices and increased purchasing power. This could result in a great amount of food wastage that could create an environmental disaster. In order to get rid of all possible long-term negative impacts to the environment, Qatar has tried to avoid short-term strategies resulting from the blockade by adapting different strategies. For instance, the country has emphasized recycling and food waste management to be one of the important objectives and issue of priority to the state of Qatar. Raising awareness about the environment and society is becoming a necessity and the food waste is not individuals based problem. Therefore, involving all the stakeholders through the entire supply chain will help improve policy implication. Thus, a closed panel discussion was held on March 31, 2019, to discuss the reduction of food wastage transferred to the landfill and to develop policies and strategies to reduce food wastage in Qatar. To formulate policies and recommendations, the collaboration of academics from several disciplines with the administrators who work in similar fields is required. Therefore, the panel brought together academics from Qatar University from different fields such as health, environment, religion, economic, and social science, as well as administrators from the Ministry of Municipality and Environment, and representatives from the private sectors and charities.

Goals of the closed panel discussion

1. Coordination, cooperation and participation between different institutions in the relevant disciplines through conducting research and projects. This would prevent duplication, develop a unified accurate database, and avoid the issues of inconsistent data.
2. Efficient management of financial resources related to the projects and research that are of high priorities to governmental institutions.
3. Discussion of the issues from multiple perspectives to provide a comprehensive understanding of the problem. This facilitates interaction and resolution. It also saves time and effort, especially because the data from the government agencies are often not readily accessible.

RESULTS AND DISCUSSION

There are several ways to minimize and dispose food wastage. The rules and regulations governing the safety of food waste treatment are important. Food redistribution practice by charities to favor underprivileged people is a famous practice worldwide. Reynolds estimated that, if the quantities of food wasted were rescued by charities, a number of 921 people could be supported in Australia (Reynolds et al., 2015).

Charitable organizations in Qatar have played a prominent role in the humanitarian activities to preserve food and to reduce waste. Some charities have thought to raise awareness and to promote a culture of food preservation by delivering excess food to beneficiaries in accordance with the best international quality and safety standards. “Hifz alNiema” and “Wahab” are non-profit organizations that collect the food leftovers from hotels, supermarkets, and restaurants to deliver it to ones in need. These projects seek to address the extravagance in the society. They reduce the waste of surplus food and redistribute meals after ensuring their validity. The food is stored in safe, healthy conditions for distribution to poor families and low-income workers (Sheikh Eid Charity Foundation, 2008). Other initiative includes Amwaj, a pilot project in Mesaieed that converts organic waste into compost and other materials. Reducing the phenomenon of wasted food is a social responsibility issue that should be addressed in homes, schools, universities, and other institutions. These initiatives require sustained government support and encouragement. Additional food redistribution, recycling, and waste reduction community initiatives are needed (Vittuari et al., 2016).

Ongoing initiatives by the Ministry of Municipality and Environment

In the State of Qatar, there are some institutional efforts to preserve the environment and to support food security. This is manifested in the food waste reduction initiatives and the projects and smart technologies that have been designed to create a clean environment and societal awareness. The following are examples of the ongoing initiatives by the Ministry of Municipality and the Environment:

1. Public awareness campaigns, e.g., those that coincide with the camping season.
2. Penalties, including fines, for improper waste disposal, e.g., dumping garbage in public places. Information about the waste penalties has made available in local radio, newspapers, and in other media.
3. “Oun”, a smartphone application launched by the Ministry of Municipality and Environment to help the public in some services such as sewage collection, manage, and rodent control.
4. The use of methane-fueled machines in the landfill to reduce considerable amount of methane and hence reduce emissions.
5. The use of methane-fueled vehicles to transport the waste to the landfill.
6. Redesigning and engineering the construction of landfills to enhance its capacity.
7. Planting 100 trees from seven types of trees, including the acacia, to absorb soil salinity and research is currently underway to study “Marmar” trees to develop a natural plant of oxygen production.
8. Some private factories have been converting food waste into animal feeds and fish food.
9. The largest Plant in the Middle East that recycles waste and converts it into compost follows the Ministry of Municipality and Environment. The State of Qatar decided to achieve 100% self-sufficiency in compost production, which used to be imported from India and Pakistan.

The results of the closed panel discussion

Academics from several disciplines at Qatar University contributed to the design of policies to reduce the amount of food waste transferred to the landfill. The two-hour discussions focus on the following agendas:

1. Promote new food behaviors and attitudes.
2. Increase awareness of food waste and its negative impacts.
3. Enact strong legislation and impose penalties on wastes.
4. Promote the concept and principles of food waste recycling.
5. Encourage community participation, such as school and university students, in reducing food waste.
6. Encourage research in food waste management in the State of Qatar and assist decision-makers and policy makers in estimating food waste.

Policies and recommendations for reducing the amount of food transferred to landfills

The panel's outcomes drafted a proposal of policies and strategies that can be circulated among the relevant authorities. The panel approved the following policies and recommendations:

1. Create a national committee with a mandate to address food waste. Its mission would be to redefine the national consumption culture at the state level through a "National Food Consumption Charter."
2. Publish comprehensive analysis of policies and recommendations about food waste management in a book titled "*Public Policies in Qatar*".
3. Focus on institutional waste rather than just household waste by targeting the sources.
4. Increase awareness and provide training on waste classification and individual recycling with consideration of long-term nature of behavioral and social change.
5. Design a religious–educational communication strategy to connect religion to the environment and community.
6. Reduce food waste in Ramadan's feeding projects such as eco-friendly Ramadan tents.
7. Create incentives and/or penalties to reduce food waste.
8. Include educational and training programs on food waste reduction in the school curricula.

The panel discussion took place when the time of Ramadan was approaching and a significant number of tents spread around the country to provide food to underprivileged individuals for religious reasons. Based on Policy (6), the participants decided to implement a pilot project entitled "Ramadan Eco-friendly Tents" that aimed to reduce the huge amount of food wastage collected from both tents and households in Ramadan. The charities supervised the process of food waste collection. Then, waste treatment center and recycling that belongs to the Ministry of Municipality and Environment recycled the food waste to compost, in order to use it for agriculture production. Though this kind of projects is small in nature, they could make significant contribution to achieving sustainable development and increasing community awareness about the environment and environmental risk management.

Conclusion

The United Nations Sustainable Development Goals (SDGs) 2030 draws attention to the most pressing issues of the past decade mainly: population growth, climate change, soil degradation, water scarcity, and food security. Moreover, feeding the growing population requires more food production while minimizing food waste. The issue of food waste is largely rampant in Qatar because of many factors such as the dramatic increase in population, the rapid agricultural expansion and Qatar's decision to achieve self-sufficiency provoked by the blockade from its main food importers; this is in addition to the fact that Qatar has limited capacity of the land to absorb waste and to replenish natural resources at the same time. It is very important therefore for Qatar to balance the fast growth and environmental protection by insuring sustainable production and consumption pattern to achieve sustainable development. The political and economic impacts of the blockade by its neighbors, has initially had a soon theoretically shocking impact. With the wise leadership of the State and the will of its people, the State of Qatar was able to reverse this situation in the first year, by resourcing and enhancing the country's agricultural potentials. In order to avoid the trap of short-run strategies, the state has drawn attention to the importance of adopting sustainable technologies, increasing recycling, and converting food waste into compost for distribution to farms, gardens, and households to support food production. Charitable and support organizations have been encouraged to work on the sustainable development projects that have national priority. In this study, the stakeholders who are concerned with food waste came together to come up with proposed effective policies and recommendations to reduce the amount of food wasted in Qatar, that is, household or/and institutional waste that is transferred to the landfill aiming to alleviate the threats to the arable land and the risk of groundwater pollution. Generally, literature shows that religious belief, cultural attitudes, socio-economic status, and working conditions are the main drivers of food waste in Qatar. Therefore, better understanding of attitudes and behaviors towards food waste is very important to formulate sustainable management policies. Finally, the collaboration between sectors are very important; the academic institutions can take care of supervision and consultations and the administrators from different sectors may adapt action plans according to the need and priorities of the countries national strategies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abdelaal AH (2017). Food Waste Generation and its Potential Management at Education City, Qatar. Hamad Bin Khalifa University (Qatar), ProQuest Dissertations Publishing, 2017. 10283865.

Abiad MG, Meho LI (2018). Food loss and food waste research in the Arab world: A systematic review. Food Security 10(2):311-322.

Adema S (2016). Food Waste Woes in Qatar. [online] EcoMENA. Available at: https://www.ecomena.org/food-waste-in-qatar/ [Accessed 11 Dec. 2019].

Aktas E, Sahin H, Oledinma A, Huda AKS, Irani Z, Sharif AM, Wout T (2017). A consumer behavioral approach to food waste. Journal of

Enterprise Information Management 31(5):658-673.
Al-Thani MA, Al-Mahdi N, Al-Kareem H, Barakat D, Al-Chetachi W, Tawfik A (2017). An Overview of food patterns and diet quality in Qatar: findings from the national household income expenditure survey. Cureus *9*(5):e1249.
Baiga BM, Al-Zahrania K, Schneider H, Straquadine GS, Mourad M (2018). Food waste posing a serious threat to sustainability in the Kingdom of Saudi Arabia – A systematic review. Saudi Journal of Biological Sciences (26):1743-1752.
Bos-Brouwers HEJ, Soethoudt JM, Canali M, Östergren K, Amani P, Aramyan L, Sijtsema SJ, Korhonen O, O'Connor, C. (2014). Drivers of current food waste generation, threats of future increase and opportunities for reduction. Bologna: FUSIONS N FP7-KBBE-2012-6-311972.https://research.wur.nl/en/publications/drivers-of-current-food-waste-generation-threats-of-future-increa
Buzby J, Hyman J (2012). Total and per capita value of food loss in the United States. Food Policy 37(5):561-570.
Elagroudy S, Warith MA, Elzyat M (2016). Municipal Solid Waste Management and Green Economy. Global Young Academy. Jager – str 22/23.10117. Berlin: Germany.
Edelstein S (2011). Food, cuisine, and cultural competency for culinary, hospitality, and nutrition professionals. Sudbury, Mass: Jones and Bartlett Publishers.
Flanagan K, Lipinski B, Goodwin L (2018). Champions 12.3: Reviewing and providing helpful input on draft versions of this publication. Available at: https://champions123.org/target-12-3. (Accessed February 2019) Non-profit group of experts dedicated to the achievements of the goal 12.3 of SDG by 2030.
Food and Agricultural Organization (FAO), IFAD and WFP (2015a). The State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. Rome, FAO. (www.fao.org/publications).
Food and Agricultural Organization (FAO) (2015b). Global initiative on Food waste and Food loss Reduction. United Nation Publication.
Food and Agricultural Organization (FAO) (2018). Food loss, waste and The Right to Adequate Food: Making the Connection. Rome.48 pp license: CC BY.NC.SA3.0 IGO.
General Secretariat for Development Planning (2008). Qatar National Vision 2030 July 2008, Doha-Qatar at www.planning.gov.
Ministry of Environment and Municipality (2018). The sixth national report of biodiversity in Qatar. Department of Protection and Biodiversity Life. Doha, Qatar. Available at https://www.cbd.int/doc/nr/nr-06/qa-nr-06-ar.pdf
Qatar News Agency. (2018). Qatar's achievements in Food Security. Ministry of Municipality and Environment. Accessed online on March 6. Available at http://www.mme.gov.qa/cui/view.dox?id=1440&contentID=5612&siteID=1
Mu'azu ND, Blaisi NI, Naji AA, Abdel-Magid IM, AlQahtany A (2019). Food waste management current practices and sustainable future approaches: A Saudi Arabian perspectives. Journal of Material Cycles and Waste Management 21(3):678-690.
Planning and Statistics Authority (2018). Second National Development Strategy 2018–2022, General Secretariat for Development Planning statistical reports publications. Qatar-Doha. https://www.psa.gov.qa/en/pages/default.aspx
Planning and Statistics Authority (2017). Qatar's voluntary national review 2017: Sustainable development goals 2030, high-level political forum, Doha, Qatar. Presented by S. Al-Nabit, 19 July 2017.
Richer RA (2014). Sustainable development in Qatar: Challenges and opportunities, Q Science Connect 2014:22 http://dx.doi.org/10.5339/connect.2014.
Reynolds CJ, Piantadosi J, Boland J (2015). Recycling Food from the Organics Waste Steam to Feed the Food Insecure: An Economic and Environmental of Australia food Rescue Option Using Environmentally Extent Waste Input-output analysis. Sustainability 7(4):4707-4726.
Schanes K, Doberics K, Gozet B (2018). Food Waste Matters: A Systematic Review of Household Practices and other Policy Implications. Cleaner Production 182 p.
Sheikh Eid Charity Foundation (2008). Save the Grace Project. Available from: http://www.eidcharity.net/ar/site/web/index.php?page=article&id=514#.XI11ofl5yHs
Sillitoe P, Misnad S (2014). Sustainable Development: An appraisal focusing on the Gulf Region. New York: Berghan Books.
Thyberg KL, Tonjes DJ (2016). Drivers of Food Wastage and their Implications for Sustainable Policy Development. Resources Conservation and Recycling 106(2016):110-123.
Qatar National Food Security Program (QNFSP) (2012). Sustainable water planning for food security Dr. Patrick Linke .Gulf Water Conference, Doha, 22 April 2012 https://www.wstagcc.org/WSTA_10th_Conference/S1_L5_Sustainable_water.
United Nation (2015). Transforming our world: the 2030 agenda for sustainable development A/RES/70/1 plan of action. Retrieved from https://sustainabledevelopment.un.org/content/documents
Vittuari M, Gaiani S, Politano A, Timmermans AJM, Bos-Brouwers HEJ (2016). Policy options to stimulate social innovation initiatives addressing food waste prevention and reduction: WP/Deliverable: WP3, Deliverable D3.3. Bologna: Food & Biobased Research Wageningen UR. https://research.wur.nl/en/publications/policy-options-to-stimulate-social-innovation-initiatives-address

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Technical efficiency and its determinants in sugarcane production among smallholder sugarcane farmers in Malava sub-county, Kenya

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The aim of the study was to determine the farm level technical efficiency and its determinants among smallholder sugarcane farmers in Malava Sub-county, Western Kenya. Primary data were collected using questionnaires from a sample of 384 farmers through systematic random sampling. The study applied stochastic frontier analysis and Tobit regression analysis using computer software STATA. The results found that technical efficiency of sugarcane farmers ranges from almost zero to 0.9829, with mean value of 0.7069, implying that an average farmer could increase sugarcane productivity by 29.31% at the existing level of resources. Maximum likelihood estimate of technical efficiency depicted that the use of fertilizer, labour, seed-cane and farm size are positive and significant at 1% level in determining technical efficiency. Tobit regression analysis showed that education, farming experience, family size, credit access and extension services were positive and significant in contributing to technical efficiency. However, age of the farmer, farm distance from home and contract engagement was negatively influencing technical efficiency. The study recommends the Kenyan government to formulate policies that ensure provision of quality extension services, increased credit access and education among smallholder sugarcane farmers. The results also recommended the need for a review of the existing contract engagement policies among sugarcane farmers.

Key words: Technical efficiency, stochastic frontier analysis, tobit, sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is one of the major crops grown in the world due to its multiple uses in daily life of any nation including nutritional and economic sustenance. Sugarcane contributes to about 80% of the total sugar produced in the world (Rumánková and Smutka, 2013). Brazil is the largest producer of sugarcane in the world with an annual production of about 768,678,382 metric tonnes which is followed by India that produces 348, 448,000 metric tonnes per year (FAOSTAT, 2016). Other countries which have shown high production of sugarcane are China and Thailand whose annual production is 123,059,739 and

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100,100,000 metric tonnes respectively (FAOSTAT, 2016).

African countries contribute about 5% of the total world sugar of which 80% is contributed by Sub-Saharan African countries (Travella and Oliveira, 2017). The major Sub-Saharan African countries where sugarcane crop is grown are South Africa, Sudan, Swaziland, Zambia, Mauritius and Kenya. These countries accounts for more than half of African total sugarcane production (Travella and Oliveira, 2017).

In Kenya, sugarcane is extensively planted in Western part of the Country. Production of sugarcane in Kenya is one of the major agricultural activities contributing to the national economic growth alongside tea, coffee, horticultural crops and maize (Waswa et al., 2012). The contribution of the Kenyan sugarcane sector towards the total agricultural gross domestic product (GDP) is about 15% with 25% of the Kenyan people relying directly and indirectly on sugarcane production for their living (Wekesa et al., 2015). Malava Sub-County which is one of the areas where sugarcane is the main cash crop has the highest number of people who depend on sugarcane activity for their living (Kenya Sugar Board, 2014). This Sub-County has two milling factories which are West Kenya Sugar Factory and Butali Sugar Mills.

However, despite the importance of sugarcane sector to the Kenyan economy, production of sugarcane has been deteriorating over the years (Mulianga et al., 2015). On average, the current production of sugarcane is about 60.52 tonnes per hectare (Kenya Sugar Board, 2014) which is low as compared to 90.86 tonnes per hectare in the year 1996 (Wolfgang and Owegi, 2012). Currently, the domestic demand is higher than production capacity in the Country whereby the production is about 550,000 tonnes of sugar per year against the domestic consumption of about 800,000 tonnes of sugar per year (Wawire and Ouma, 2013). As such, the Kenyan government has been heavily investing in this sector in order to obtain the optimum production and become self-sufficient in sugar production. However, this objective has never been met since the potential output is still not achieved in most of the sugarcane growing areas. Kenya being a developing Country is however constrained by production resources. For this reason, the achievement of technical efficiency at farm level would be the best complement to all efforts in attaining the optimum and self-sufficiency in sugarcane production. Efficiency in agricultural production refers to the choice of using the limited agricultural resources in an optimal way. The scope of production in crop farming can be sustained through efficient use of scarce resources in the economy. It has been widely argued that efficiency is the center of farm production (Awunyo-Vitor et al., 2016; Severini and Sorrentino, 2017). The objective of this study was therefore to determine technical efficiency and the effect of selected socioeconomic factors on efficiency among smallholder sugarcane farmers in Malava Sub-county.

MATERIALS AND METHODS

Description of study area

The study was conducted in Malava Sub-County which is one of the twelve Sub-counties of Kakamega County in Kenya. Malava Sub-County is mainly located in Lower Midland (LM) Zone 2-3 and Upper Midland (UM) Zone 4 Agro-ecological zones (Jaetzold et al., 2005) where the main economic activity is the growing of sugarcane as a cash crop. The area experiences two distinct rainy seasons. Long rain is experienced from March to July while short rains occur from September to December, with a short dry season that occur from January to February. This Sub-County has seven administrative units (Wards) which are; East Kabras, West Kabras, Chemuche, Manda-Shivanga, South Kabras, Butali-Chegulo and Shirugu-Mugai (IEBC, 2017).

Sample procedure and sample size

The sample size for this study was 384 respondents who were determined through Fischers formula given by Kothari (2004) as indicated in Equation 1.

$$\mathrm{n} = \frac{z^2(p)(q)}{\varepsilon^2} \quad (1)$$

Where, n is the sample size, z is equal to 1.96 which is the tabulated Z value for 95% confidence level, p is the sample proportion where 0.5 is the highest that can produce at least the desired precision while ε is the margin of error which is 0.05 since the estimate of the study will be within 5% of the true value.

Using Equation 1 above and assuming 50% probability that the respondent has the characteristic being measured, the sample size was determined as shown below;

$$\mathrm{n} = \frac{1.96^2(0.5)(1-0.5)}{0.05^2} = 384 \quad (2)$$

All the seven administrative units (Wards) in Malava Sub-county were purposively selected due to their agrarian potential for sugarcane production. The sample size of respondents from each administrative unit was selected through a proportional sampling allocation technique (Cochran, 1977) as shown below:

$$n_i = \frac{N_i \times \mathrm{n}}{\mathrm{N}} \quad (3)$$

Where, n_i is the number of sugarcane farmers interviewed in the selected wards, N_i is the total number of the sugarcane farmers in the selected Ward, n is the sample size for the study while N is the total number of sugarcane farmers in the area of study.

A systematic random sampling technique was applied to select farmers to be interviewed in each Ward.

Method of data collection

This study used structured questionnaire to collect primary data from respondents on sugarcane production. Trained enumerators were employed to facilitate the process of data collection under the supervision of the researcher. Detailed information from the selected farm households were collected on demographic and socio-economic factors, farm characteristics, input use, production, institutional and policy related variables. The survey was carried out from July to August, 2019.

Data analysis

The study applied both descriptive and econometric statistics to achieve its objective. Descriptive analysis such as mean, standard deviation, minimum, maximum, percentage and frequency counts were used to summarize socio-economic and demographic characteristics of the respondents, input and output variables, and frequency distribution of technical efficiency levels. Econometric techniques such as stochastic frontier analysis technique and tobit regression were applied to analyze technical efficiency (TE) among the selected households and the effect of the selected socioeconomic factors on TE.

Analytical framework

Several approaches have been developed to estimate efficiency of farms including econometric and mathematical programming approaches. However, there are two frontier model that are commonly applied; the Stochastic Frontier Model (SFM) and Data Envelopment Analysis (DEA). The choice of a specific model depends on the objective of the study, kind of data and assumptions (Erkoc, 2014). SFM has been commonly used in determination of agricultural efficiency since DEA has been widely criticized due to its assumption that all deviation from the frontier are associated with inefficiency. These assumptions are hard to be accepted due to inherent variability of agricultural production as a result of weather variation, pest and disease outbreak (Coelli et al., 2005). SFM which was first introduced by Aigner et al. (1977) is prefered due to its ability to measure efficiency in the presence of statistical noise. This model has got two error terms where one accounts for the existing measurement error in production and the other one is as a result of the estimation of frontier production function. According to Aigner et al. (1977), the parametric frontier is presented as:

$$Y_i = f(X_i, \beta) + V_i - U_i \tag{4}$$

Where, V_i is the error component which accounts for the measurement error in the output variable due to the weather, combined effect of the unobserved input on production, errors in the observation and measuring of data and U_i is the error component that accounts for the existence of inefficiency in production. Y_i is the quantity of output, X_i refers to quantity of inputs, βs are the unknown parameters to be estimated, which represents elasticities of inputs while f represent the production frontier function.

The estimated technical efficiency of i^{th} farmer is determined as the ratio of the observed output for the i^{th} farm relative to the potential output. This can be illustrated as shown in Equation 5.

$$TE_i = \frac{Y}{Y^*} = \frac{exp(X_i\beta + V_i - U_i)}{exp(X_i\beta + V_i)} = \exp(-U_i) \tag{5}$$

Where, Y is the observed output and Y^* is the potential or frontier output.

Literature has revealed that stochastic frontier model has been broadly used to determine efficiency in agriculture. For instance, Kassa et al. (2019), Dube et al. (2018), Mamo et al. (2018) and Getahun and Geta (2017) used SFM to determine the technical efficiency levels in production of teff, potato, wheat and barley respectively in Ethiopia. The technique was also applied by Yegon et al. (2015) to evaluate the technical efficiency of smallholder soybean production in Kenya.

Model specification for technical efficiency

The current study applied stochastic frontier model to determine technical efficiency within the framework of Cobb-Douglas production function. Following the specification of the stochastic Cobb-Douglas production model, the data was fitted as below:

$$\ln Y_i = \beta_0 + \beta_1 lnX_1 + \beta_2 lnX_2 + \beta_3 lnX_3 + \beta_4 lnX_4 + V_i - U_i \tag{6}$$

Where, ln = logarithm to base e, β_0= constant which represents the intercept of production function, β_1 to β_4 = unknown parameter that were established which are also the output elasticities of amount of fertilizer, labour, seed-cane and farm size respectively. Y_i = quantity of sugarcane in tonnes, V_i = two sided random error representing stochastic effect beyond farmer's control, measurement errors and other statistical noise and U_i = a non-negative random variable representing technical inefficiency of sugarcane farmer. X_1, X_2, X_3 and X_4 are the amounts of fertilizer, labour, seed-cane and farm size respectively.

Following Coelli et al. (2005) and Bi (2004), the model given in the Equation 6 was estimated using the maximum likelihood estimates (MLE). MLE provides the rationale estimates for unknown parameter (β), gamma (Υ) and sigma squared (σ^2).

Model specification for the effect of socioeconomic factors on technical efficiency

The relationship between socioeconomic factors and technical efficiency was analyzed using tobit regression model since technical efficiency has a lower limit of zero and an upper limit of one. Tobit model was applied as indicated in Equation 7.

$$TE_i = \delta_0 + \delta_1 Z_1 + \cdots + \delta_{11} Z_{11} + \omega \tag{7}$$

Where, TE_i= technical efficiency, δ_0 is the intercept of the function while δ_1, δ_2 … δ_{11} are unknown scalar parameters to be estimated. $Z_1, Z_2, Z_3, Z_4, Z_5 Z_6, Z_7 Z_8, Z_9, Z_{10}$ and Z_{11} are age, gender, education, family size, farming experience, credit access, farm distance from home, extension services, contract engagement, soil testing before planting and farm record keeping respectively. ω is the error term which is assumed to be normally distributed.

Validity of model assumptions

The hypothesis of homoscedasticity and no multicollinearity in the data set were tested for the validity of model assumptions. Breusch-Pagan and Variance Infation Factors (VIF) were applied respectively to test for the presence of heteroscedasticity and multicollinearity in the data set.

Test of heteroscedasticity

Heteroscedasticity refers to a situation where the assumption that the classical linear regression model has equal variance of residuals is violated. There exists several tests for heteroscedasticity detection such as the Koeker Basset, the White's and the Breusch-Pagan tests among others (Gujarati and Porter, 2009). This study used the Breusch-Pagan with null hypothesis of constant variance for heteroscedasticity. Breusch-Pagan is a chi-squared test whereby if the statistical test gives a p-value that is below suitable threshold of 0.05 then the null hypothesis of homoscedasticity is rejected (Gujarati and Porter, 2009).The calculated chi square value was 0.39, with a p-value of 0.5308 which is greater than 0.05 indicating homoscedasticity in the data set.

Test for multicollinearity

The problem of multicollinearity occurs when one or more of the explanatory variables indicate a linear combination of other variables. This problem can result to wrong signs in the estimated regression coefficients and smaller t-ratios thereby having wrong conclusions. A strong correlation coefficient may be an indicator of this problem and can be examined further by computing VIF for each of the independent variables (Rabe-Hesketh and Everitt, 2000). Following Chatterjee and Price (1991), when values of VIF are greater than 10 or when a mean of the factors (1/VIF) is considerably greater than 1, then there is a problem of multicollinearity which calls for concern. Accordingly, values of VIF were calculated for explanatory variables and were ranging from 1.09 to 3.60 with a mean of 1.85. Furthermore, the mean values of the factors (1/VIF) ranged from 0.278 to 0.919. Multicollinearity was therefore not a problem among the explanatory variables.

RESULTS AND DISCUSSION

Demographic and socio-economic characteristics of the sampled households

Table 1 shows descriptive results of demographic and socio-economic characteristics of selected smallholder sugarcane farmers. The average size of the family in the area of study was 6 people with a minimum of 1 and a maximum of 13 persons implying the availability of labour among smallholder sugarcane farmers. The result showed that on average, respondents have 16 years of experience in sugarcane farming implying that most farmers could provide reliable information and have deep understanding of sugarcane farming. Years of experience amongst respondents ranged from 1 year to 36 years.

Both the youth and elderly were involved in sugarcane farming whereby, majority of respondents (72.66%) were between 21 and 50 years of age which is the most productive age group with active farmers. On the other hand, 27.34% of the respondents were above 50 years of age implying that some areas had less active farmers involved in sugarcane production.

The study indicated that 71.61% of the respondents were male while 28.39% were female indicating that the sugarcane crop is important for both gender. However, most of the respondents were male indicating that decisions in sugarcane production at farm level are mostly made by male gender who are the head of the household. This therefore confirms the worldwide situation whereby women are significantly involved in sugarcane farming activities mainly as casuals but not land owners given their limited access to agricultural resources (Fonjong and Mbah, 2007).

The study indicated that majority of the farmers had formal education where 36.20% of the respondents had secondary education and 15.89% had tertiary education. This high percentage of farmers with formal education imply that majority of farmers were capable of increasing sugarcane productivity through quick understanding of trainings given on the crop management such as best practices and the adoption of new sugarcane production techniques.

Results demonstrated that only 42.19% of the respondents required credit in their production. The majority representing 57.81% of the respondents did not require credit in their production. This imply that majority of farmers were capable of purchasing inputs for sugarcane production and that lack of finance was probably not a limiting factor to most of the smallholder farmers. However, for those who required credit for production, only 64.81% got the credit that they requested for while 35.19% did not get the credit. This imply that some farmers who were in need of credit could not access credit services to enable them purchase production inputs and increase farm productivity.

Majority of respondents (73.96%) have their sugarcane farms less than 1 kilometer from home, making it easier for management and supervision of the farm. Additionally, short distance of sugarcane farms from home implies that help from the family in terms of labour and crop security can easily be provided. However, some farmers (26.04%) had their farms located over 2 km from home making it difficult for proper farm management. The study showed that only 42.97% of the farmers have access to extension services with majority having no access implying that new technologies in sugarcane farming are not disseminated to most farmers. It was however noted that most farmers who have no access to extension services are non-contracted and comprise the majority (65.89%) in the study area.

Only 16.67% of the respondents carry out soil testing before planting of sugarcane. This implies most farmers are not able to know the types and amount of nutrients that are lacking in their soils for enhanced productivity. Knowledge on the soil nutrient status would guide the farmers on the type of fertilizer to apply. Most of the farmers representing 59.38% do not keep records on revenues generated and expenses incurred in the farm activities. This implies that most of the farmers could not determine whether their enterprises were profitable or not.

Descriptive statistics for production variables

The summary statistics for the variables used in estimation of production function and technical efficiency are presented in Table 2. The production function and technical efficiency for this study were estimated using four types of inputs which are the amount of fertilizer, labour, farm size and seed-cane.

The findings in Table 2 shows that on average small scale sugarcane farmers produce 18.69 tonnes of sugarcane per acre which is below the national average yield of about 24 tonnes per acre (Kenya Sugar Board, 2014). This indicates that farmers in the area of study are

Table 1. Demographic and socioeconomic characteristics of the respondents.

Variable	Mean		Std. Dev	Min.	Max.
Family size	6		3.25	1	13
Farming experience	16		8.69	1	36
	Categories		**Frequency**	**Percentage**	
Ages of respondents	21 – 30 years		55	14.32	
	31 – 40 years		89	23.18	
	41 – 50 years		135	35.16	
	Above 50 years		105	27.34	
Gender of respondents	Male		275	71.61	
	Female		109	28.39	
Level of Education of respondents	No formal education		48	12.50	
	Primary		136	35.42	
	Secondary		139	36.20	
	Tertiary		61	15.89	
Credit access	Required credit	Yes	162	42.19	
		No	222	57.81	
	Got credit	Yes	105	64.81	
		No	57	35.19	
Farm distance from home	Less than 1 Km		284	73.96	
	2 – 4 Km		71	18.49	
	Over 4 Km		29	7.55	
Get extension services	Yes		165	42.97	
	No		219	57.03	
Contract engagement	Yes		131	34.11	
	No		253	65.89	
Soil test before planting	Yes		64	16.67	
	No		320	83.33	
Farm record keeping	Yes		156	40.62	
	No		228	59.38	

Table 2. Descriptive statistics for the model variables.

Variable	Obs	Mean	Std. Dev.	Min	Max
Amount of fertilizer (Kgs per acre)	384	308.29	138.85	50	650
Labour (man days per acre)	384	20.58	5.5767	7	41
Sugarcane cuttings (tonnes per acre)	384	2.27	1.20	0.5	9
Farm size (acres)	384	2.80	2.58	0.25	33
Sugarcane yield (tonnes per acre)	384	18.69	10.00	1.5	63

producing below their production potential. The minimum yield of sugarcane obtained is 1.5 tonnes per acre and the maximum is 63 tonnes per acre implying that farmers have a potential of producing up to 63 tonnes per acre. The average values for fertilizer, labour and seed-cane are 308.29 kg, 20.58 man days and 2.27 tonnes per acre

Table 3. Stochastic frontier production function results.

Variable	β-coef.	Std. Err.	Z-Value	P>|z|
Lnfertilizer	0.267***	0.0308	8.67	0.000
Lnlabour	0.626***	0.0774	8.08	0.000
Lnseed cane	0.155***	0.0279	5.57	0.000
lnfarm size	0.146***	0.0232	6.26	0.000
Constant	-0.407**	0.192	-2.12	0.034
Usigma	-1.028***	0.0781	-13.16	0.000
Vsigma	-6.154***	0.419	-14.70	0.000
Diagnostic test				
Sigma u	0.598	0.0233585	25.60	0.000
Sigma v	0.0461	0.0096507	4.78	0.000
Lambda (λ)	12.973	0.0269179	481.95	0.000
Sigma2 (σ^2)	0.360			
Gamma (Υ)	0.994			
Log likelihood	-101.136			
Prob > chi2 = 0.0000				

***significant at 1% and **significant at 5%.

respectively. The average farm size allocated to sugarcane production for households was 2.80 acres. This implies that sugarcane in the area of study is on average grown in small scale farms.

Estimation of parameters of the frontier production function

Table 3 shows the findings of the stochastic frontier analysis. The parameters of fertilizer, labour, seed-cane and farm size were found to be significant at 1% level with the estimated β-coefficients of 0.267, 0.626, 0.155 and 0.146 respectively. The results imply that 1% increase in the amount of fertilizer used increases sugarcane output by 0.267% and 1% increase in labour use increases sugarcane output by 0.626%. Moreover, an increase of improved seed-cane by 1% would increase output by 0.155%. On the other hand, 1% increase in farm size increases sugarcane yield by 0.146%. The results are in line with the economic theory of production and concur with the findings by Wawire and Ouma (2013) who found out those sugarcane farmers were not maximizing their production yields.

The findings on the effect of farm size on sugarcane production in the current study were in line with those of Khan et al. (2010) and Baruwa and Oke (2012) in Bangladesh and Nigeria respectively. However, these results were in contradiction with the results by Tchale (2009) which showed a negative influence of farm size on technical efficiency in Malawi. The latter study however associated the negative effect with operating beyond the optimal scale of the land where production was carried out on larger farms than what a farmer could manage. Thus, in Kenya the size of sugarcane farms can still be managed and increase in sugarcane farm area would increase production. However, farm expansion should be carried out with care as Anyaegbunam et al. (2012) found out in their study that farm size may inversely increase with technical efficiency. Since all the four inputs were positive and significant, it is indicated that these factors significantly determine sugarcane output in the study area.

The findings in Table 3 indicate that the value of lambda (λ) is 12.973 indicating that in total deviation 12.973% difference between observed and potential yield is due to the inefficiency among the sampled respondents. The parameter gamma (γ) value is 0.994 which is very close to one. This parameter is usually associated with the two error terms of the stochastic frontier function (Battese and Coelli, 1995). This parameter measures the deviation of the output from the frontier caused by the effect of inefficiency and it equals to $\sigma^2\mu/(\sigma^2 v+\sigma^2\mu)$ whereby $\sigma^2\mu$ and $\sigma^2 v$ represent the variances related to technical inefficiency and statistical noise respectively. The values therefore indicated that 99.4% variations in the composite error terms was caused by inefficiency effects. Additionally, the estimated value of sigma squared (σ^2) is 0.3597, which is significantly greater than zero, indicating the appropriateness of the model. The log likelihood statistic also shows the appropriateness of the model given it is significant at 1% level and the large

Table 4. Frequency distribution of technical efficiency estimates.

Technical efficiency range	Frequency	Percentage
0.0 – 0.20	12	3.13
0.21 – 0.40	30	7.81
0.41 – 0.60	54	14.06
0.61 – 0.80	142	36.98
0.81 -0.99	146	38.02
Mean (0.7069)		
Minimum (0.000465)		
Maximum (0.9829)		

Table 5. Tobit regression analysis results.

Variable	Coef.	Std. err.	t-value	P value
Age	-0.0726***	0.0155	-4.70	0.000
Gender	0.0109	0.0190	0.58	0.564
Education	0.0213**	0.0108	1.98	0.049
Family size	0.0240***	0.00403	5.95	0.000
Farming experience	0.00429**	0.00177	2.41	0.016
Credit access	0.0596***	0.0203	2.94	0.003
Farm distance from home	-0.0982***	0.0140	-7.02	0.000
Extension services	0.105***	0.0192	5.46	0.000
Contract engagement	-0.0938***	0.0213	-4.41	0.000
Soil testing before planting	0.0476**	0.0241	1.97	0.049
Farm record keeping	0.0153	0.0199	0.77	0.442
Constant	0.797***	0.0572	13.95	0.000
Sigma	0.161***	0.00582		
Log likelihood		155.53		
Prob > chi2 = 0.0000				

***significant at 1% and **significant at 5%.

absolute value of Log Likelihood ratio of -101.136.

Technical efficiency among sugarcane farmers

The results of the frequency estimates of the technical efficiency are shown in Table 4. The findings indicated that majority of respondents recorded below 0.81 level of technical efficiency. This shows that most of the smallholder sugarcane farmers are technically inefficient. The results also showed that farmers are operating at an average technical efficiency of 0.7069 ranging from a minimum of 0.000465 to a maximum of 0.9829. This wide variation in technical efficiency estimates indicates that majority of the farmers are inefficiently utilizing their resources in the production process and there are opportunities for increasing their current yield by improving technical efficiency. An average farmer is operating at 70.69% below the production frontier due to inefficiency effects. This complemented the results from the hypothesis testing showing that on average, the frontier production is not yet attained due to significant inefficiency effects. This could be attributed to misuse and/or wastage of inputs. Similar results were reported by Kassa et al. (2019) and Nyagaka et al. (2010).

Factors affecting technical efficiency among sugarcane farmers

Table 5 shows the tobit regression results for the relationship between the selected socioeconomic factors and technical efficiency. The log likelihood statistic which determines the appropriateness of the model indicates that the model is applicable given its significant chi-square (p<0.000) and the large absolute value of Log Likelihood ratio of 155.53.

The findings presented in Table 5 shows that, the level of education, farming experience and soil testing before planting are positive and significant at 5% level. Family size, access to extension services and access to credit are positive and significant at 1% level. However, age of the farmer and contract engagement were found to be negative and significant at 1% level. Gender and farm record keeping were positive but insignificant at all levels.

Age variable depicted a negative effect on technical efficiency where an increase of age by 1% would reduce technical efficiency by 0.0726%. This showed that the older a farmer become, the higher the technical inefficiency in sugarcane production. Age of the farmer can take a positive sign when older farmers are willing to adopt improved methods thus increasing technical efficiency effects or when knowledge, skills and the experience gained during their years of farming contribute in reducing inefficiency. This variable can take a negative sign like in the current study, indirectly showing that older farmers are resistant to adopt improved technologies and that they lack mental and physical capacity to efficiently participate in sugarcane production. Similar results were found by Khan and Saeed (2011) who argued that older farmers are less technically efficient than younger farmers, showing that the more the younger farmers get educated the more efficient they become. On the contrary, Getahun and Geta (2017) and Binam et al. (2004) assumed that when farmers get old, they become more experienced and efficient. Then again, higher technical efficiency is attained by the age group which have more interest in the type of crop being cultivated (Thabethe and Mungatana, 2014).

The level of education is positive and significant indicating that 1% increase in the level of education would increase technical efficiency by 0.0213%. This relationship is significant at 1% level. This means that when farmers are educated on the suitable techniques of farming as well as resource use, they become more efficient. This finding concur with those of Weir and Knight (2007) who found out that there was a positive relationship between the level of education and efficiency among small scale farmers. A study by Sulaiman et al. (2015) on resource use efficiency among sugarcane farmers in Nigeria indicated that farmers who are more educated quickly acquire new technologies and produce more output which is closer to the production frontier.

Family size indicated a positive relationship with the technical efficiency as expected. From Table 5, it is shown that 1% increase in family size increases the technical efficiency by 0.024%. Large family size is associated among other factors with availability of cheap labour. Sugarcane production is a labour intensive activity and hence a large family size is assumed to provide cheap labour. These results concur with those of Mailena et al. (2014), Sulaiman et al. (2015) and Ahmad et al. (2018). However, the results by Kadiri et al. (2014) showed a negative relationship between family size and technical efficiency of paddy rice production in Nigeria. On the other hand, Ali and Jan (2017) and Getahun and Geta (2017) showed that there was insignificant effect of this variable on technical efficiency. This variable therefore needs more research on its effect on technical efficiency in order to make a reliable conclusion.

The findings on farming experience revealed a positive relationhip with technical efficiency. An increase in the level of experience by 1% increases sugarcane yield by 0.00429%. High farming experience is associated with increased proficiency in the processes of farm production and hence inreased productivity. Similar results were found by Nyagaka et al. (2010) in their analysis of economic efficiency in Irish potato production in Kenya. Mulwa et al. (2014) and Mburu et al. (2014) showed the same relationship between farming experience and efficiency among smallholder maize farmers in Western Kenya and Nakuru District in Kenya respectively.

Credit access revealed a positive and significant relationship with technical efficiency among sugarcane farmers. Access to credit is an important source of capital which enables smallholder sugarcane producers to purchase production inputs on time thereby increasing farm productivity. It enables the farmer to adopt new technologies and practices through easing farmers liquidity constraints (Ike and Inoni, 2006). This variable was hypothesized to have a positive effect on technical efficiency which was confirmed by findings. The findings were similar to those by Kibaara (2005) and Sulaiman (2015) who found a positive relationship between access to credit and technical efficiency.

Extension services showed a positive and significant relationship with technical efficiency where a farmer could increase technical efficiency 10.5% by adopting these services. This implied that access to extension services by sugarcane farmers contribute to technical efficiency in production of sugarcane. The positive effect of extension services on technical efficiency could be linked to the information and knowledge received by sugarcane farmers which complement the trainings. These findings were consistent with those of Nchare (2007) and Simonyan et al. (2011). In contrast, Ezeh et al. (2012) found out that extension services had a negative effect on technical efficiency which was not expected and they recommended further research to be conducted on the same.

Farm distance from home showed a negative relationship with technical efficiency implying that nearer farms can be efficiently managed as compared to farther farms. The more the distance of sugarcane farm from home, the more the difficulty in farm management andhence low productivity. The findings were in line with those of Mamo et al. (2018). Contract engagement also showed a negative relationship with technical efficiency. These findings on the contract engagement concur with those of Waswa et al. (2012), Sopheak (2015) and Musungu and Sorre, (2017). The negative effect on technical efficiency may be attributed among other factors to increased input prices and harvesting of canes before maturity. On the contrary, the results by Hu (2013) and Igweoscar (2014) showed a positve and significant effect of contract engagement on technical efficiency. This variable therefore needs more investigation since farmers enter into contract engagement with the aim of increasing their productity which the current study has revealed otherwise.

Soil test before planting is an important practice which helps farmers to identify the type of nutrients needed in the soils as well as the type of crops appropriate in the area. The study showed a positive relationship of this variable with technical efficiency as expected. It showed that adoption of this practice increases technical efficiency by 4.76%. The results are consistent with the recommendations by Jamoza et al. (2013) and Amolo et al. (2017).

Conclusion

The results of this study showed that smallholder sugarcane farmers are inefficient with a mean technical efficiency of 0.7069. There is high variation of technical efficiency between smallholder sugarcane farmers in the Country. The maximum likelihood estimates indicated that fertilizer, labour, seed-cane and farm size make significant contribution in improving the productivity of sugarcane among smallholder farmers. The study tested a null hypothesis that socioeconomic factors have no effect on technical efficiency among smallholder sugarcane farmers. The findings revealed that age, education, farming experience, family size, access to extension services, access to credit, contract engagement and soil testing before planting were significantly affecting technical efficiency. Therefore, the null hypothesis is rejected in favor of the alternative that socioeconomic factors have effect on technical efficiency among smallholder sugarcane farmers.

Reccommendations

The findings of the study revealed that there exist an opportunity to increase sugarcane production at the existing level of inputs use and level of technology. The study therefore came up with the following recommendations to guide farmers, policy makers as well as researchers for further investigations.

1. The Kenyan government should ensure the provision of quality extension services to smallholder sugarcane farmers for increased productivity since this variable was found to have great positive impact on productivity of sugarcane among smallholder farmers.
2. Contract engagement is meant to improve productivity of farmers. However, this study has revealed that contract engagement is negatively affecting technical efficiency. As such, the Kenyan government should review policies on contract engagement with contract service providers to change this situation.
3. Some of the farmers in the area of study achieved high yield and obtained high technical efficiency and hence such farmers can be used effectively to illustrate the usefulness of good farming practices in order to reduce the gap that exists between the most technically efficient and the most inefficient farmers.
4. Sugarcane farmers should establish a formal and active association to represent their right interest so as to help them to acquire new and current information about sugarcane cultivation, access to credit, technical supports and rights on contract engagement from the government and other stakeholders like sugar factories.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmad N, Sinha DK, Singh KM, Mishra RR, Singh SP (2018). Resource Use Efficiency in Sugarcane Production in Bihar (India): A Stochastic Frontier Analysis. Quarterly Journal of Science, Agriculture and Engineering 8(665):45-51.
Aigner DK, Lovell CK, Schmidt P (1977). Formulation and Estimation of Stochastic Frontier Production Function Models. Journal of Agricultural Economics 26(1):21-37.
Ali A, Jan AU (2017). Analysis of Technical Efficiency of Sugarcane Crop in Khyber Pakhtunkhwa: A Stochastic Frontier Approach. Sarhad Journal of Agriculture 33(1):69-79.
Amolo RA, Sigunga DO, Owuor PO (2017). Evaluation of soil properties of sugarcane zones and cropping systems for improved productivity in Western Kenya. International Journal of Agronomy and Agricultural Research 11(3):11-16.
Anyaegbunam HN, Nto PO, Okoye BC, Madu TU (2012). Analysis of determinants of farm size productivity among small-holder cassava farmers in South East agro ecological zone, Nigeria. American Journal of Experimental Agriculture 2(1):74-80.
Awunyo-Vitor D, Wongnaa CA, Aidoo R (2016). Resource Use Efficiency Among Maize Farmers in Ghana. Agriculture and Food Security 5(1):241-248.
Baruwa OI, Oke J (2012). Analysis of the technical efficiency of small-holder Cocoyam farms in Ondo State, Nigeria. Tropicultura 14:36-40.
Battese GE, Coelli TJ (1995). A model of Technical Inefficiency Effect in stochastic Frontier Production for Panel Data. Empirical Economics 20:325-345.
Bi H (2004). Stochastic Frontier Analysis of a Classic Self- Thinning Experiment. Journal of Austral Ecology 29(4):408-417.
Binam JN, Tonye J, Akoa M (2004). Factors Affecting the Technical Efficiency among smallholder farmers in the Slash and Burn Agricultural Zone of Cameroon. Journal of Food Policy 29(3):531-545.
Chatterjee S, Price B (1991). Regression Analysis by Example (2nd ed.). New York: Wiley.
Cochran WG (1977). Sampling Techniques (3rd Edition ed.). New York: John Wiley and Sons.
Coelli J, Rao P, Christopher J, Donnell O, Battese E (2005). An

Introduction to Efficiency and Productivity Analysis (2nd ed.). New York, USA: Springer Science and Business Media pp. 53-67.
Dube AK, Ozkan B, Ayele A, Idahe D, Aliye A (2018). Technical efficiency and profitability of potato production by smallholder farmers: The case of Dinsho District, Bale Zone of Ethiopia. Journal of Development and Agricultural Economics 10(7):225-235.
Erkoc TE (2014). Estimation Methodology of Economic Efficiency: Stochastic Frontier Analysis vs Data Envelopment Analysis. International Journal of Academic Research in Economics and Management Sciences 1(1):443-449.
Food and Agriculture Organization of the United Nations (FAOSTAT) (2016). Available at: http://faostat.fao.org/site/567/desktopDefault.aspx?PageID=567#ancor.
Fonjong NL, Mbah FA (2007). The fortunes and misfortunes of women maize producers in Ndop, Cameroon and the Implications for gender roles. Journal of International Women's Studies 8(4):211-230.
Getahun W, Geta E (2017). Technical efficiency of smallholder barley farmers: The case of Welmera district, Central Oromia, Ethiopia. African Journal of Agricultural Research 12(22):1897-1905.
Gujarati DN, Porter DC (2009). Basic Econometrics (4th ed.). New York: Mc Graw Hill Inc.
Hu W (2013). Effect of Contract Farming on the U.S. Crop Farmers' Average Return. Agricultural Economics (Zemědělská ekonomika) 59(5):195-201.
Igweoscar O (2014). Effect of Contract Farming on Productivity and Welfare of Cassava - Based Farmers in South Eastern Nigeria. European Journal of Business and Management 6(7):2222-2839.
Ike PC, Inoni O (2006). Determinants of Yam Production and Economic Efficiency Among Smallholder Farmers in South Eastern Nigeria. Journal of Central European Agriculture 44(6):102 -119.
Independent Electoral and Boundaries Commission (IEBC) (2017). The revised preliminary report of the proposed boundaries of constituencies and wards. Available at: https://www.iebc.or.ke/resources/?Boundary_Delimitation
Jaetzold R, Schmidt H, Hornetz B, Shisanya C (2005). Farm Management Handbook of Kenya (2nd ed., Vol. II). Nairobi: Ministry of Agriculture, Kenya, in Cooperation with the German Agency for Technical.
Jamoza JE, Amolo RA, Muturi SM (2013). A Baseline Survey on the Status of Sugarcane Production Technologies in Western Kenya. International Society of Sugarcane Technologist Conference. Kenya Sugar Research Foundation pp. 2-10
Kadiri FA, Eze CC, Orebiyi JS, Lemchi JI, Ohajianya DO, Nwaiwu IU (2014). Technical Efficiency in Paddy Rice Production in Niger Delta Region of Nigeria. Global Journal of Agricultural Research 2(2):33-43.
Kassa MD, Demissie WM, Batu MM (2019). Smallholders' technical efficiency of teff production in Ethiopia. African Journal of Agricultural Research 4(33):1641-1648.
Kenya Sugar Board (2014). Cane Census Report. Agriculture Department, Nairobi.
Khan A, Huda FA, Alam A (2010). Farm household technical efficiency: A study on maize producers in selected areas of Jamalpur district in Bangladesh. European Journal of Social Sciences 14(2):262-271.
Khan H, Saeed I (2011). Measurement of technical, allocative and economic efficiency of tomatoe farmers in Northern Pakistan. International Conference on Management, Economics and Social Sciences (ICMESS). Bangkok.
Kibaara BW (2005). Technical Efficiency in Kenyan's Maize Production: An Application of The Stochastic Frontier Approach. MSc. thesis. Colorado State University, Colorado.
Kothari CR (2004). Research Methodology: Methods and Techniques(2nd ed). New Age International Publishers pp. 103-147
Mailena L, Shamsudin MN, Radam A, Mohamed Z (2014). Efficiency of Rice Farms nd its Determinants: Application of Stochastic Frontier Analysis. Trends in Applied Sciences Research 9(7):360-371.
Mamo T, Getahun W, Chebil A, Tesfaye A, Debele T, Assefa S, Solomon T (2018). Technical efficiency and yield gap of smallholder wheat producers in Ethiopia: A Stochastic Frontier Analysis. African Journal of Agricultural Research 13(28):1407-1418.
Mburu S, Ackello-Ogutu C, Mulwa R (2014). Analysis of Economic Efficiency and Farm Size: A Case Study of Wheat Farmers in Nakuru District, Kenya. Economics Research International 48(13):89-101.
Mulianga B, Ogeda I, Mwanga D (2015). Assessing the Impact of Climate Change on Sugarcane Productivity in Kibos - Miwani, Kenya. Agricultural and Forest Meteorology 16(4):169-175.
Mulwa R, Emrouznejad A, Muhammad L (2014). Economic Efficiency of Smallholder Maize Producer in Western Kenya: A DEA Meta-Frontier Analysis. International Journal of Operational Research 4(3):250-267.
Musungu V, Sorre MB (2017). Contract Sugarcane Farming and its Effect on Household Income among Smallholder Farmers in Busia County, Kenya. International Journal of Science and Research Methodology 7(4):201-209.
Nchare A (2007). Analysis of Factors Affecting Technical Efficiency of Arabica Coffee Producers in Cameroon. Africa Economic and Research Consortium 163(6):501-509.
Nyagaka DO, Obare GA, Nguyo WN (2010). Economic Efficiency of Smallholder Irish Potato Producers in Nyandarua North District, Kenya. African Journal of Agricultural Research 5(11):1179-1186.
Rabe-Hesketh S, Everitt B (2000). A Handbook of Statistical Analyses using STATA (2nd ed.). Chapman and Hall/CRC.
Rumánková L, Smutka L (2013). Global Sugar Market – The Analysis of Factors Influencing Supply and Demand. ACTA Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis 53(2):463-472.
Severini S, Sorrentino A (2017). Efficiency and Coordination in the EU Agri-Food Systems. Agricultural and Food Economics 5(1):341-348.
Simonyan JB, Umoren BD, Okoye BC (2011). Gender Differentials In Technical Efficiency Among Maize Farmers In Essien Udim Local Government Area, Nigeria. International Journal of Economics and Management Science 1(2):17-23.
Sopheak K (2015). The Effect of Rice Contract Farming on Smallholder Farmers' Income in Combodia. Journal of Agricultural Economics 3(2):43-51.
Sulaiman M, Abdulsalam Z, Damisa M, Siewe F (2015). Resource Use Efficiency in Sugarcane Production in Kaduna State, Nigeria: An Application of Stochastic Frontier Production Function. Asian Journal of Agricultural Extension, Economics and Sociology 7(2):1-11.
Tchale H (2009). The Efficiency of Smallholder Agriculture in Malawi. Africa Journal of Agricultural and Resource Economics 3(2):101-121.
Thabethe L, Mungatana E (2014). Estimation of Technical, Economic and Allocative Efficiencies in Sugarcane Production in South Africa: A Case of Mpumalanga Growers. Journal of Economics and Sustainable Development 5(16):86-96.
Travella SR, Oliveira D (2017). Sugarcane in Africa. Retrieved from www.vib.be/en/about-biotech-news/Documents/vib_fact_ sugarcane_EN_2017_1006_LR_single.pdf
Waswa F, Gweyi-Onyango P, Mcharo G (2012). Contract Sugarcane Farming and Farmer's Income in the Lake Basin, Kenya. Journal of Applied Biosciences 52:3685-3695.
Wawire NW, Ouma VO (2013). Assessment of Profitability in Sugarcane Production Using Cost Benefit Analysis and Net Present Value Techniques in Kenya. East African Agricultural and Forestry Journal 79(4):243-250.
Weir S, Knight J (2007). Production Externalities of Education: Evidence from Rural Ethiopia. Journal of African Economies 16(1):134-165.
Wekesa R, Onguso MJ, Nyende B, Wamocho LS (2015). Sugarcane in Vitro Culture Technology: Opportunities for Kenya's Sugar Industry. African Journal of Biotechnology 14(47):3170-3178.
Wolfgang F, Owegi F (2012). Sweetening Kenya's Future –The Challenges of the Sugar Industry. Available at: http://blogs.worldbank.org/africacan/sweetening-kenya-s-future-the-challenges-of-the- sugar-industry
Yegon PK, Kibet LK, Lagat JK (2015). Determinants of Technical Efficiency in Smallholder Soybean Production in Bomet district, Kenya. Journal of Development and Agricultural Economics 7(5):190-194.

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Production of banana bunchy top virus (BBTV)-free plantain plants by *in vitro* culture

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Banana Bunchy Top Disease (BBTD) caused by the Banana Bunchy Top Virus (BBTV) is one of the most important banana diseases in the Democratic Republic of Congo. This study focused on the production of BBTV-free plantain seedlings from infected banana plants. A total of 10 suckers from the French plantain Litete (*Musa* AAB) and the False Horn plantain Libanga Likale (*Musa* AAB) with advanced BBTD symptoms were collected. Meristematic apices excised from those suckers were cultured *in vitro* and subcultured five times. The presence of BBTV was evaluated by the Triple-Antibody Sandwich Enzyme-linked Immunosorbent Assay (TAS-ELISA). The BBTV was confirmed in all suckers prior to *in vitro* culture but 73.3% of Litete plantlets and 66.6% of Libanga Likale plantlets regenerated from meristematic tissues were virus-free. This indicates that *in vitro* culture is a simple tool to generate BBTV-free plantains.

Key words: Banana bunchy top virus (BBTV), *in vitro* tissue culture, plantains

INTRODUCTION

The Banana Bunchy Top Disease (BBTD) is one of the most devastating diseases in banana and plantain, sometimes causing 100% yield losses (Qazi, 2016). About 20 virus species belonging to 5 families have been reported to infect banana and plantain worldwide (Kumar et al., 2015). The most economically important viruses of banana are Banana Bunchy Top Virus (BBTV, genus Babuvirus, family Nanoviridae), several species of Banana Streak Viruses (BSVs, genus Badnavirus, family Caulimoviridae) and Banana Bract Mosaic Virus (BBrMV, genus Potyvirus, family Potyviridae). Of minor significance are Abaca Bunchy Top Virus (ABTV, genus Babuvirus), Abaca Mosaic Virus caused by a distinct strain of Sugarcane Mosaic Virus (SCMV) designated as SCMV-Ab (genus Potyvirus), Banana Mild Mosaic virus (BanMMV), and Banana virus X (BVX) both unassigned

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members in the family Betaflexiviridae, and Cucumber Mosaic Virus (CMV, genus Cucumovirus, family Bromoviridae). Viruses are a major concern to banana and plantain production because of their effects on yield and quality, and as constraints to the international exchange of *Musa* germplasm. Direct losses are incurred from reduced production, and indirect losses are associated with maintaining plant health, including the production of virus-free planting material. BBTV is among the top 10 viruses worldwide in terms of economic impact (Rybicki, 2015). The BBTV is transmitted by the banana aphid *Pentalonia nigronervosa* Coquerel. Its transmission efficiency is affected by temperature, the stage of life of the vector and plants during the period of collection (Anhalt et al*.,* 2008). The long-range diffusion of BBTD, however, is more closely related to the transport of infected plant material (Qazi, 2016). In sub-Saharan Africa, BBTV was first reported in the Democratic Republic of Congo (DR Congo) in the 1950s (Kumar et al*.,* 2011). It has spread throughout the country (Mukwa et al*.,* 2014). Recently, 16 BBTV isolates from the former Orientale and South Kivu provinces (North-east and central DR Congo) were compared as part of a global distribution study of BBTV, revealing a large human contribution to its dispersal over long distances (Stainton et al*.,* 2015). In DR Congo, BBTV is present in all its 11 old provinces (Kumar et al., 2011; Mukwa et al*.,* 2014; Ngama et al*.,* 2014). Farmers collect suckers from infected symptomless plants to establish new fields thereby spreading further the disease and encountering heavy yield losses. There is thus a clear need to provide farmers with virus-free planting material.

In infected plants, virus particles might be omnipresent but it is hypothesized that at least part of the meristem is virus-free. *In vitro* culture of meristems has the potential to multiply precisely these virus-free cells to amounts that allow plant regeneration from it and therefore to deliver virus-free plants. Bananas (bananas and plantains) constitute a crop that plays a major role in food security in the Democratic Republic of Congo (DR Congo). Indeed, they are rich in energy, mineral salts (potassium, calcium, phosphorus) and vitamins A, B and C. The production of bananas and plantains of DR Congo occupies the 10th position in the world. Compared with other food products, their production comes second to cassava. In addition, bananas and plantains play a role in improving the income of the population because of their high market value (Dhed'a et al., 2019).

The aim of this study is to clean plantain plants, a starchy banana of the *Musa* AAB subgroup which is widely cultivated in the Congo basin and in West Africa, from BBTV by *in vitro* culture to regenerate healthy plants free of BBTV and confirm this by TAS-ELISA.

MATERIALS AND METHODS

The plant material consisted of young suckers (30-40 cm in height) of two plantain (*Musa* AAB) varieties, 'Litete' (Figure 1) which is a French type plantain and 'Libanga Likale' (Figure 2), a False Horn type plantain.

Suckers of the two cultivars were collected around Kisangani (DR Congo) town on plants with visual symptoms of BBTD. The severity of the disease in the field was scored using a scale of 0 - 5 (0: No symptoms, 1: presence of streaks on the leaf, 2: presence of streaks on the pseudostem, 3: discoloration of the leaf keeping its normal size, 4: reduced leaf size and 5: bushy appearance at the top or Bunchy top) (Niyongere et al*.,* 2011; Ngama et al*.,* 2014). Only suckers with advanced stages of BBTV (4 and 5) were collected (Figure 2).

A total of 10 infected suckers were collected from 5 'Litete' and 5 'Libanga Likale' tufts. All suckers were tested using TAS-ELISA and were confirmed as positives. The TAS-ELISA method used involved BBTV extraction from the leaves, incubation and addition of monoclonal antibody and antibody coupled to alkaline phosphatase B in the presence of positive and negative BBTV controls. All the processes were conducted in the laboratory of the Faculty of Science of the University of Kisangani (UNIKIS).

In vitro cultures of infected plants were established on standard media with mineral salts (Murashige and Skoog, 1962) (Figure 4). This medium was enriched with 30 g/l of sucrose, 2 g/l of gelrite, nicotic acid (0.5 mg/l), pyridoxine (0.4 mg/l), thiamine (0.5 mg) and 2 mg/glycine and supplemented with a 10 μM 6-benzylaminopurine (BAP) and 1 μM of indole acetic acid (IAA) according to Banerjee et al. (1985, 1986) and Vuylsteke (1989) (Figure 3).

Each cultivar was subcultured 5 times at one month intervals. The *in vitro* plants were regenerated and acclimatized in the screenhouse for two months until the plantlets reached a size of 20 cm and then tested twice for BBTV by TAS-ELISA. Data were analyzed by R Software (3.1.3).

RESULTS

The two plantain cultivars were put *in vitro* and subcultured 5 times (Figure 4). There was no difference in bud proliferation between both cultivars. Indeed after the first subculture, Libanga Likale produced 7.2 proliferating buds compared to 5.2 for Litete, a non-significant difference (p-value = 0.3455; t = 1.0025). After the fifth subculture, Libanga Likale produced 8.6 proliferating buds compared to Litete which produced 11.2 proliferating buds, a non-significant difference (p-value = 0.3287; t = 1.0442).

After *in vitro* culture, the banana plants were regenerated and all the samples analyzed. Of the 30 Libanga Likale plants produced, 10 were positive and 20 negative; also, Litete produced 8 positive and 22 negative plants. The BBTV-free plants grew fast unlike plants infected with BBTV (Table 1 and Figure 5).

The results in Table 1 show that the *in vitro* culture could clean 73.3% Litete and 66.6% Libanga Likale plants. Overall, this technique cleaned 70.0% of all plants studied.

DISCUSSION

The interest of this work lies in the development of a propagation technique of healthy plants that will contribute to the improvement of the production of banana, making it possible to improve the food security in


(a) (b)

Figure 1. Cultivars used for in vitro culture (a) Litete and (b) Libanga Likale.


Level 4 Level 5

Figure 2. Typical banana bunchy top disease symptoms.

Kisangani, DR Congo.

Plantain cultivars responded quickly to *in vitro* culture as already after one subculture, the number of buds was 5.2 and 7.2 for Litete and Libanga Likale respectively. The number of proliferating buds increased by the fifth subculture with the number of buds more than doubled in Litete (11.2) while the number of buds for Libanga Likale increased less drastically (8.6). Reyes et al. (2017) found *in vitro* proliferation rates of 1.95-2.20 in plantains, while Korneva et al. (2013) found a 0.8 proliferation rate for

Figure 3. Stages of cultivation. A: Removal of all old non-meristematic tissues. B: Explant with reduced size and washed under running water. C: Disinfection successively by immersing the explant in alcohol 70% for 15 s, and the solution of calcium hypochlorite 30% for 20 min. The explant is then rinsed with sterile distilled water three times. D: Removal of the foliar tissues one after the other until extraction of the meristematic apex. E: Putting the explant in the in vitro culture medium. F: Transfer of tubes containing the explants into the culture chamber. G: Proliferation of the buds after a minimum of two weeks. H: Subculture (separation of buds and their transfer to a new medium). I: Regeneration of buds to rooted seedlings. J and K: Transfer of vitro plants to pots.

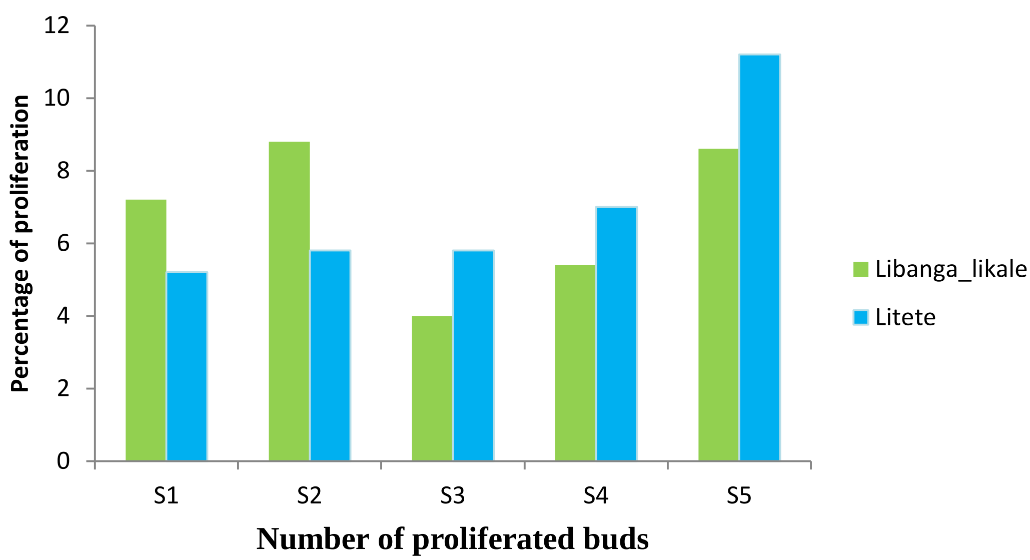

Figure 4. Number of buds produced in vitro during 5 subcultures (S1- S5) of the two plantain cultivars, Litete and Libanga Likale.

Table 1. Health status of Libanga Likale and Litete after *in vitro* culture.

Cultivars	Plants tested	Positive plants	Negative plants	Remediation rate (%)
Libanga Likale	30	10	20	66.6
Litete	30	8	22	73.3
Total	60	18	42	70.0

Figure 5. Banana bunchy top virus-free plantlet (left), and infected plantlet (right).

plantain and 1.86 for banana. Our results are also in line with Dhed'a (1992), who showed that 10 µM BAP increased the proliferation rates in *in vitro* with 3.5 for the plantain cultivar Three Hand Planty (*Musa* AAB) with 18.6, 12.4 and 21.4 for the cooking banana cultivars Bluggoe, Cardaba and Saba (*Musa* ABB) and 7.1 for the dessert banana cutivar Yangambi Km5 (*Musa* AAA). Roels et al. (2005) obtained a proliferation rate of 3-5 in the dessert Cavendish (AAA) subgroup. The virus detection by TAS-ELISA showed that 73.3% of Litete and 66.6% of Libanga Likale were found to be BBTV free after plant regeneration. Our results are in line with Morel and Martin (1952), who by taking meristematic spikes of dalhias obtained dahlias free from the mosaic of dalhias and the spotted wilt virus which are caused by RNA viruses. On the other hand, it is by using the meristem culture that Wang and Hu (1980) managed to eliminate more than 70 known diseases in more than 40 different species. Sweet et al. (1979) obtained a high level of purification from the "Nepo" viruses (RRV = Raspberry ringspot, AMV = Arabis mosaic virus) by coupling thermotherapy and meristem culture, whereas meristem culture alone was sufficient to eliminate cucumber mosaic (CMV). Mosella et al. (1980) obtained 57% plants free from N.R.S.V (Sharka necrotic ringspot virus) and 72% for Sharka starting with 0.4 -0.8 mm explants. Panis et al. (2001) also found that 37.9% of banana and plantain plantlets regenerated from cryopreserved proliferation meristems tested negative for ELISA. However, since possible remediation mechanisms are not fully understood, the most likely assumptions are: - absence of vascular connections between the meristem and the underlying tissues; viruses must progress symplastically or apoplastically rather than vascularly to reach the meristem, which is slower; an actively growing meristem can therefore "escape" the viruses,- absence of plasmodesmes at the meristem, hence slowing propagation symplastically, intense competition between meristematic cells under active division and viral particles for nucleoproteins,- presence of inhibiting substances,- in case of excision of the meristem, temporary unavailability of enzymes necessary for viral replication; this unavailability is a function of the size of the meristem, and is therefore longer as the meristem is small. It has been observed that small meristems that contain viruses can regenerate healthy plants. Our results on sanitation show that it is possible to obtain a high level of plants sanitized by in vitro culture.

In vitro culture of BBTV infected plantain cultivars is a simple tool to obtain virus free clean planting material from plants with advanced symptoms. With only five subcultures, we obtained 73.3% virus-free plants in the Litete cultivar and 66.6% in the cultivar Libanga Likale. Hence it is hypothized that more virus-free plants can be obtained in either cultivar by either increasing the number of subcultures or increasing the concentration of BAP, as Dhed'a et al. (1991) showed that 100 µM BAP increases drastically the proliferation rate. Since the proliferation rate varies a lot between different banana cultivars with a different genomic background, we also speculate that the rates of cultivars becoming virus-free also vary within the banana subgroup.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Anhalt MD, Almeida RP (2008). Effect of temperature, vector life stage, and plant access period on transmission of banana bunchy top virus to banana. Phytopathology 98:743-748.

Banerjee N, De Langhe E (1985). A tissue culture technique for rapid clonal propagation and storage under minimal conditions of Musa (Banana and plantain). Plant Cell Reports 4:351-354.

Banerjee N, Vuylsteke D, De Langhe E (1986). Meristem tip culture of *Musa:* histomorphological studies of shoot bud proliferation. In Plant tissue culture and its Agricultural Applications (Withers L.A., Alderson P.G., eds). London, UK: Butterworth pp. 139-147.

Dhed'a DB, Adheka GJ, Onautshu OD, Swennen R (2019). La culture des bananiers et plantains dans les zones agroécologiques de la République Démocratique du Congo, Presse Universitaire UNIKIS, Kisangani 72 p.

Dhed'a D (1992). Culture de suspensions cellulaires embryogéniques et régénération en plantules par embryogénèse somatique chez le bananier et le bananier plantain *(Musa* spp.). PhD thesis, KU Leuven, Belgium 171 p.

Dhed'a D, Dumortier F, Panis B Vuylsteke D (1991). Plant regeneration in cell suspension cultures of cooking banana cv Bluggoe (*Musa* sp.), Fruits 46:125-135.

Korneva S, Flores J, Santos E, Piña F, Mendoza J (2013). Plant regeneration of plantain 'Barraganete' from somatic embryos using a temporary immersion system. Biotecnología Aplicada 30:267-270.

Kumar PL, Hanna R, Alabi OJ, Soko MM, Oben TT, Vangu GHP, Naidu RA (2011). Banana bunchy top virus in sub-Saharan Africa: investigations on virus distribution and diversity. Virus Research 159:171-182.

Kumar PL, Selvarajan R, IskraCaruana ML, Chabannes M, Hanna R (2015). Biology, etiology, and control of virus diseases of banana and plantain. Advances in Virus Research 91:229-69.

Mosella LCh, Signoret PA, Nard RJO (1980). Sur la mise au point de techniques de microgreffage d'apex en vue de l'élimination de deux types de particules virales chez le pêcher (*Prunus persica*, Batseh). Academy of Sciences 290:287-290.

Morel G, Martin C (1952). Guérison de dahlias atteints d'une maladie à virus. C.R. Academy of Sciences 235:1324-1325.

Mukwa LFT, Muengula M, Zinga I, Kalonji A, IskraCaruana ML, Bragard C (2014). Occurrence and distribution of banana bunchy top virus related agro-ecosystem in south western Democratic Republic of Congo. American Journal of Plant Sciences 5:647-658.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Plant Biology 15(3):473-497.

Ngama F, Ibanda B, Komoy J, Lebisabo C, Muhindo H, Walunkonka F, Wembonyama J, Dhed'a B, Lepoint P, Sivirihauma C, Blomme G (2014). Assessing incidence, development and distribution of banana bunchy top disease across the main plantain and banana growing regions of the Democratic Republic of Congo. African Journal of Agriculture 9(34):2611-2623.

Niyongere C, Ateka E, Losenge T, Blomme G, Lepoint P (2011). Screening Musa genotypes for banana bunchy top disease resistance in Burundi. Acta Horticulturae 897:439-447.

Panis B, Helliot B, Reyniers K, Locicero A, Vandewalle M, Muylle H, Michel C, Lepoivre P, Swennen R (2001). Assessment of cryopreservation for Cucumber Mosaic Virus (CMV) eradication in banana plantlets. Belgian Plant Tissue Culture Group Journal 11:8.

Qazi J (2016). Banana bunchy top virus and the bunchy top disease. Journal of General Plant Pathology 82:2-11.

Reyes G, García J, Piña F, Mendoza J, Sosa D, Noceda C, Blasco M, Flores J (2017). In vitro proliferation and cryoconservation of banana and plantain elite clones. Journal of Horticultural Research 25(2):37-47.

Roels S, Escalona M, Cejas I, Noceda C, Rodriguez R, Canal MJ, Sandoval J, Debergh P (2005). Optimization of plantain (Musa AAB) micropropagation by temporary immersion system. Plant Cell, Tissue and Organ Culture 82:57-66.

Rybicki AP (2015). A top ten list for economically important plant viruses. Archives of Virology 160:17-20.

Stainton D, Martin D, Muhire B, Lolohea S, Halafihi M, Lepoint P, Blomme G Crew KS, Sharman M, Kraberger S, Dayaram A, Walters M, Collings DA, Mabvakure B, Lemey P, Harkins G, Thomas JE, Varsani A (2015). The global distribution of Banana bunchy top virus reveals little evidence for frequent recent, human-mediated long distance dispersal events. Virus Evolution 1:1. doi:10.1093/ve/vev009.

Sweet JB, Constantine DR, Sparks TR (1979). The elimination of three viruses from Daphne spp. by thermotherapy and meristem excision. Journal of Horticultural Science 54:323-326.

Vuylsteke D (1989). Shoot-tip culture for the propagation, conservation and exchange of *Musa* germplasm. Practical manuals for handling crop germplasm *in vitro* 2. IBPGR. Rome, Italy 62 p.

Wang PJ, Hu CY (1980). Regeneration of virus-free plants through in vitro culture. Advances in Biomedical Engineering 18:61-99.

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Influence of supplementary hoe weeding on the efficacy of ButaForce® for lowland rice (*Oryza sativa* L.) weed management

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Field experiments to determine the influence of supplementary hoe weeding on the efficacy of ButaForce® (N-(butoxymethyl)-2-chloro-N-2,6-dimethyl acetanilide) for low land rice (*Oryza sativa* L.) weed management was conducted at the Faculty of Agriculture Teaching and Research Farm of the University of Port Harcourt during the early cropping seasons of 2018 and 2019. Seven treatments were used for the experiment namely: ButaForce® at 1.5 L/ha + SHW (21 DAS), ButaForce® at 2.0 L/ha + SHW (21 DAS), ButaForce® at 2.5 L/ha + SHW (21DAS), ButaForce® at 3.0 L/ha (recommended rate), weed-free (weekly weeding), hoe weeded twice at 21 and 42 DAS and weedy check. The treatments were laid out in a Randomized Complete Block Design (RCBD) with three replicates. Results from the study showed that weed-free check (weekly weeding) was more effective in weed control in lowland rice. It also gave the highest growth and yield attributes over all other treatments. Weed suppression and rice performance was better in plots treated with ButaForce® at 2.5 L/ha + SHW (21 DAS) than in other supplementary hoe weeding. The economic analysis showed that although hoe weeded plots had higher yields, the profit obtained from them were lower when compared with the supplementary hoe weeding and ButaForce® at 3.0 L/ha. Among all the weed control treatment, plots treated with ButaForce® at 2.5 L/ha with supplementary hoe weeding gave the highest profit. Since the highest profit was recorded in plots treated with ButaForce® at 2.5 L/ha with supplementary hoe weeding, it is therefore recommended to rice farmers in the study area.

Key words: Hoe weeding, lowland rice, supplementary, weed management, economic analysis.

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the family of Poaceae and is a staple cereal crop in Nigeria. In Nigeria, it is grown in almost all agro-ecological zones as it forms one important cereal crop cultivated by farmers. Although rice is cultivated in almost all the agro-ecological zones in Nigeria, the cultivated area seemed to be small and the average rice farm holding is between 1 and 2 hectares (Akpokodje et al., 2001). Globally rice production records

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Table 1. Herbicide used in the study.

Common Name	Trade name	Formulation	Manufacturer	Main marketing agent in Nigeria
Butachlor	ButaForce ®	50%	Syngenta	Syngenta Nigeria limited

Table 2. Chemical name of the herbicide used in the study.

Herbicide name	Chemical name
ButaForce ®	N-(butoxymethyl)-2-chloro-N-2,6-dimethyl acetanilide

showed that of the 14.6 million metric tonnes of paddy rice produced annually on 7.3 million ha of land in Africa, Nigeria's production moved from 3.7 million metric tonnes in 2017 to 4.0 million metric tonnes in 2018 and with this slight increase it became the largest producer of rice in Africa (Oduntan, 2019) despite the increase, its yield remain moderate. Multifarious factors constrain rice production in Nigeria among which is ineffective weed control methods. Yield loss between 75 and 100% in rice as a result of uncontrolled weed growth has been reported by Akobundu (2011) and Imeokparia (2011). The elimination of weed competition at different stages of crop growth is critical and can be achieved manually or with the use of herbicides. However, both methods have their shortcomings. Hoe weeding is associated with drudgery and some weed species can develop resistance with the continuous use of herbicide (Udensi et al., 2017). As a result, herbicide application must be supplemented with hoe weeding in an integrated manner, to effectively control weeds in rice (Akobundu, 1987). The few reports on effectiveness of ButaForce® on weed control and performance of crops such as wheat and rice had been reported by Singh et al. (2016) and Hassan et al. (2017). No one weed control method has proved to be effective hence; this study tends to identify the efficacy of ButaForce supplemented with hoe weeding compared with the commonly adopted hoe weeding (weekly weeding and hoe weeded at 21 and 42DAS) and ButaForce® at 3.0 L/ha.

MATERIALS AND METHODS

Experimental site

The field trials were conducted during the 2018 and 2019 rainy seasons at the Teaching and research farm of the University, Port Harcourt, Rivers State, (latitude 04° 54 538'N, and longitude 006° 55 329'E; 17 m above sea level), Nigeria. The site had an average rainfall between 2500 – 4000 mm and a mean temperature of 27°C, relative humidity of 78% and Nwankwo and Ehirim, 2010). The area has two seasons (wet and dry). The wet season has double rainfall peaks with two cropping seasons in the area: early from March to July and late from August to December. The experimental site had been planted to mixed crops of maize, pepper and watermelon before commencement of the experiment. The dominant weed species found in the experimental site was identified with a weed handbook (Akobundu et al., 2016). These weeds were: *Ageratum conyzoides* Linn *Aspilia africana* (Pers.) C.D. Adams. *Chromoleana odorata* (L.) R.M. King & Robinson, *Cleome rutidosperma* DC. *Cyperus esculentus* Linn.. *Mariscus alternifolius* Vahl., *Mitracapus villosus* (Sw.) DC., *Oldenlandia corymbosa* Linn. and *Panicum maximum* Jacq.

Soil analysis

Soil samples were collected before planting operations at a depth of 0-15 cm deep using an auger of 10 cm in diameter at ten different points from the experimental site. The samples collected was air-dried at ambient temperature for two weeks and pulverized to facilitates laboratory analysis and for the removal of plant debris. The dry pulverized samples was assessed through a 2 mm mesh sieve and analyzed for physicochemical properties using standard methods (IITA, 1982).

Rice variety used

The rice variety used was (UPIA 2) and UPIA is an acronym for University of Port Harcourt, International Rice Research Institute and AGRA. It has an outstanding characteristic of high yield and tolerance to iron toxicity and African rice gall midge, matures between 110 - 120 days with a potential yield of 8.0 t/ha. The seeds were obtained from rice seed banks at the Teaching and Research Farm of University of Port Harcourt, Rivers State.

Herbicide used

ButaForce herbicide was used for the study. The herbicide was obtained at an Agrochemical store in Port Harcourt, Rivers State. The common name of the herbicide, its formulation, manufacturer and main marketing agent in Nigeria is shown in Table 1 and the chemical name Table 2.

Treatment and experimental design

Seven treatments were used for the experiment, which are itemized below:

(i) ButaForce ® at 1.5 L/ha + SHW (21 DAS)
(ii) ButaForce ® at 2.0 L/ha + SHW (21 DAS)
(iii) ButaForce ® at 2.5 L/ha + SHW (21 DAS)
(iv) ButaForce ® at 3.0 L/ha
(v) Weed free (weekly weeding)
(vi) Weeding twice at 21 DAS and 42 DAS

(vii) No weeding

These treatments were replicated three times to give a total twenty - one experimental plots, arranged in a Randomized Complete Block Design (RCBD).

Cultural details

The experimental land area of 29 m × 12 m (348 m^2) of approximately 0.03 ha was cleared manually, stumps and excess vegetation packed away from the plots. The experimental area was divided into three blocks while each block was further divided into seven plots making it a total of twenty one plots. Each plot size was 3 m × 3 m. The plots were separated by 1 m while the blocks were separated with a pathway of 1 m. Planting was done on the 14^{th} and 15^{th} May 2018 and 2019 respectively. The seeds were sown at a spacing of 30 cm × 30 cm with three seeds per hole and later thinned to one seedling at fourteen days after sowing (14 DAS) to give a plant population of 100 plants /plot which is equivalent to (111,111plants/ha). One day after sowing (1DAS) , twelve plots were sprayed with ButaForce ® at 1.5, 2.0 and 3.0 L/ha using a hand-operated CP3 knapsack sprayer calibrated to deliver approximately 240 L/ha spray volume at a pressure of 210 kpa with red polijet nozzle (swath width½m). Supplementary hoe weeding was carried at 21 DAS in plots that were treated with ButaForce ® at 1.5, 2.0 and 3.0 L/ha. Three plots were manually weeded with a hoe twice at 21 DAS and 42 DAS while another 3 plots were hoe weeded weekly. Basal application of urea fertilizer at 97.8 kg/ha was carried out at 21 DAS. This was done because the soil sample from the experimental site was found to be deficient of nitrogen (0.10 and 0.11% in 2018 and 2019 respectively) when compared to the critical level of nitrogen (0.15%) of southeastern soil established by Ibedu et al. (1988). Harvesting was carried out on 17^{th} and 18^{th} of September in 2018 and 2019 respectively with the use of sickle.

Data collection

Weed growth characteristics

Weed density and weed dry weight: Weed samples were collected at 21, 42, 63 and 84 DAS by placing 50 cm × 50 cm quadrats diagonally per plot twice. The weeds within each quadrat were removed by hand, counted and expressed in no/m^2. The weed dry weight was carried by using the same quadrat technique as weed density. The weeds were removed within the quadrat, sun dried to constant weight, weighed with an electronic scale, and expressed in g/m^2.

Weed control efficiency

Weed control efficiency was determined by using the method of Subramanian et al. (1991) as:

$$\text{WCE (\%)} = \frac{DWT\ of\ no\ weeding\ \ plot - DWT\ of\ treated\ \ plot}{DWT\ of\ no\ weeding\ \ \ plot} \times 100$$

Where:
WCE (%) = Weed control efficiency
DWT = Dry weight

$$\text{WI} = \frac{yield\ \ from\ weed\ \ free\ \ check - yield\ \ from\ treated\ \ plot}{yield\ \ from\ the\ weed\ \ free\ \ check} \times 100$$

Subramanian et al. (1991)

Where: WI = weed index

Rice performance

Five plants from the middle row of each plot were randomly selected and tagged and used to determined plant height, number of tillers, number of leaves, and leaf area index.

Plant height

The height of each tagged plant was taken at 4 intervals (21, 42, 63 and 84 DAS) using a meter ruler. Plant height was determined by placing a meter ruler at the soil surface to the tip of the flag leaf of each tagged plant and the mean calculated and recorded in cm.

Number of productive tillers

The number of tillers was obtained by counting starting from 21 to 84 DAS.

Number of leaves

This was done by counting the number of leaves per plant.

Leaf area index (LAI)

Leaf area index was determined by the following equation below:

LAI = T A x N /GA,

Where, TA = Total leaf area /plant N = number of plants/ gross plot, GA= Gross plot Area (Remison, 1997).

Panicle length

This was done by randomly from five panicles selected from harvested produce in each plot. It was measured from the neck-node to the tip of the apical grain and their average was taken as per panicle length.

Panicle weight (g)

The panicles selected for measuring length were weighed on an electrical weighing balance and then mean was worked out.

Paddy yield

The grains obtained after threshing and winnowing of the produce from each gross plot were sun dried, weighed per gross plot with a scale and the weight was expressed in kilogram per hectare (kg/ha).

Economic assessment

The economic assessment was done by using partial budgeting (Okoruwa et al., 2005).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) at 5% level of probability using GENSTAT 12^{th} Edition while treatments mean

Table 3. Physiochemical properties of the experimental site before planting.

Soil properties	2018	2019
Physical properties		
Sand (%)	84	82
Silt (%)	4	3
Clay (%)	12	15
Textural class	Loamy sand	Loamy sand
Chemical properties		
pH (H_{2O})	5.8	5.9
Total organic carbon (%)	1.17	1.19
Total nitrogen (%)	0.10	0.11
Available P (mg/kg)	14	15
Cation exchangeable capacity (cmol/kg)		
Ca	3.15	3.16
Mg	3.03	3.05
Na	1.35	1.12
K	3.05	3.03

Table 4. Rainfall (mm) data at the experimental sites during 2018 and 2019 cropping seasons.

Month	2018	2019
May	255	288.80
June	358	401.83
July	410	218.69
August	339	202.69
Total	1362	1112.01

Source: Department of Geography and Environmental Management, University of Port Harcourt.

were separated by using the least significant difference (LSD).

RESULTS

Soil analysis

The physiochemical characteristics of the soil before planting in both years are presented in Table 3. The soil of both years of study was loamy sand, slightly acidic with a moderate organic carbon content, available Phosphorus (P), exchangeable Potassium (K), Magnesium (Mg), Calcium (Ca) and low in total Nitrogen.

Rainfall

Table 4 shows the amount of rainfall data in 2018 and 2019 cropping seasons. The total amount of rainfall in 2018 cropping season (1362 mm) was higher than that of 2019 (1112.01 mm) by 22.48%.

Weed growth characteristics

Weed density

The effect of supplementary hoe weeding on the efficacy of Butaforce on weed density of low land rice is shown in Table 5. The treatments differed significantly ($P < 0.05$) throughout the sampling intervals (21, 42, 63 and 84 DAS). The highest weed density was recorded in no weeding plots throughout the observation periods except at 21DAS in 2018. The lowest weed density was recorded in plots that were weekly weeded throughout the periods of observation. All the ButaForce® rates with a supplementary hoe weeding had similar weed density at all the sampling intervals. Though at 21 DAS there were no significant differences between the supplementary hoe weeding and the recommended rates of ButaForce® at 3.0 L/ha at that period of sampling in both years, but all the herbicide plots that were supplemented with hoe weeding had lower weed density than the recommended rates of ButaForce® at 3.0 L/ha.

Table 5. Effect of supplementary hoe weeding on the efficacy of ButaForce® on weed density (no/m^2) of lowland rice.

Treatment	**21 DAS**	**42 DAS**	**63 DAS**	**84 DAS**
2018				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	86	97	143	207
ButaForce® at 2.0 L/ha + SHW (21 DAS)	81	80	115	183
ButaForce® at 2.5 L/ha + SHW (21 DAS)	69	80	111	160
ButaForce at 3.0 L/ha	47	205	253	303
Weed free (weekly weeding)	0	0	0	0
Hoe weeded at 21 and 42 DAS	103	89	54	76
No weeding	90	325	400	459
LSD (P=0.05)	55.36	85.4	93.5	93.2
2019				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	31.3	79.3	179	229
ButaForce® at 2.0 L/ha + SHW (21 DAS)	27.7	61.3	133	130
ButaForce® at 2.5 L/ha + SHW (21 DAS)	14.0	50.7	116	120
ButaForce at 3.0 L/ha	7.3	97.3	207	233
Weed free (weekly weeding)	0	0	0	0
Hoe weeded at 21 and 42 DAS	36.7	108	83	120
No weeding	159.3	216.7	353	390
LSD (P=0.05)	48.52	56.97	119.2	88.7

SHW = Supplementary hoe weeding, DAS = Days after sowing.

Table 6. Effect of supplementary hoe weeding on the efficacy of ButaForce® on weed dry weight (g/m^2) of lowland rice.

Treatment	**21DAS**	**42DAS**	**63DAS**	**84DAS**
2018				
ButaForce® 1.5 L/ha + SHW (21 DAS)	10.0	2.3	17.3	19.3
ButaForce® 2.0 L/ha + SHW (21 DAS)	7.3	1.0	13.7	16.7
ButaForce® at 2.5 L/ha + SHW (21 DAS)	5.3	0.8	10.9	16.0
ButaForce® at 3.0 L/ha	2.8	20.7	24.0	38.0
Weed free (weekly weeding)	0.0	0.0	0.0	0.0
Hoe weeded at 21 and 42 DAS	8.7	1.5	1.0	1.2
No weeding	7.3	37.3	36.7	42.7
LSD (P=0.05)	11.3	11.12	23.06	21.64
2019				
ButaForce® 1.5L/ha + SHW (21 DAS)	1.30	8.00	12.87	114.3
ButaForce® 2.0 L/ha + SHW (21 DAS)	1.10	5.53	9.00	104.1
ButaForce® at 2.5 L/ha + SHW (21 DAS)	1.07	3.77	7.33	68.3
ButaForce® at 3.0 L/ha	1.00	11.87	16.33	121.7
Weed free (weekly weeding)	0.00	0.00	0.00	0.00
Hoe weeded at 21 and 42DAS	2.37	1.53	2.50	70.3
No weeding	2.23	126.13	265.00	360.3
LSD (P=0.05)	0.179	0.679	2.331	38.59

SHW = Supplementary hoe weeding, DAS = Days after sowing.

Weed dry weight

The effect of supplementary hoe weeding on the efficacy of ButaForce® on weed dry weight of low land rice is shown in Table 6. The treatments differed significantly ($P < 0.05$) throughout the sampling intervals (21, 42, 63 and 84 DAS) on weed dry weight. Plots that were weeded weekly had the lowest weed dry weight when compared

Table 7. Effect of supplementary hoe weeding on the efficacy of ButaForce® on weed control efficiency (%) of lowland rice.

Treatment	21 DAS	42 DAS	63 DAS	84 DAS
2018				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	-36.99	94.0	64.40	40.1
ButaForce® at 2.0 L/ha + SHW (21 DAS)	0.00	96.7	73.20	56.9
ButaForce® at 2.5 L/ha + SHW (21DAS)	27.40	98.2	75.3	68
ButaForce® at 3.0 L/ha	61.64	44.50	34.60	11.01
Weed free (weekly weeding)	100	100	100	100
Hoe weeded at 21 and 42 DAS	-19.17	94.4	95.8	93.8
No weeding	0	0	0	0
LSD (P=0.05)	1.206	26.08	52.13	37.45
2019				
ButaForce® at 1.5 L/ha + SHW(21DAS)	41.39	93.66	95.14	68.26
ButaForce® at 2.0 L/ha + SHW (21 DAS)	50.32	95.61	96.00	71.10
ButaForce® at 2.5 L/ha + SHW(21 DAS)	51.90	97.01	97.23	81.05
ButaForce® at 3.0 L/ha	55.16	90.59	93.83	66.23
Weed free (weekly weeding)	100	100	100	100
Hoe weeded at 21 and 42 DAS	-6.20	98.90	99.02	89.73
No weeding	0.00	0.00	0.00	0.00
LSD (P=0.05)	2.427	0.566	0.807	0.812

SHW = Supplementary hoe weeding, DAS = Days after sowing.

to other treatments in both years of study. The highest weed dry weight was produced in weedy plots at the four periods of observations except at 21 DAS in 2018 where the dry weight was statistically on par with plots that were hoe weeded twice. All the ButaForce® rates with a supplementary hoe weeding had similar weed dry weight at all the sampling intervals. Though at 21DAS there were no significant differences between the supplementary hoe weeding and the recommended rates of ButaForce® at 3.0 L/ha at that period of sampling in both years, but all the herbicide plots that were supplemented with hoe weeding had lower weed dry weight than the recommended rates of ButaForce® at 3.0 L/ha.

Weed control efficiency

The effect of supplementary hoe weeding on the efficacy of ButaForce®on weed control efficiency of low land rice is shown in Table 7. The treatments differed significantly on weed control efficiency in both years of experimentation. Weed control efficiency was higher in plots that were hoe weeded weekly in all the sampling periods when compared to other treatments in both years of study. Weed control efficiency was lower in weedy plots throughout the sampling periods in both years of study except at 21 DAS where it was higher in plots with ButaForce® at 1.5 L/ha + SHW (21 DAS) and Hoe weeded at 21 and 42 DAS in 2018 and Hoe weeded at 21 and 42 DAS in 2019.

Rice performance

Plant height

The effect of supplementary hoe weeding on the efficacy of ButaForce® on plant height of low land rice is shown in Table 8. All the weed control treatments significantly ($P < 0.05$) affected rice height at the various sampling periods. Plants grown on weekly weeded plots grew taller than that of other treatments at 21, 42, 63 and 84 DAS. All the plots that received one supplementary hoe weeding at 21 DAS had identical plant heights in both years of study. Plots treated with ButaForce® at 3.0 L/ha at 21 DAS grew taller when compared to those plots that received one supplementary hoe weeding in both years. Plants in the weedy plots grew shorter throughout the sampling periods but it was at *par* with that of hoe weeded plots in both years of study at 21 DAS.

Leaf area index

Table 9 shows the effect of supplementary hoe weeding on the efficacy of ButaForce® on leaf area index of low land rice. The leaf area index differed significantly in all

Table 8. Effect of supplementary hoe weeding on the efficacy of ButaForce on plant height (cm) of lowland rice.

Treatment	21DAS	42 DAS	63DAS	84DAS
2018				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	21.17	40.09	53.81	62.39
ButaForce® at 2.0 L/ha + SHW (21 DAS)	22.12	42.03	54.71	62.55
ButaForce® at 2.5 L/ha + SHW (21 DAS)	24.23	43.30	55.19	65.89
ButaForce® at 3.0 L/ha	25.97	40.00	52.81	51.00
Weed free (weekly weeding)	27.37	52.83	65.00	70.21
Hoe weeded at 21 and 42 DAS	11.99	43.00	58.89	64.11
No weeding	11.33	23.00	38.72	46.00
LSD (P=0.05)	1.417	4.107	4.938	15.12
2019				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	25.31	41.8	55.14	59.23
ButaForce® at 2.0 L/ha + SHW (21 DAS)	25.33	43.8	55.71	59.53
ButaForce® at 2.5 L/ha + SHW (21 DAS)	25.59	46.2	56.19	60.22
ButaForce® at 3.0 L/ha	25.92	41.6	53.81	58.34
Weed free (weekly weeding)	27.75	51.8	68.33	69.20
Hoe weeded at 21 and 42 DAS	11.41	48.3	61.22	64.20
No weeding	11.38	35.4	40.06	44.87
LSD (P=0.05)	7.689	12.99	5.034	6.416

SHW = Supplementary hoe weeding, DAS = Days after sowing.

Table 9. Effect of supplementary hoe weeding on the efficacy of ButaForce® on leaf area index of lowland rice.

Treatment	21 DAS	42 DAS	63 DAS	84 DAS
2018				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	0.03	0.34	1.74	2.17
ButaForce® at 2.0 L/ha + SHW (21 DAS)	0.03	0.42	1.57	2.38
ButaForce® at 2.5 L/ha + SHW (21 DAS)	0.03	0.67	1.58	3.01
ButaForce® at 3.0 L/ha	0.04	0.62	0.63	0.95
Weed free (weekly weeding)	0.06	0.73	2.16	3.25
Hoe weeded at 21 and 42DAS	0.03	0.54	1.62	2.67
No weeding	0.03	0.21	0.67	1.01
LSD (P=0.05)	0.02	0.035	1.06	1.83
2019				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	0.02	0.29	0.69	1.15
ButaForce® at 2.0 L/ha + SHW (21 DAS)	0.03	0.31	0.91	1.28
ButaForce® at 2.5 L/ha + SHW (21 DAS)	0.04	0.43	1.08	1.47
ButaForce® at 3.0 L/ha	0.06	0.40	0.60	0.92
Weed free (weekly weeding)	0.06	0.52	1.2	1.62
Hoe weeded at 21 and 42 DAS	0.02	0.30	0.60	1.13
No weeding	0.01	0.18	0.48	0.33
LSD (P=0.05)	0.033	0.468	0.466	1.016

SHW = Supplementary hoe weeding, DAS = Days After Sowing.

the sampling periods in both years of study. Plots hoe weeded weekly consistently produced the greatest leaf area index at 21, 42, 63 and 84 DAS in both years of study. The lowest leaf area index was observed in

Table 10. Effect of supplementary hoe weeding on the efficacy of ButaForce® on number of tiller (no/plant).

Treatment	21 DAS	42 DAS	63 DAS	84 DAS
2018				
ButaForce® at 1.5 L/ha + SHW (21 DAS	0.47	4.67	18.00	23.00
ButaForce® at 2.0 L/ha + SHW (21 DAS	0.59	6.00	19.33	25.67
ButaForce® at 2.5 L/ha + SHW (21 DAS	0.83	6.67	21.00	27.67
ButaForce® at 3.0 L/ha	1.07	3.67	13.33	17.67
Weed free (weekly weeding)	1.1	7.67	23.00	30.00
Hoe weeded at 21 and 42 DAS	0.00	6.00	19.00	27.33
No weeding	0.00	1.67	8.67	13.00
LSD (P=0.05)	0.07	0.571	1.520	1.758
2019				
ButaForce® at 1.5 L/ha + SHW (21 DAS)	0.53	3.00	14.00	20.33
ButaForce® at 2.0 L/ha + SHW (21 DAS)	0.67	3.76	17.33	22.33
ButaForce® at 2.5 L/ha + SHW (21 DAS)	0.93	4.56	18.33	25.00
ButaForce® at 3.0 L/ha	1.20	3.34	12.67	16.00
Weed free (weekly weeding)	1.26	5.78	19.00	29.00
Hoe weeded at 21 and 42DAS	0.00	4.00	17.00	27.00
No weeding	0.00	1.75	6.00	11.00
LSD (P=0.05)	0.093	0.776	1.363	1.722

SHW = Supplementary hoe weeding, DAS = Days after sowing.

weedy plots. Although plots treated with ButaForce® at 3.0 L/ha tended to have the greatest leaf area index at 21 DAS but the leaf area index did not differed significantly from that plots that received supplemented one hoe weeding in 2018. While in 2019 plots treated with ButaForce® at 3.0 L/ha differ significantly from that of plots treated with ButaForce® at 1.5 L/ha with a supplementary hoe weeding (21 DAS) but statistically similar with that of ButaForce® at 2.0 L/ha + SHW (21DAS). In the 2018 cropping season, all the plots with one supplementary hoe weeding did not differ significantly from one another at all sampling intervals except at 42 DAS. However, in 2019 there were no significant differences among the supplementary hoe weeding throughout the sampling periods.

Number of tillers

Table 10 shows the effect of supplementary hoe weeding on the efficacy of ButaForce® on number of tillers on low land rice. There were significant differences in the number of tillers among the weed control treatments at the various intervals of sampling in both years of experimentation. The highest number of tillers was recorded in weekly weeding plots throughout the sampling intervals. The weedy plots had the lowest number of tillers at the various sampling intervals but at 21 DAS it has the same values on the number of tillers with plots that were hoe weeded twice in both sampling periods. At 21 DAS, plots that were treated at the recommended rates of ButaForce® at 3.0 L/ha had higher numbers of tillers that differed from those with supplemented hoe weeding plots.

Panicle length and panicle weight

Table 11 shows the effect of supplementary hoe weeding on the efficacy of ButaForce® on panicle length and panicle weight. There were significant differences among the weed control treatment on panicle length in both years of study. The weekly weeded plots had the longest length of panicle while the weedy plots had the shortest length in both years of study. Panicle length was longer in Plots treated with ButaForce® at 2.5 L/ha than the others supplementary hoe weeding Panicle weight was heavier in weekly weeding and lighter in weedy check. Plots treated with ButaForce® at 2.5 L/ha had a heavier weight of panicle than other plots that received one supplementary weeding.

Yield and weed index

Table 12 shows the effect of supplementary hoe weeding on the efficacy of ButaForce® on yield and weed index of low land rice. There were significant differences

Table 11. Effect of supplementary hoe weeding on the efficacy of ButaForce® on panicle length and panicle weight.

Treatment	Panicle length (cm)	Panicle weight (g/plant)
2018		
ButaForce® at1.5 L/ha + SHW (21 DAS)	22.00	8.00
ButaForce® at2.0 L/ha + SHW (21 DAS)	22.67	9.00
ButaForce®at 2.5 L/ha + SHW (21 DAS)	24.33	11.33
ButaForce® at 3.0 L/ha	19.00	6.67
Weed free (weekly weeding)	25.00	14.33
Hoe weeded at 21 and 42DAS	24.33	13.67
No weeding	10.33	5.33
LSD (P=0.05)	1.714	1.326
2019		
ButaForce® at1.5L/ha + SHW (21 DAS)	19.00	6.00
ButaForce® at2.0L/ha + SHW (21 DAS)	19.33	7.67
ButaForce® at2.5L/ha + SHW (21 DAS)	21.67	10.33
ButaForce® at 3.0L/ha	18.67	5.67
Weed free (weekly weeding)	23.33	11.33
Hoe weeded at 21 and 42 DAS	20.00	10.33
No weeding	8.00	3.33
LSD (P=0.05)	1.608	1.751

SHW = Supplementary hoe weeding, DAS = Days after sowing.

Table 12. Effect of supplementary hoe weeding on the efficacy of ButaForce® on paddy yield and weed index of lowland rice.

Treatment	Paddy yield (kg/ha)	Weed index (%)
2018		
ButaForce® at 1.5 L/ha + SHW (21 DAS)	2720	27.71
ButaForce® at 2.0 L/ha + SHW (21 DAS)	2740	27.18
ButaForce® at 2.5 L/ha + SHW (21 DAS)	2783	26.96
ButaForce® at 3.0 L/ha	2600	38.02
Weed free (weekly weeding)	2883	0.00
Hoe weeded at 21 and 42 DAS	2863	19.37
No weeding	502	86.67
LSD (P=0.05)	129.8	2.359
2019		
ButaForce® at 1.5L/ha + SHW (21 DAS)	2396.30	9.77
ButaForce® at 2.0L/ha + SHW (21 DAS)	2400.3	8.46
ButaForce® at 2.5L/ha + SHW (21 DAS)	2468.0	6.68
ButaForce® at 3.0 L/ha	2233.30	15.62
Weed free (weekly weeding)	2646.70	0.0
Hoe weeded at 21 and 42 DAS	2470.3	6.66
No weeding	425.00	83.94
LSD (P=0.05)	20. 48	1.377

SHW = Supplementary hoe weeding, DAS = Days After Sowing.

among the weed control treatments on paddy yield in both years. In 2018, the weekly weeded plots recorded significantly higher yields, which was comparable to the weeding twice plots and three supplementary hoe

Table 13. Economic evaluation of the different weed control treatments for the production of lowland rice.

Treatment	Cost of production (₦/ha)	Sale Revenue (₦/ha)	Profit (₦/ha)
2018			
ButaForce® at 1.5 L/ha + SHW (21 DAS)	271,222	1,088000	816,778
ButaForce® at 2.0 L/ha + SHW (21 DAS)	272,800	1,096000	823,200
ButaForce® at 2.5 L/ha + SHW (21 DAS)	274,378	1,113200	838,822
ButaForce at 3.0 L/ha	238,467	1,040000	801,533
Weed free (weekly weeding)	581,000	1,153200	572,200
Hoe weeded at 21 and 42 DAS	346,000	1,145200	799,200
No weeding	221,000	200,800	-20,200.
2019			
ButaForce® at 1.5 L/ha + SHW (21 DAS)	270,725	1,078335	807,610
ButaForce® at 2.0 L/ha + SHW (21 DAS)	272,300	1,080135	807,835
ButaForce® at 2.5 L/ha + SHW (21 DAS)	273,875	1,110600	836,725
ButaForce at 3.0 L/ha	238,465	1,004985	766,520
Weed free (weekly weeding)	581,000	1,191015	610,015
Hoe weeded at 21 and 42 DAS	346,000	1,111365	765,635
No weeding	221,000	191,250	-29750

SHW = Supplementary hoe weeding, DAS = Days After Sowing, calculation of sale revenue is based on N400/ kg in 2018 and 450/kg in 2019 at Choba market, Port Harcourt.

weedings at 21DAS while in 2019 it was comparable to hoe weeded plots at 21 and 42 DAS treated with ButaForce® at 2.0L/ha and 2.5 L/ha with one supplementary hoe weeding each, respectively.

Weed index differed significantly among the weed control treatments in both years. The highest weed index was recorded in weedy plots while the lowest was recorded in the weekly weeded plots. Weed index in 2018 cropping season in all the supplementary hoe weeded plots were comparable.

Economic assessment

The economic evaluation of the different weed control treatments for the production of lowland rice is presented in Table 13. The highest cost of production was recorded in plots that were manually hoe weeded weekly at 21 and 42 DAS in both years of study while the weedy plots had the lowest cost of production. Sale revenue was higher in plots weeded weekly and weeded twice at 21 and 42 DAS of both years while the lower revenue was produced in plots in the weedy check. The highest profit in both years of study was obtained in plots treated with ButaForce® at 2.5 L/ha with supplementary hoe weeding (21 DAS) while the weedy check had the lowest profit with negative values which signified no gain or loss.

DISCUSSION

The physiochemical characteristics of the soil before planting showed that the soil was sandy loam, slightly acidic with moderate organic carbon content, available P, exchangeable K, Mg, Ca and Na but was low in nitrogen content when compared to their critical levels (Ibedu et al., 1988). The low value of N obtained from the soil could be attributed to excessive rainfall, and leaching of nutrients and high temperature.

All the weed control treatment significantly reduced weed infestation judging from their lower weed density and weed dry weight when compared to weedy check probably due to their effectiveness in controlling weeds. Weed density and dry weight were low in weekly weeded plots as a result of the constant weekly hoe weeding. Unweeded plots had the highest weed density and weed dry weight probably because no treatment was applied to them. However, at 21 DAS, plots labeled as hoe weeded at 21 and 42 DAS were not weeded before collecting weed data as at that period, hence it was weedy as the no weeding plots. Among the plots that received supplementary hoe weeding, ButaForce® at 2.5 L/ha had the lowest weed density and dry weight than the others probably because it was applied at a higher rate. ButaForce® at 3.0 L/ha applied without supplementary hoe weeding could only have effective control of weeds at 21 DAS. As the rice growth stages progress there was a gradual decline of the herbicide rate in controlling weeds probably due to decrease of the herbicide concentration by gradual dissipation of the herbicide from the soil due to leaching. Although weed density and dry weight were reduced in all the plots that received supplementary hoe weeding, the reduction was more pronounced with ButaForce® at higher rates of 2.5L/.ha.

Imoloame (2017) reported similar findings that integration of herbicide rate with one supplementary hoe weeding provided better weed control in maize. Peer et al. (2013) also noted similar finding in other crops that herbicide supplemented with hoe weeding gave adequate weed control.

Generally, weed control efficiency was higher in weekly weeded plots when compared to other treatments probably as a result of constant weeding which made it free from weeds. The significantly higher plant height obtained in the weekly weeded plots could be attributed to efficient and effective weed control of the treatment. This is in line with Akbar et al. (2011) who reported taller plants in weed- free plots than in weedy plots. Leaf area index was higher in weekly weeded plots. High leaf area index indicates that the crop had a good canopy cover which shade out weeds from sunlight from penetrating the soil surface which could have stimulated weed growth. The weed - suppressing ability for other crops due to crop canopies has been reported by Busaari (1996) and Binang et al. (2016). The weedy plot had the lowest leaf area index which implies poor canopy formation which allows sunlight to penetrate the soil surface to stimulate rapid weed germination and weed growth. The highest tiller number and tiller dry weight was recorded in the weed -free plot while the lowest tiller number and tiller dry weight was recorded in the weedy plot. The number of tillers observed in the weekly weeded plots might be attributed to high weed control efficiency of the treatment as a result of reduced weed pressure.

Panicle length and panicle weight were longer and heavier in weekly weeded plots when compared to other treatments probably as a result of the weed-free condition of the plots. The rice plant was able to out-compete the weeds for available growth resources. The paddy yield was higher in weekly weeded plots probably as a result of no weed competition and higher leaf area index which produced good canopy closer for capturing sunlight for photosynthesis which promotes more yield. The high tillers produced from the weekly weeded plots also smothered the weeds giving the rice crop a competitive advantage. The weedy plots had the lowest paddy yield probably as a result of severe weed competition for water, carbon dioxide, sunlight, and space. Uncontrolled weed growth resulted to a paddy yield loss of 86.67 and 83.94% in 2018 and 2019 cropping seasons respectively. This result is in collaboration with that of Rodenburg and Johnson (2009) who reported 28- 89% yield loss in direct – seeded lowland rice due to uncontrolled weed growth. The yield variation observed in both years might be attributed to differences in rainfall. Rainfall was higher in 2018 than in 2019 and this could be the probable reason for the higher yield recorded in 2018 than in 2019.

The differences observed in the sale revenue of the various weed control treatments could be attributed to differences in yield. Although plots hoe weeded weekly and weeding twice had the highest sale revenue, their cost of production was higher than others probably as a result of expensive labour involved due to scarcity during the time of the weed control. This finding is in line with that of Adigun and Lagoke (2003) who noted that the cost of hoe weeding is expensive. The highest profit was obtained in plots treated with 2.5 L/ha +SHW, followed by 2.0 L/ha + SHW and ButaForce® at 1.5 L/ha + SHW, and ButaForce® at 3.0 L/ha without supplementary hoe weeding in both years of study probably because their cost of production was lower than that of hoe weeded plots. Imoloame (2009) also observed a similar finding that herbicide use in most crops production is more profitable than manual hoe weeding.

Conclusion

Weekly weeding plots had the lowest weed index and highest weed control efficiency. The performance of lowland rice was better in weed-free plots than other treatments. Weed suppressive ability was better in plots treated with ButaForce® at 2.5 L/ha with supplementary hoe weeding than other supplementary hoe weeding in both years; judging from it high weed control efficiency and lower weed index. SHW enhanced the yield in plots treated with ButaForce® rate lower than the recommended rate of 3.0 L/ha with about a 6% yield advantage and a 3.0% profit margin in 2018 while in 2019 they had 8.43% yield advantage and a 6.63% profit margin. Since ButaForce® at 2.5 L/ha with supplementary hoe weeding had the highest profit, it is therefore recommended to rice farmers in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adigun JA, Lagoke STO (2003). Weed control in transplanted rain fed and integrated tomato in the Nigeria Savanna. Nigerian Journal of Weed Science 16:23-29.

Akbar N, Ehsanullah KJ, Ali MA (2011). Weed management improves yield and quality of direct seeded rice. Australian Journal of Crop Science 5(6):688-694.

Akobundu IO, Ekeleme F, Agyakwa CW, Ogazie CA (2016). A hand book of West African Weeds. 3rd ed. International Institute of Tropical Agriculture, AFKAR printing and publishing company limited.

Akobundu IO (2011). Weed Control in Direct-seeded Lowland Rice under Poor water Control Conditions. Weed Research 21:273-278.

Akobundu IO (1987). Weed Science in the Tropics: Principle and practices. Wiley inter science. Chischester, pp. 33-35.

Akpokodje G, Lanco F, Erentein O (2001). The Nigerian Rice economy in a competitive World: Constraints, opportunities and strategic choices. Nigeria's Rice Economy: State of the Art. WARDA, Bouake, Cote d Ivoire. November, 2001. pp. 7-38.

Binang WB, Shiyam JO, Uko AE, Ntia J, Okpara DA, Ojikpong TO Ntun OE , Ekeleme F (2016). Influence of Gender and Spacing on Weed Smothering Potentials of Fluted Pumpkin (*Telfairia occidentalis* Hook F.) in Southeastern Nigeria. Journal of Applied Life Sciences International 8(4):1-10.

Busaari LD (1996). Influence of row spacing on weed control in soybean in southern Guinea Savanna of Nigeria. Nigerian Journal of Weed Science 9:17-23.

Hassan K, Ibrahim M, Mustapha AM (2017). Effects of spacings and Butalachlor levels on Weed Control, Growth and Yield of NERICA 1 Rice (ORYRA SATIVA L. X ORYZA GLABERRIMA L.). Journal of Agricultural Sciences 62(4):361-369.

Ibedu MA, Unambra RPA, Udealor A (1988). Soil management strategies in relation to farming system development in the south eastern agricultural zone of Nigeria. Paper presented at the National Farming system Research Workshop, Jos, Plateau State, Nigeria, pp. 26-29.

IITA (1982). Selected methods of soil and plant analysis. International Institute of Tropical Agriculture .Manual Series No. 7 Ibadan, Nigeria.

Imeokparia PO (2011). Control of Cut grass (*Leersia hexandra*) in Direct seeded Lowland Rice at Badeggi. Agronomy Seminar, Ahmadu Bello University, Zaria.

Imoloame EO (2017). Evaluation of Herbicide Mixtures and Manual Weed Control Methods in Maize (*Zea mays*) Production in the Southern Guinea Agro – Ecology of Nigeria. Nigerian Journal of Weed Science 23:73-84.

Imoloame EO (2009). Effects of pre and post emergence herbicides on weed infestation and productivity of (*Sesamum indicum* L.) in a Sudan Savanna zone of Nigeria. Ph.D Thesis, Department of Crop Production University of Maiduguri, Maiduguri, P. 145

Nwankwo CN, Ehirim CN (2010).Evaluation of aquifer characteristics and ground water quality using geoelectric method in Choba, Port Harcourt. Journal of Scholars Research Library 2:396-403.

Oduntan JB (2019). Improving the Quality of Rice Production in Nigeria through Technology Transfer. The Nigerian Voice (TNV) May 2.

Okoruwa VO, Obadaki FO, Ibrahim G (2005). Profitability of beef cattle fattening in the cosmopolitan city of Ibadan, Oyo State. Moor Journal of Agricultural Research 6(1):45-51.

Peer FA, Badrul lone BA, Qayoom SA, Khanday BA, Singh P, Singh G (2013). Effect of weed control methods on yield and yield attributes of Soyabeans. African Journal of Agricultural Research 8(48):6135-6614.

Remison SU (1997). Basic Principles of Crop Physiology. Sadoh Press (Nig.) Benin City.

Rodenburg J, Johnson DE (2009). Weed management in rice-based cropping systems in Africa. Advances in Agronomy 103:149-218.

Singh VP, Joshi N, Bisht N, Kumar A, Satyawali K, Singh RP (2016). Impact of various doses of butachlor on weed growth, crop yield of rice, microbial population and residual effect on wheat crop. International Journal of Science Environment and Technology 5(5):3106-3114.

Subramanian S, Ali AM, Kumar RJ (1991). All about weed control. Kalyani Publishers, New Delhi– 110002, India P. 315.

Udensi EU, Egbage O, Omovbude S (2017). Tolerance of egusi-melon [*Citrillus colocynthis* (L). schrad] and susceptibility of weeds to Primextra. Journal of Applied Bioscience 110:10747-10760.

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Selection efficiency of yield based drought tolerance indices to identify superior sorghum [*Sorghum bicolor* (L.) Moench] genotypes under two-contrasting environments

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Drought is the most significant environmental calamity on sorghum in Ethiopia and hence improving yield under drought is a major goal of plant breeding. This study was designed to introgress drought tolerant genes into adapted varieties through marker-assisted backcrossing and select based on tolerance indices. Sixty-one backcrossed lines and along with their nine parental lines were evaluated under full-irrigation and water-limited condition in Alpha lattice design with three replications. Yield-based drought tolerance indices including stress tolerance index (STI), mean relative performance (MRP), geometric mean productivity (GMP), harmonic mean (HM), mean productivity(MP), tolerance index (TOL), stress susceptible index(SSI), yield stability index (YSI) and yield index (YI)were calculated based on yield obtained from the two moisture regimes. Results showed that genotypes differed significantly in yield and their indices. Mean grain yields that varied widely in stressed (1.1 to 4.42 t ha^{-1}) and full-irrigation (2.25 to 5.71 t ha^{-1}) were 1.93 and 3.7 t ha^{-1}, respectively. Of the backcrossed lines, four (BC_2F_3_ETSC_16258,BC_2F_3_ETSC_16216, BC_2F_3_ETSC_16257, and BC_2F_3_ETSC_16213) were top yielding in stressed conditions with values of 4.42, 3.5, 3.1, and 2.83 t ha^{-1}, respectively. These progenies also showed consistently higher values of STI, MRP, GMP, HM, MP, YSI, and YI and lower values of SSI and TOL indicating less sensitive to stress. The correlation and principal component analyses also revealed STI, MRP, GMP, HM, MP and YI showed highly significant positive correlation among themselves and yield in both environments, indicating their suitability for identifying superior genotypes. Overall, STI, MRP, GMP, HM and MP indices can be efficiently exploited to screen drought tolerance or superior genotype(s) under both moisture conditions.

Key words: Coefficient of correlation, drought tolerance indices, principal component, clusters analysis.

INTRODUCTION

Sorghum, *Sorghum bicolor* (L.) Moench is an important cereal crop in many parts of the world grown for food, feed, and industrial purposes (Reddy, 2017; Visarada and Aruna, 2019). It is one of the most important dry land food crops grown in marginal lands and dietary food for more than half a billion poor and most food insecure people living in the sub-tropical and semi-arid regions of Africa and Asia (FAO, 2017).Sorghum is produced in intensive and commercialized in developed world with average yields of 3-5 t ha^{-1} largely used for feed, while, in the developing countries, it is grown in low-input, extensive production systems, with productivity of being 1 t ha^{-1} mostly for food (Kumar, 2016; Reddy, 2017). Ethiopia is the sixth largest producer of sorghum in the world after USA, Nigeria, Mexico, Sudan and India and the third in Africa behind Nigeria and Sudan (FAO, 2017) with sorghum contributing 16.89% of the total annual cereal grains production occupying approximately 1.9 million ha of land (CSA, 2018). Sorghum takes the third largest share of all cereals grown in Ethiopia next to tef [*Eragrostistef* (Zucc.) Trotter] and maize (*Zea mays* L.) be it in hectare or volume of total annual national production (CSA, 2018). It provides more than one third of the cereal diet and acts as a principal source of food, feed, income and beverages for millions of the resource-poor people (MoA, 2018) dwelling in marginal areas where drought is the primary production constraint (Amelework et al., 2015; Mera, 2018; Teshome and Zhang, 2019; Wagaw, 2019).

Despite the potential and multitude uses of sorghum, however, the full genetic potential of the crop cannot be harnessed particularly in tropical and sub-tropical Africa including Ethiopia because of limitations simultaneously imposed by attacks from biotic and abiotic constraints. Of the abiotic constraints, drought is an important limiting factor for sorghum production in most parts of the world including Ethiopia, ultimately influencing yield and quality (Harris et al., 2007; Kassahun et al., 2010; Sabadin et al., 2012; Reddy et al., 2014; Madhusudhana, 2015; Amelework et al., 2015, Sory et al., 2017; Mera, 2018; Teshome and Zhang, 2019; Wagaw, 2019). Yield loss due to drought in the tropics alone exceeds 17% of well-watered production, reaching up to 60% in severely affected regions (Ribaut et al., 2002; Sharma and Lavanya, 2002). In Ethiopia, where more than 50% of the total area is semi-arid, insufficient, unevenly distributed, and unpredictable rainfall is usually experienced in drier parts of the country (Amelework et al., 2015; Mera, 2018; Teshome and Zhang, 2019).It is manifested by delay in onset, dry spell after sowing, drought during critical crop stage and too early stop. It is frequently observed that drought is occurring at more frequent intervals-every two years during recent years. For instance, between 1960 and 1990 there were six droughts in the country, but between 1990 and 2014 there were nine droughts (Mera, 2018) caused up to complete annihilation of sorghum and other crops affecting millions of people. This showed that climate change makes increasing production much more challenging. Recent reports also declare that the intensity and frequency of droughts are expected to increase, resulting in decreased food production and food security and increased vulnerability of the crop to drought (Bates et al., 2008; Wassmann et al., 2009; Mera, 2018; Teshome and Zhang, 2019).

Among the drought management strategies, genetic manipulation of the crop to improve tolerance is preferred because of its sustainability and feasibility particularly to the resource-poor (Singh, 2002; Keneni, 2007).Breeding for drought-tolerant crops largely depends on the availability of the genetic resources for tolerance, reliable screening techniques, identification of genetic components of tolerance (Blum, 2011), successful genetic manipulation of the desired genetic backgrounds, and ultimate development of drought-tolerant cultivars with acceptable agronomic and quality-related traits (Araus and Cairns, 2014). The relative yield performance of genotypes under drought stressed and non-stressed environments can be used as an indicator to identify drought resistant varieties in breeding program for drought prone areas (Raman et al., 2012; Mohammadi, 2016). Based on their comparative yield performance in stress and non-stress environments genotypes were categorized in four groups; genotypes with high performance under both moisture regimes (group A), high yield in non-stress conditions (group B), high yield in stress conditions (group C), and low yield under both moisture regimes (group D) (Fernandez, 1992). In this regard, several drought indices that are based on drought resistance or susceptibility of genotypes have been suggested and computed between yield under stress and optimal conditions. Drought indices which provide a measure of drought based on loss of yield under drought conditions in comparison to normal conditions have been used for screening drought tolerant genotypes.

Thus, many authors have been reported that the relative merits of different indices for screening of genotypes to drought based on their comparative yield performance in stress and non-stress environments. These include; stress tolerance index (STI) and geometric mean productivity (GMP) (Fernandez 1992), stress susceptibility index (SSI) (Fischer and Maurer, 1978), tolerance index (TOL) (Hossain et al., 1990), mean productivity (MP) (Rosielle and Hamblin, 1981), yield index (YI) (Gavuzzi et al., 1997), yield stability index(YSI) (Bouslama and Schapaugh, 1984), harmonic mean (HM) (Schneider et al., 1997), and mean relative performance (MRP) (Osmanzai, 1994). However, the different indices have different levels of precision, making comparisons between genotypes difficult. It is generally presumed that good performance under both irrigated and drought conditions leads to high values of STI, MP, HM, MRP, GMP, YSI and YI and generally low values of

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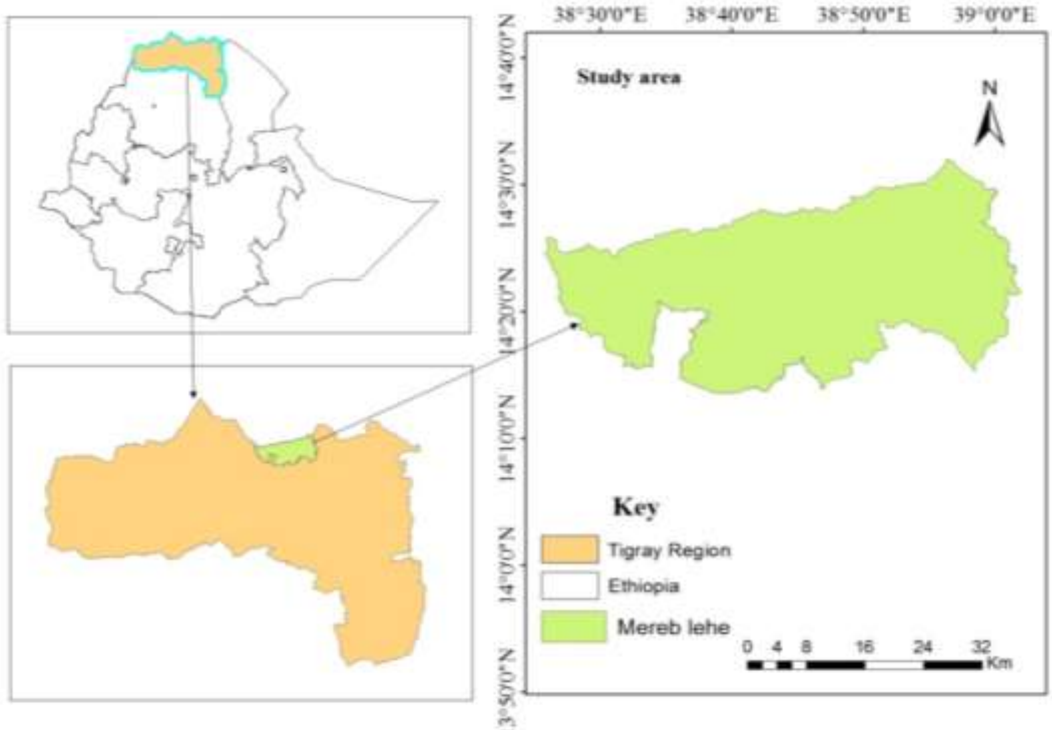

Figure 1. Map of Mereblekhe district in Tigrai Regional State, Ethiopia.

TOL and SSI. To improve sorghum yield and its stability in stress environments, there is a need to identify selection indices able to distinguish high yielding sorghum genotypes in these conditions. However, very limited work has been reported for sorghum from Ethiopia. The study was, therefore, aimed at introgression of drought tolerance genes into adapted varieties through marker-assisted backcrossing and assesses the efficiency of indices to identify drought tolerance in sorghum, so that suitable lines can be recommended for cultivation in drought prone areas of Ethiopia.

MATERIALS AND METHODS

Description of study area

Field experiments were conducted in Rama Kebele of Mereblekhe District in central zone of Tigrai, Ethiopia (Figure 1). The location was selected based on the potential of sorghum grown and availability of irrigation. The site is situated at 14° 23' 39″ N latitude and 038° 48' 90″ E longitude. Rama is found at an altitude of 1389 meter above sea level, with average minimum and maximum temperatures ranging from 22 to 38°C, respectively, during the study time (December 2018 to May 2019).

Genetic materials

The parental lines used for this backcrossing program were one donor parent "B35" and eight recurrent parents which are released varieties and known farmers' cultivars (Tseadachimure and Wediaker [local landraces]; Dekeba, Gambella 1107, Macia, Meko, Melkam, and Teshale [released varieties] (Table 1). The donor parent is known for post-flowering drought tolerant and it has been used as source of tolerant genes to drought by the inter-intra-national sorghum breeding programmes. B35 is a 3-gene dwarf genotype, BC_1 derivative of IS12555 accession, a durra from Ethiopian and is known for its stay green (Rosenow et al., 1983, 2002) with a type-A stay-green-delayed onset of leaf senescence (Thomas and Smart, 1993; Thomas and Howarth, 2000). It is well characterized for its stay green and several research groups (Tuinstra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu et al., 2000; Sanchez et al., 2002) have identified a number of stay green QTL involving B35. B35 is early maturing, long in stature, has short compact panicle with copious number of infertile branches; purple genotype with small seeds covered by glumes, dry leaf midrib and relatively low yield potential (Srinivas et al., 2009; Kassahun et al., 2010). The recurrent parents are generally high yielding under optimum moisture conditions (MoA, 2018) and popular amongst the farmers but susceptible to terminal drought.

Development of backcross lines

A series of crosses and backcrosses were performed to introgress drought tolerant genes from the known donor parent (as pollen source) into adapted varieties (seed parents). The donor parent was crossed to the selected adapted varieties to generate F_1 plants using hand pollination method at Melkassa Agricultural Research Center (MARC), Ethiopia. The F_1 plants were backcrossed to the respective recurrent parents to generate BC_1F_1 progenies. Then after the progenies selected was backcrossed to the recurrent parent to generate BC_2F_1 following by twice selfing (BC_2F_3). The generated sixty-one BC_2F_3 progenies and nine parental lines were evaluated for

Table 1. The genotypes used for marker-assisted backcrossing.

S/N	Variety	Pedigree	Year of release	Center of release
1	Melkam	WSV-387	2009	MelkassaARC
2	Teshale	3443-2-0P	2002	Srinka/MelkassaARC
3	Gambella 1107	Gambella 1107	1976	Melkassa ARC
4	Dekeba	ICSR 24004	2012	Melkassa ARC
5	Macia	Macia	2007	Melkassa ARC
6	Meko-1	M-36121	1997	Melkassa ARC
7	Tseadachimure	Local	-	-
8	Wediaker	Local	-	-
9	B35	IS12555	-	

ARC= Agricultural Research Center.

their drought tolerant and other agronomic characteristics.

Experimental design and treatments

The field trials were consisted of 61 BC_2F_3, one donor parent and eight recurrent parents. The field trials were conducted under well-watered and water-limited conditions arranged in an incomplete block design (Alpha lattice design) with three replications. The well-watered trial was irrigated well throughout the season, so that, essentially, no moisture stress occurred at any stage of the crop development. Conversely, the limited irrigation (stress) trial was irrigated well during the early growth stages with irrigation withheld after anthesis. These conditions are ideal for evaluating the expression of stay green traits under terminal moisture-deficit condition and to study its relation with other important agronomic characters. The trials were planted in the same date, and adjacent to each other. The experimental units were two-row, with each row 4 m long, plant to plant spacing was 0.15 and 0.75 m space between rows. Fertilizer (NPS) was applied at a rate of 100 kg ha^{-1} at planting and urea at rate of 50 kg ha^{-1} on split based (at planting and knee height). All agronomic management practices other than the treatment were applied uniformly to ensure good crop stand. The crop was protected from leaf feeding/sucking insect pests such as aphids, stem borers and fall armyworm by following the recommended plant protection measures. The insecticides used were Karate 5% EC, Darate 5%, and Bestfield 360 EC based on the manufacturer recommendation rate that is, 300, 300, and 400 mm ha^{-1}, respectively.

Data collection

The yield of sorghum lines were obtained from the stressed and non-stressed irrigation conditions to screen superior genotypes based on the different henceforth drought indices.

(1) Stress susceptibility index (SSI) (Fischer and Maurer, 1978)

Stress Susceptibility Index (SSI) $= \frac{[1-\left(\frac{Ys}{Yp}\right)]}{1-SI}$;

$$SI=[1-\left(\frac{\overline{Y}s}{\overline{Y}p}\right)]$$

(2) Mean relative performance (MRP) (Osmanzai, 1994)

$$MRP = \frac{Ys}{\overline{\overline{Ys}}} + \frac{Yp}{\overline{\overline{YP}}}$$

(3) Tolerance index (TOL) (Hossain et al., 1990)

Yp-Ys

(4) Mean productivity (MP) (Rosielle and Hamblin, 1981)

$$MP = \frac{Yp + Ys}{2}$$

(5) Harmonic mean (HM) (Schneider et al., 1997)

$$HM = \frac{2(Yp*Ys)}{Yp+Ys}$$

(6) Geometric mean productivity (GMP) (Fernandez, 1992)

$$GMP = \sqrt{(Yp)(Ys)}$$

(7) Stress tolerance index (STI) (Fernandez, 1992)

$$STI = \frac{(Yp)(Ys)}{(\overline{Yp})^2}$$

(8) Yield index (YI) (Gavuzzi et al., 1997)

$$YI = \frac{Ys}{\overline{\overline{Ys}}}$$

(9) Yield stability index (YSI) (Bouslama and Schapaugh, 1984)

$$YSI = \frac{Ys}{Yp}$$

Where, Ys = yield in stress conditions, Yp = yield in irrigated conditions, $\overline{Y}$s= mean yield of all genotypes under stress conditions, $\overline{Y}$p = mean yield of all genotypes in irrigated conditions and SI = Stress intensity.

Data analysis

The analysis of variance, coefficients of correlations, principal component (PC) analysis and cluster analysis were carried out using the R software version 3.6.1 (R Core Team, 2019).Genotype differences in yield and indices were analysed by residual maximum likelihood algorithm (ReML) as suggested (Patterson and Thompson, 1971) analysis using R. The relevant number of clusters in the data set was determined by an R package NbClust, available from the comprehensive R archive network (CRAN) at http://CRAN.R-project.org/package=NbClust (Charrad et al., 2014).

RESULTS AND DISCUSSION

Yield performance

The analysis of variance for grain yield grown under both moisture regimes indicated the presence of a considerable genotypic variation, indicating differential responses to different environmental conditions, thereby suggesting the possibility of selecting better-performing genotypes under both production environments. Mean grain yields that varied widely in water-limited (1.1 for BC_2F_3_ETSC_16218 to 4.42 t ha^{-1} for BC_2F_3_ETSC_16258) and full-irrigation conditions (2.25 for B35 to 5.71 t ha^{-1} for Dekeba) were 1.93 and 3.7 t ha^{-1}, respectively (Table 2). This showed that an increase of 47.8 % in yield productivity under the later compared to the former. The grain yield under optimum condition revealed that most of recurrent parents showed highest yield compared to the majority of the developed lines. Among the developed lines with higher yield and statistically similar to the recurrent parents were BC_2F_3_ETSC_16214, BC_2F_3_ETSC_16216, BC_2F_3_ETSC_16251, BC_2F_3_ETSC_16235, BC_2F_3_ETSC_16139, BC_2F_3_ETSC_16258, BC_2F_3_ETSC_16257, BC_2F_3_ETSC_16242, and BC_2F_3_ETSC_16223 indicating the potential of these lines under optimum production environments. On the other hand, the developed backcrossed lines showed highest grain yield under stressed condition. Of the 61 lines, four were the top yielding under stressed conditions; BC_2F_3_ETSC_16258, BC_2F_3_ETSC_16216, BC_2F_3_ETSC_16257, and BC_2F_3_ETSC_16213 with a yield of 4.42, 3.5, 3.1, and 2.83 t ha^{-1}, respectively. The yield under water-stressed conditions (Ys) had good association with yield obtained under non-stressed conditions (Yp), indicating the possibilities of obtaining potential lines for both moisture regimes. For example, backcrossed lines with a good yield performance under both irrigation conditions were BC_2F_3_ETSC_16258, BC_2F_3_ETSC_16216, BC_2F_3_ETSC_16257, BC_2F_3_ETSC_16251, and BC_2F_3_ETSC_16141 (Table 2). The consistence performances of the backcrossed lines in the two contrasting (non-stress *vis-à-vis* stress) environments represent very nearly the same character, determined nearly by the same set of genes (Falconer, 1989). This may probably have the advantage of the possibilities to forecast the performance of genotypes under one condition on the basis of performance obtained under another and can assist breeders in deciding variety development and allocation of the scarce resources (Keneni, 2007). Therefore, indirect selection for such conditions based on the results of optimum conditions may be efficient (Brennan and Byth, 1979; Rosielle and Hamblin, 1981). However, this needs to be supported by a large data from the multi-location-year experiments as many authors disproved the concept that stipulates cultivars selected under favorable environments also suitable to the unfavorable ones (Ceccarelli and Grando, 1996; Banziger and Edmeades, 1997; Banziger et al., 1997; Banziger and Lafitte, 1997) because it is practically impossible to collect together genes responsible for superior performance in all environments into a single genotype (Annicchiarico, 2002).

Drought tolerance indices

The ANOVA for the quantitative selection indices differed significantly for all indices namely SSI, MRP, MP, HM, GMP, STI, YI, TOL and YSI (Table 2). The mean values of each tolerance indices ranged from the highest 1.61 for BC_2F_3_ETSC_16235 to the lowest 0.12 for BC_2F_3_ETSC_16258, 3.48 for BC_2F_3_ETSC_16258 to 1.19 for B35, 4.5 for BC_2F_3_ETSC_16258 to 1.7 for B35, 4.52 for BC_2F_3_ETSC_16258 to 1.47 for B35, 4.52 for BC_2F_3_ETSC_16235 to 1.58 for B35, 1.72 for BC_2F_3_ETSC_16258 to 0.18 for BC_2F_3_ETSC_16215, 2.22 for BC_2F_3_ETSC_16258 to 0.54 for BC_2F_3_ETSC_16218, 3.33 for BC_2F_3_ETSC_16235 to 0.42 for BC_2F_3_ETSC_16258, and 4.27 for BC_2F_3_ETSC_16235 to 0.98 for BC_2F_3_ETSC_16258 in that order. The highest values of SSI and TOL belonged to lines; BC_2F_3_ETSC_16235, BC_2F_3_ETSC_16218, BC_2F_3_ETSC_16238, BC_2F_3_ETSC_16249, BC_2F_3_ETSC_16242, BC_2F_3_ETSC_16217 and BC_2F_3_ETSC_16139, whereas lower values related to BC_2F_3_ETSC_16258, BC_2F_3_ETSC_16229, BC_2F_3_ETSC_16247, BC_2F_3_ETSC_16213, BC_2F_3_ETSC_16252, BC_2F_3_ETSC_16216, BC_2F_3_ETSC_16149, BC_2F_3_ETSC_16239, BC_2F_3_ETSC_16230, and BC_2F_3_ETSC_16227. For instance, line BC_2F_3_ETSC_16235 with both greater SSI and TOL values had grain yield of 4.68 and 1.32 t ha^{-1} under full-irrigation and water-limited, respectively; therefore, was identified as highly sensitive to moisture stress after anthesis. In contrast, the lower value of SSI and TOL belonged to BC_2F_3_ETSC_16258 with grain yield of 4.57 t ha^{-1} under full-irrigation and 4.42 t ha^{-1} in water-limited condition. Therefore, this line is less sensitive to stress. This means that the greater SSI and TOL values, the greater sensitivity to stress, thus a smaller value of these indices is favored, agreeing with other reports (Rosielle and Hamblin, 1981; Ghasem and

Table 2. Estimates of stress tolerance attributes under full-irrigation and water-limited based on yield of seventy sorghum genotypes.

SN	Genotypes	Yp	Ys	SSI	TOL	MRP	MP	HM	GMP	STI	YI	YSI
1	B35	2.25	1.12	1.11	1.12	1.19	1.70	1.47	1.58	0.25	0.56	0.50
2	BC_2F_3_ETSC_16139	4.61	1.65	1.32	2.99	2.12	3.15	2.30	2.67	0.57	0.83	0.40
3	BC_2F_3_ETSC_16140	3.47	1.47	1.35	2.04	1.73	2.51	2.02	2.25	0.45	0.74	0.39
4	BC_2F_3_ETSC_16141	4.06	2.47	0.82	1.68	2.34	3.23	3.08	3.16	0.88	1.24	0.63
5	BC_2F_3_ETSC_16142	5.43	2.35	1.20	3.15	2.69	3.91	3.15	3.49	0.91	1.18	0.46
6	BC_2F_3_ETSC_16143	2.69	1.92	0.70	0.87	1.72	2.32	2.27	2.30	0.43	0.96	0.68
7	BC_2F_3_ETSC_16144	4.02	2.37	0.88	1.63	2.28	3.16	2.86	3.01	0.77	1.19	0.60
8	BC_2F_3_ETSC_16145	2.95	1.78	0.77	1.10	1.71	2.37	2.20	2.28	0.37	0.89	0.66
9	BC_2F_3_ETSC_16146	3.36	1.52	1.09	1.86	1.69	2.45	1.94	2.17	0.39	0.76	0.51
10	BC_2F_3_ETSC_16147	3.59	1.62	1.24	2.06	1.80	2.60	2.11	2.33	0.45	0.81	0.44
11	BC_2F_3_ETSC_16148	3.54	1.90	0.98	1.65	1.91	2.69	2.42	2.54	0.57	0.95	0.56
12	BC_2F_3_ETSC_16149	3.10	2.12	0.58	1.06	1.93	2.62	2.49	2.56	0.54	1.07	0.74
13	BC_2F_3_ETSC_16150	2.95	1.13	1.32	1.89	1.39	2.05	1.48	1.72	0.30	0.56	0.40
14	BC_2F_3_ETSC_16210	3.23	1.55	1.11	1.70	1.68	2.41	2.07	2.23	0.41	0.77	0.50
15	BC_2F_3_ETSC_16211	2.77	1.89	0.82	1.01	1.71	2.32	2.24	2.29	0.43	0.95	0.63
16	BC_2F_3_ETSC_16212	4.16	1.99	1.08	2.27	2.14	3.06	2.63	2.83	0.68	1.00	0.51
17	BC_2F_3_ETSC_16213	3.94	2.83	0.46	1.27	2.47	3.32	3.03	3.15	0.80	1.42	0.79
18	BC_2F_3_ETSC_16214	4.93	2.06	1.25	2.98	2.36	3.44	2.78	3.08	0.82	1.03	0.43
19	BC_2F_3_ETSC_16215	2.36	1.28	1.10	1.26	1.26	1.76	1.57	1.66	0.18	0.64	0.50
20	BC_2F_3_ETSC_16216	4.76	3.50	0.52	1.31	3.05	4.11	3.93	4.02	1.32	1.76	0.77
21	BC_2F_3_ETSC_16217	4.03	1.61	1.32	2.56	1.89	2.78	2.13	2.41	0.47	0.80	0.40
22	BC_2F_3_ETSC_16218	3.36	1.07	1.48	2.24	1.47	2.23	1.64	1.90	0.28	0.54	0.33
23	BC_2F_3_ETSC_16219	4.23	1.76	1.25	2.42	2.07	3.02	2.39	2.67	0.58	0.88	0.44
24	BC_2F_3_ETSC_16220	3.96	1.89	1.18	2.03	2.08	2.98	2.52	2.74	0.57	0.95	0.47
25	BC_2F_3_ETSC_16221	3.35	2.46	0.58	0.85	2.18	2.93	2.80	2.86	0.64	1.24	0.74
26	BC_2F_3_ETSC_16222	3.28	1.51	0.98	1.61	1.68	2.42	2.05	2.22	0.41	0.75	0.56
27	BC_2F_3_ETSC_16223	4.29	1.85	1.15	2.35	2.13	3.10	2.53	2.79	0.64	0.93	0.48
28	BC_2F_3_ETSC_16224	3.63	1.17	1.36	2.42	1.58	2.40	1.63	1.95	0.29	0.59	0.39
29	BC_2F_3_ETSC_16225	3.38	1.74	1.07	1.61	1.80	2.56	2.22	2.38	0.45	0.87	0.52
30	BC_2F_3_ETSC_16226	4.15	2.21	0.95	1.87	2.28	3.22	2.76	2.97	0.73	1.11	0.57
31	BC_2F_3_ETSC_16227	2.88	1.79	0.70	0.94	1.71	2.36	2.09	2.21	0.43	0.90	0.68
32	BC_2F_3_ETSC_16228	4.22	1.95	1.17	2.26	2.17	3.12	2.60	2.85	0.62	0.98	0.47
33	BC_2F_3_ETSC_16229	3.14	2.45	0.36	0.59	2.09	2.79	2.70	2.74	0.56	1.24	0.84
34	BC_2F_3_ETSC_16230	2.80	1.78	0.68	0.98	1.67	2.30	2.13	2.21	0.36	0.89	0.69
35	BC_2F_3_ETSC_16231	3.06	1.61	1.00	1.37	1.65	2.34	2.05	2.18	0.39	0.81	0.55
36	BC_2F_3_ETSC_16232	2.72	1.69	0.75	1.02	1.58	2.18	2.03	2.10	0.32	0.85	0.66
37	BC_2F_3_ETSC_16233	3.05	1.29	1.05	1.71	1.52	2.21	1.73	1.94	0.35	0.65	0.53
38	BC_2F_3_ETSC_16234	3.43	1.60	1.10	1.82	1.78	2.56	2.11	2.32	0.43	0.80	0.50
39	BC_2F_3_ETSC_16235	4.68	1.33	1.61	3.33	1.98	3.03	2.00	2.45	0.55	0.67	0.27
40	BC_2F_3_ETSC_16236	3.33	1.37	1.34	2.02	1.62	2.37	1.91	2.13	0.40	0.69	0.39
41	BC_2F_3_ETSC_16237	3.51	1.36	1.32	2.17	1.66	2.45	1.88	2.13	0.38	0.68	0.40
42	BC_2F_3_ETSC_16238	3.29	1.11	1.48	2.28	1.44	2.16	1.61	1.86	0.29	0.55	0.33
43	BC_2F_3_ETSC_16239	2.93	2.00	0.62	0.86	1.80	2.45	2.31	2.37	0.53	1.00	0.72
44	BC_2F_3_ETSC_16240	3.64	1.92	1.07	1.76	1.96	2.76	2.47	2.60	0.62	0.96	0.52
45	BC_2F_3_ETSC_16241	3.48	1.96	1.01	1.61	1.93	2.70	2.42	2.56	0.54	0.98	0.54
46	BC_2F_3_ETSC_16242	4.32	1.50	1.33	2.83	1.91	2.87	2.19	2.50	0.49	0.75	0.40
47	BC_2F_3_ETSC_16243	2.89	1.70	0.85	1.16	1.64	2.28	2.05	2.16	0.34	0.86	0.62
48	BC_2F_3_ETSC_16244	3.15	1.27	1.39	1.93	1.50	2.20	1.75	1.95	0.33	0.63	0.38
49	BC_2F_3_ETSC_16245	3.58	1.67	1.18	1.89	1.83	2.63	2.24	2.42	0.46	0.83	0.47
50	BC_2F_3_ETSC_16246	3.67	1.60	1.22	2.07	1.84	2.66	2.16	2.39	0.52	0.80	0.45
51	BC_2F_3_ETSC_16247	2.72	2.22	0.45	0.48	1.87	2.47	2.39	2.43	0.47	1.11	0.80

Table 2. Contd

52	BC_2F_3_ETSC_16248	3.66	2.44	0.74	1.20	2.26	3.08	2.84	2.95	0.78	1.22	0.66
53	BC_2F_3_ETSC_16249	3.65	1.42	1.36	2.29	1.74	2.56	1.91	2.18	0.43	0.71	0.38
54	BC_2F_3_ETSC_16250	3.41	1.45	1.27	1.95	1.68	2.45	2.00	2.20	0.41	0.73	0.42
55	BC_2F_3_ETSC_16251	4.70	2.26	1.21	2.48	2.44	3.49	2.99	3.23	0.86	1.14	0.46
56	BC_2F_3_ETSC_16252	3.07	2.32	0.46	0.70	2.04	2.74	2.58	2.65	0.58	1.17	0.79
57	BC_2F_3_ETSC_16253	3.75	2.24	0.83	1.43	2.11	2.94	2.70	2.81	0.63	1.13	0.62
58	BC_2F_3_ETSC_16254	2.49	1.52	0.92	0.95	1.42	1.97	1.85	1.90	0.29	0.76	0.58
59	BC_2F_3_ETSC_16255	3.65	1.48	1.29	2.05	1.74	2.56	2.04	2.27	0.44	0.74	0.42
60	BC_2F_3_ETSC_16256	3.76	2.01	1.06	1.69	2.04	2.88	2.46	2.64	0.70	1.01	0.52
61	BC_2F_3_ETSC_16257	4.52	3.09	0.75	1.37	2.77	3.77	3.63	3.69	1.20	1.55	0.66
62	BC_2F_3_ETSC_16258	4.57	4.42	0.12	0.14	3.48	4.49	4.52	4.52	1.72	2.22	0.95
63	Dekeba	5.71	2.82	1.07	2.89	3.01	4.29	3.72	3.98	1.16	1.42	0.52
64	Gambella1107	4.66	2.18	1.26	2.38	2.40	3.45	2.98	3.21	0.87	1.10	0.43
65	Macia	4.75	2.62	0.92	2.11	2.60	3.66	3.32	3.48	0.95	1.32	0.58
66	Meko	4.85	2.55	0.88	2.29	2.59	3.67	3.27	3.46	0.92	1.28	0.60
67	Melkam	4.38	1.99	1.12	2.32	2.20	3.17	2.68	2.91	0.64	1.00	0.49
68	Teshale	3.42	2.29	0.79	1.15	2.09	2.84	2.74	2.79	0.65	1.15	0.64
69	Tseadachimure	4.25	2.54	0.75	1.65	2.45	3.40	3.13	3.25	0.83	1.28	0.66
70	Wediaker	5.18	2.66	1.04	2.57	2.74	3.88	3.53	3.71	1.07	1.34	0.53
	Mean	3.7	1.9	1	1.8	1.99	2.8	2.4	2.6	0.6	0.97	0.54
	LSD	1.56	1.03	0.67	1.7	0.73	1	1.02	0.98	0.48	0.52	0.3
	CV (%)	23.6	29.5	37.4	30.4	20	19.7	23.2	20.7	20.7	29.6	31.2

Farshadfar, 2015).On the other hand, selection based on TOL with minimum yield reduction under stress condition in comparison with non-stress condition failed to identify the most tolerant genotypes (Farshadfar et al., 2013). Similar to TOL, stress susceptibility index (SSI), genotypes with highest values were considered as genotypes with high drought susceptibility and poor yield stability in both moisture regimes. With regard to yield stability index (YSI) backcrossed lines with higher values were related to BC_2F_3_ETSC_16258, BC_2F_3_ETSC_16229, BC_2F_3_ETSC_16143, BC_2F_3_ETSC_16216, BC_2F_3_ETSC_16249, BC_2F_3_ETSC_16141, BC_2F_3_ETSC_16247, and BC_2F_3_ETSC_16221 and were also the most stable under stress and non-stress conditions. The lowest values of SSI and TOL as well as the highest values of YSI indicated that SSI, TOL, and YSI indices were able to identify genotypes with higher yields under drought stress rather than under non-stress conditions.

The tolerance indices MRP, GMP, STI, HM, MP and YI measure the higher stress tolerance and yield potential. Accordingly, the highest and consistent values across all indices belonged to the four backcrossed linesBC_2F_3_ETSC_16258, BC_2F_3_ETSC_16216, BC_2F_3_ETSC_16257, and BC_2F_3_ETSC_16142 and therefore, they were the most tolerant progenies based on all quantitative indices. These lines were the most tolerant genotypes and also had lower values of SSI and TOL (Table 2). Conversely, the lowest values for all quantitative indices related to B35, BC_2F_3_ETSC_16215, BC_2F_3_ETSC_16150, BC_2F_3_ETSC_16254, BC_2F_3_ETSC_16238, BC_2F_3_ETSC_16218, BC_2F_3_ETSC_16233 and BC_2F_3_ETSC_16244 and, therefore, some of them were stress sensitive and the other stress tolerant (B35) but with low yield potential under both moisture regimes. Generally, this study showed that quantitative indices (MRP, GMP, STI, HM, MP, and YI) were comparable for identifying superior sorghum genotypes under both environments. Different researches have also used different indices for selecting tolerant genotypes in various crops. For instances, SSI and GMP were preferable in common bean (Ramirez and Kelly, 1998), STI and GMP in maize (Khallili et al., 2004) and mung bean (Fernandez, 1992), durum wheat (Nouri et al., 2011; Mohammadi, 2016), safflower (Majidi et al., 2011; Bahramiet al., 2014), HM, YI, MP, GMP, STI in bread wheat (Khakwani et al., 2011; Dorostkar et al., 2015; Ghasemi and Farshadfar, 2015; Amare et al., 2019), Barley (Nazari and Pakniyat, 2010) and sorghum (Sory et al., 2017) implies that they were useful in identifying lines that yield well under well-watered and also relatively well in water-limited condition.

Interrelationships of the drought tolerance indices

To determine the most desirable drought tolerance criteria, the correlation coefficient between grain yield

Table 3. Correlation coefficients (r) between grain yield of sorghum genotypes under non-stressed and stressed conditions and among selection indices.

Trait	Yp	Ys	SSI	MRP	TOL	MP	HM	GMP	STI	YI
Yp										
Ys	0.52**									
SSI	0.18NS	-0.70**								
MRP	0.82**	0.91**	-0.38**							
TOL	0.66**	-0.29*	0.82**	0.12NS						
MP	0.91**	0.83**	-0.23**	0.99**	0.28*					
HM	0.71**	0.96**	-0.52**	0.98**	-0.05NS	0.94**				
GMP	0.81**	0.92**	-0.40**	1.00**	0.10NS	0.98**	0.99**			
STI	0.76**	0.92**	-0.40**	0.98**	0.05NS	0.95**	0.97**	0.98**		
YI	0.52**	1.00**	-0.70**	0.91**	-0.29*	0.83**	0.96**	0.92**	0.92**	
YSI	-0.20NS	0.72**	-0.97**	0.38**	-0.85**	0.22NS	0.53**	0.40**	0.42**	0.71**

**, * = significant at 0.01 and 0.05 respectively, NS = non-significant, STI = stress tolerance index, MRP = mean relative performance, GMP = geometric mean productivity, HM = harmonic mean, MP= mean productivity, TOL = tolerance index, SSI = stress susceptible index, YSI = yield stability index YI = yield index, Yp = mean grain yield under full-irrigation, Ys = mean grain yield underwater-limited condition.

under the well-watered (Yp), water-limited conditions (Ys), and the quantitative indices of drought tolerance were determined (Table 3). The results of the correlation analysis showed that both positive and negative associations, showing that some of the indices are generally similar and dissimilar in genotypic ranking, respectively. The correlation coefficients of grain yield under non-stressed condition (Yp) showed significant positive correlation with grain yield in the stressed environment (Ys) and all of the selection indices except for SSI and YSI. The significant positive correlations between non-stressed and stressed conditions indicated that genotypes that performed well under non-stress also performed well under stress. No significant correlations were observed between Yp and that of SSI and YSI. In the same manner, grain yield under Ys was significantly and positively correlated with all of the indices except for SSI and TOL which were significant negative correlation (Table 3). A positive correlation between TOL and Yp and the negative correlation between TOL and Ys suggested that selection based on TOL will lead to reduction of yield under well-watered conditions. Among the drought tolerant indices that showed strong positive correlation under both non-stress and stress irrigation include; MRP (r= 0.82; 0.91), MP (r=0.91; 0.83), HM (r=0.71; 0.96), GMP (r=0.81; 92), STI (r=0.76; 0.92) and YI (r=0.52; 1.00), respectively. This indicated that the six indices were comparably effective for selecting and predicting better grain-yielding genotypes under both moisture regimes, corroborating with previous reports (Ezatollah et al., 2012; Farshadfar et al., 2013; Sardouie-Nasab et al., 2015; Darzi-Ramandi et al., 2016). The negative associations of SSI and TOL with grain yield under stress indicated that genotypes with low SSI and TOL values had lower yield differences between non-stress and stress environments (Ceccarelli et al., 1998; Rizza et al., 2004; Mehammadi, 2016).SSI showed significant negative correlation with all selection indices except for TOL that showed significant positive association. Moreover, SSI showed a negative correlation with Ys while no significant correlation was detected between Yp and SSI. Thus, SSI index is suitable for identification of genotypes with low yield and tolerance to drought stress (Kharrazi and Rad, 2011). TOL had significant positive association with MP and significant negative correlation with YI and YSI. TOL was not strongly correlated with indices MRP, GMP, HM, YI, MP and STI. Thus, TOL and SSI ranked differently from the other selection. MRP showed strong significant correlation with MP, HM, GMP, STI, YI and YSI but weak with TOL. Indices of MP, YI, STI, GMP, MRP, and HM showed the existence of strong positive correlation among

Table 4. Eigenvalue, variances and eigenvectors on the first five principal components for seventy sorghum genotypes to different drought tolerant selection indices grown in under full water and stressed water condition.

Parameter	Principal components (PCs)				
	PC_1	**PC_2**	**PC_3**	**PC_4**	**PC_5**
Eigenvalue	7.736	3.129	0.082	0.023	0.014
Proportion (%)	70.3	28.4	0.7	0.2	0.1
Cumulative (%)	70.3	98. 8	99.5	99.7	99.9
Characters			Eigenvector		
Yp	0.690	0.719	-0.081	-0.006	0.008
Ys	0.977	-0.207	0.033	0.003	0.016
SSI	-0.558	0.804	0.187	0.080	0.002
MRP	0.978	0.205	-0.016	-0.004	0.012
TOL	-0.084	0.988	-0.109	0.031	0.041
MP	0.930	0.364	-0.036	-0.009	0.007
HM	0.995	0.037	0.010	0.040	-0.073
GMP	0.982	0.182	-0.008	0.024	-0.045
STI	0.972	0.148	0.156	-0.063	0.039
YI	0.977	-0.206	0.030	0.003	0.014
YSI	0.560	-0.820	-0.029	0.099	0.051

Stress susceptibility index (SSI), yield stability index (YSI), stress tolerance (TOL), mean productivity (MP), mean relative performance (MRP), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean (HM), yield index (YI), and seed yield of sorghum genotypes under non-stress (Yp) and stress (Ys) conditions.

themselves showing their similarity between these indices for genotypes ranking. According to Farshadfar et al., (2001) most suitable indices for selecting stress-tolerant cultivars is an indices which has a relatively strong correlation with the seed yield under stress and non-stress conditions. Therefore, evaluating correlations between stress tolerance indices and the seed yield in both environments can lead to identification of the most suitable indices. Close correlation between MRP and GMP (r = 1.0) that indicates these two indices are identical in genotypes ranking. YSI had strong and positive correlation with HM, GMP, STI and YI but negatively with SSI and TOL. Likewise, the highest correlation (r = 1.00) was observed between mean grain yield of genotypes under stress (Ys) and yield index (YI). So that consistent correlations were also found between SSI and TOL showing they can be used interchangeably for screening under stress condition. In conclusion, the strong significant positive correlations between HMP, GMP, MP and STI indices showed genotypes with a good performance in both conditions (Yp and Ys) displaying that they are the best indices for identification of superior genotypes agreeing with reports of Mardeh et al. (2006), Golabadi et al. (2006) and Farshadfar et al. (2012).

Principal components analysis

Principal components (PC) of the grain yield under water-limited and well-watered conditions as well as drought tolerance indices of the sorghum lines are given in Table 4. The PC analysis was performed to assess the relationships between all attributes to identify superior genotypes under the two-contrasting environments. The results showed that the first five principal components (PC_1-PC_5) accounted for 99.9% of the entire variation. The first two components grossly explained 98.8% of total variation between the variables (Figure 2). The PC_1 alone contributed the largest component score of 70.3% with high positive weight due to grain yield in the stress (Ys) (0.977), MRP (0.978), MP (0.93), HM (0.995), GMP (0.982), STI (0.972), and YI (0.977). Therefore, characters with relatively larger absolute values of eigenvector weights in PC_1 had the largest contribution to the differentiation of the genotypes into clusters. It is normally assumed that characters with larger absolute values closer to unity within the first PC influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). The second PC explained 28.4% of the total variation and with high weight corresponding to Yp (0.719), SSI (0.804) and TOL (0.988) due to lower value is preferred for the lower sensitivity to moisture stress and YSI (-0.820); therefore, it was grouped as drought sensitive. This study was in agreement with earlier reports that stated more than 99% of the total variation was explained by the first two principal components (Drikvand et al., 2012; Nouraein et al., 2013; Amare et al., 2019). They also pinpointed the high association of STI, MRP, GMP, HM, MP, and YI with higher grain yield under both conditions. Therefore, selection efforts based on these indices may be more effective. PC_1 and PC_2 were explained for grain yield

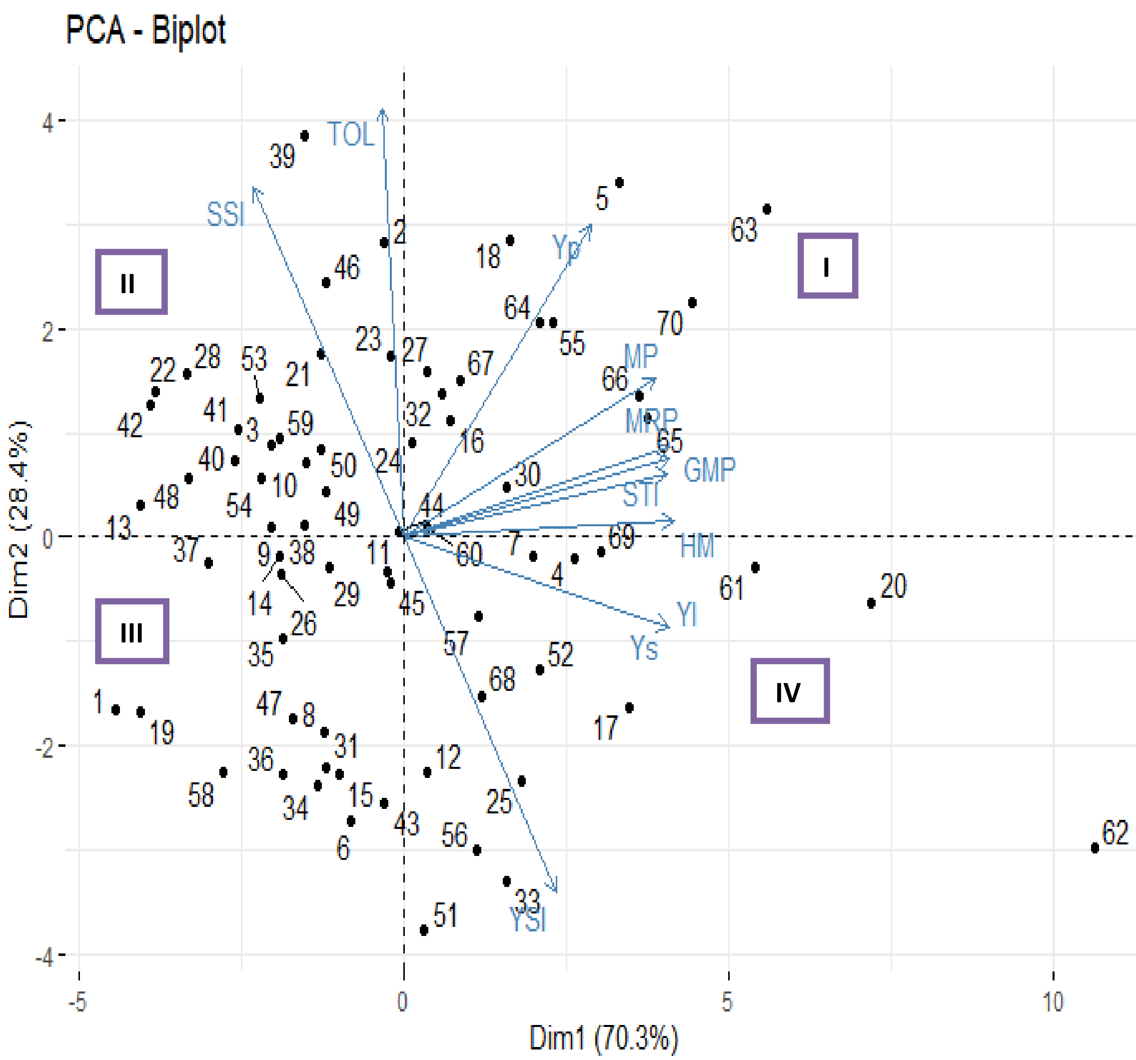

Figure 2. Biplot based on first and second components obtained from PC analysis.
NB: Numbers are indicated in the alphabetical order given in Table 2.

potential under both irrigation conditions and stress susceptibility under stressed condition, respectively. This indicates that selecting genotypes with high PC_1 and low PC_2 is suitable for both moisture regimes (Figure 2). Accordingly genotypes; 4 (BC_2F_3_ETSC_16141), 17 (BC_2F_3_ETSC_16213), 20 (BC_2F_3_ETSC_16216), 52 (BC_2F_3_ETSC_16248), 61(BC_2F_3_ETSC_16257) and 62 (BC_2F_3_ETSC_16258) with high PC_1 and low PC_2 (low sensitivity and high yield) are likely better genotypes in both environments. These genotypes also showed high values of STI, MP, MRP, YI, MP, GMP and HM as well as low values of SSI and TOL. Whereas, genotypes 5 (BC_2F_3_ETSC_16142), 18 (BC_2F_3_ETSC_16214), 55 (BC_2F_3_ETSC_16251), 63 (Dekeba), 64 (Gambella1107), 65 (Macia), 66 (Meko), and 70 (Wediaker) with both high PC_1 and PC_2 are suitable in non-stress condition because they are sensitive to terminal drought. On the other side, sorghum genotypes with both low PC_1 and PC_2 had low sensitivity to stress condition but with low yield potential and can be used in breeding programs for drought tolerance (eg. B35). Conversely, genotypes with low PC_1 and high PC_2 exhibited inferior yield performance and high sensitivity to end-season drought and therefore their cultivation and incorporating in the breeding programmes may not encouraged. Finally, the two first PCs ascertained that their discrimination and correlation between yield potential and drought sensitively agreeing with earlier reports (Thomas et al., 1995; Kaya et al., 2002; Nazari and Pakniyat, 2010; Nouri et al., 2011; Dorostkar et al., 2015; Ghasemi and Farshadfar, 2015).

Cluster analysis

Cluster analysis based on grain yield under stressed and non-stressed conditions and drought tolerance indices

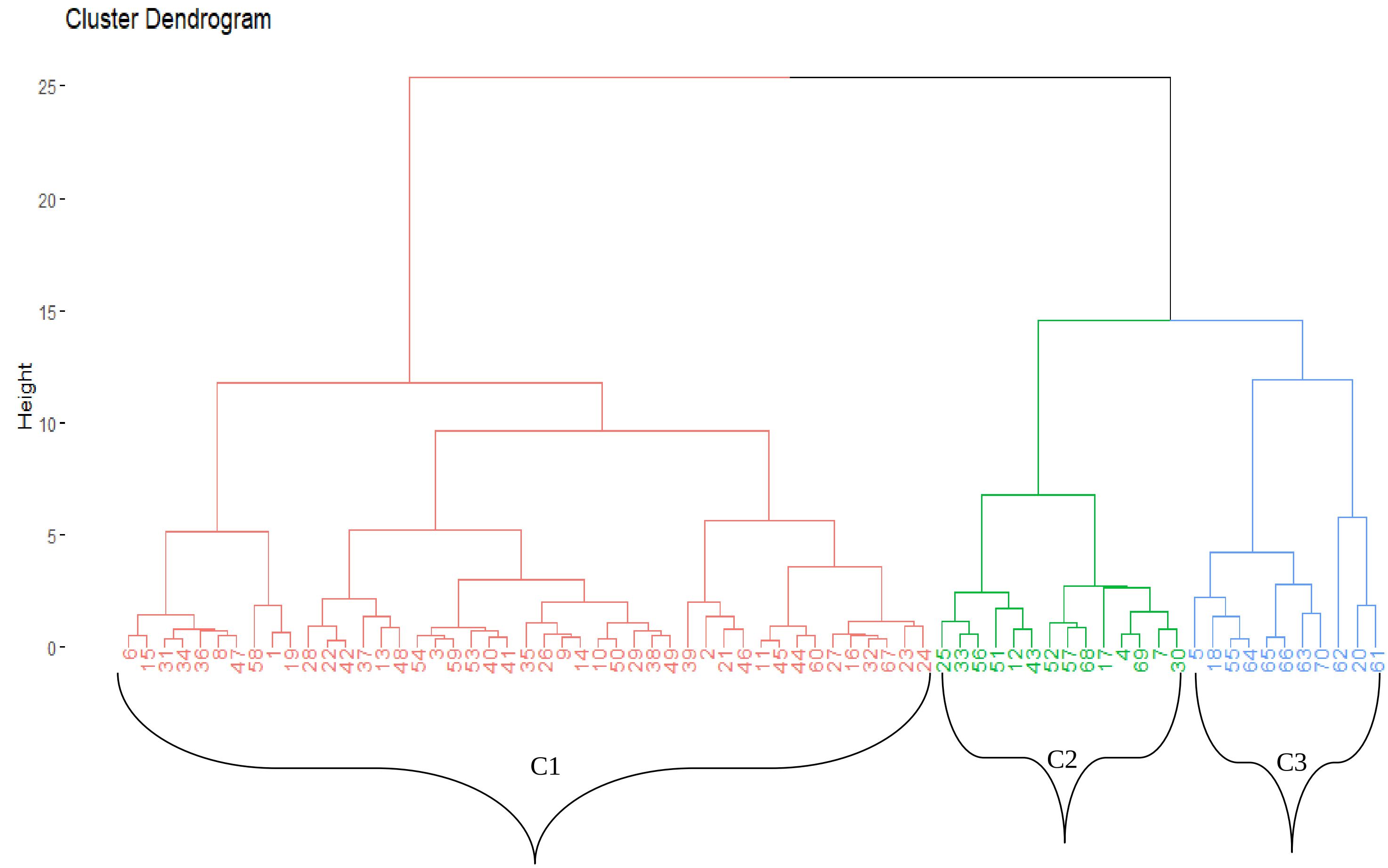

Figure 3. Cluster analysis of seventy sorghum backcrossed lines and their parental lines.

were classified into three clusters (Figure 3). Clustering indices was performed to verify the accuracy of conclusions based on their similarity by average linkage method. Clusters I, II, and III encompassed 64.3, 20 and 15.7% of the genotypes, respectively. The first cluster (C_1, n = 45) had the largest number of genotypes and was characterized by high and lowest yield under full-irrigation and water-limited condition, respectively. This cluster also showed lowest values of mean MRP, GMP, MP, STI, HM, YI and YSI, while higher values of SSI and TOL. The cluster constituted those genotypes characterized by overall inferior performances. The second cluster (C_2, n = 14) classified as intermediate in mean yield under the two-contrasting moisture regimes and high values of MRP, GMP, MP, STI, HM, YI, and YSI, with lower values of TOL and SSI.

Genotypes in cluster III (C_3, n = 11) had high grain yield both under non-stressed (4.52-4.76 t ha^{-1}) and stressed (3.1-4.42 t ha^{-1}) conditions and had the highest value of MRP, GMP, MP, STI, HM, YI and YSI, while lower values of SSI and TOL. This cluster was also superior to grand mean of all other traits averaged over all clusters, indicating that this cluster contained desirable genotypes according to yield obtained from both environments and selection indices. This study is in line with previous reports that stated genotypes can be classified adapted to moisture-stressed and non-stressed conditions using cluster analysis in various crops (Eivazi et al., 2013; Johari-Pireivatlou, 2014; Bahrami et al., 2014; Sory et al., 2017). Generally, this study showed that selection can be improved though MRP, MP, GMP, STI, and HM.

Conclusions

The results showed significant variations among the developed backcrossed lines, resulting in considerable variation in yield and drought tolerance that could be exploited in sorghum improvement. According to the correlation and principal component analysis, drought tolerance indices MRP, MP, GMP, STI, and HM, and YI are superior indices to identify genotypes that yield well under stressed and optimal conditions. YSI was also found to be more useful indices to discriminate tolerant genotypes that are stable in different conditions and produce high grain yield under stressed conditions. The progenies with high TOL and SSI had high yield only under irrigated conditions and significant yield reduction under stressed conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Amare A, Mekbib F, Tadesse W, Tesfaye K (2019). Screening of drought tolerant bread wheat (*Triticumaestivum*L.) genotypes using yield based drought tolerance indices. Ethiopian Journal of Agricultural Sciences 29(2):1-6.

Amelework B, Shimelis H, Tongoona P, Laing M (2015). Physiological mechanisms of drought tolerance in sorghum, genetic basis and breeding methods: a review. African Journal of Agricultural Research 10(31):3029-3040.

Annicchiarico P (2002). Genotype x Environment Interaction: Challenges and Opportunities for Plant Breeding and Cultivar Recommendation. FAO Plant Production and Protection Paper No. 174. Food and Agriculture Organization, Rome. P. 174.

Araus JL, Cairns JE (2014). Field high-throughput phenotyping: the new crop breeding frontier. Trends in Plant Science 19(1):52-61.

Bahrami F, Arzani A, Karimi V (2014). Evaluation of yield-based drought tolerance indices for screening safflower genotypes. Agronomy Journal 106(4):1219-1224.

Banziger M, Betran FJ, Lafitte HR (1997). Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. Crop Science 37:1103-1109.

Banziger M, Edmeades GO (1997). Predicted productivity gains from breeding maize under stressed vs. non-stressed conditions. In: Ransom JK, Palmer AFE, Zambezi BT, Mduruma ZO, Waddington SR, Pixley SR, and Jewell DC (Eds.), Maize Productivity Gains through Research and Technology Dissemination: Proceedings of the 5th Eastern and Southern Africa Regional Maize Conference (Arusha, Tanzania, 3-7 June 1996). CIMMYT, Addis Abeba, Ethiopia. pp. 136-140.

Banziger M, Lafitte HR (1997). Breeding for N-stressed environments: How useful are N-stressed selection environments and secondary traits? In: Edmeades GO, Banzinger M, Mickelson HR, Pena-Valdivia CB (Eds.), Developing drought and low N-tolerant maize, Proceedings of a Symposium, March 25-29, 1996, CIMMYT, ElBatan, Mexico. Mexico D.F., CIMMYT. pp. 401-404.

Bates BC, Kundzewicz ZW, Wu S, Palutikof JP (2008). Climate change and water.Intergovernmental Panel on ClimateChange, Geneva, 210 pp. www.ipcc.ch/pdf/technicalpapers/climate-change-water-en.pdf.Accessed 10 July 2013.

Blum A (2011). Drought resistance: is it really a complex trait? Functional Plant Biology 38:753-757.

Bouslama M, Schapaugh WT (1984). Stress tolerance in soybean.Part 1: evaluation of three screening techniques forheat and drought tolerance. Crop Science 24:933-937.

Brennan PS, Byth DE (1979). Genotype and environmental interactions for wheat yields and selection for widely adapted wheat genotypes. Australian Journal of Agricultural Research 30:221-232.

Ceccarelli S, Grando S (1996). Importance of specific adaptation in breeding for marginal conditions. In: HailuGebre, Van Leur J (eds.) Barley Research in Ethiopia: Past Work and Future Prospects. Proceedings of the first barley research review workshop. 16-19 October 2003. Addis Ababa: IAT/ICARDA. Addis Ababa, Ethiopia. pp. 34-58.

Ceccarelli S, Grando S, Impiglia A (1998). Choice of selection strategy in breeding barley for stress environments. Euphytica 103:307-318

Central Statistical Agency (CSA) (2018). Area and production of majorcrops (private peasant holdings, meher season).Statistical bulletin 586, Addis Ababa, Ethiopia. pp. 1-10.

Chahal GS, Gosal SS (2002). Principles and procedures of plant breeding: biometrical and conventional approach. Narosa Publishing House, New Delhi.

Charrad M, Ghazzali N, Boiteau V, Niknafs A (2014). NbClust Package for Determining the Best Number of Clusters. R package version 2.0.3, URL http://CRAN.R-project.org/package=NbClust 61(6):1-36.

Crasta OR, Xu WW, Rosenow DT, Mullet J, Nguyen HT (1999). Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Molecular and General Genetics MGG 262(3):579-88.

Darzi-Ramandi H, Najafi-Zarini H, Razavi K, Kazemitabar S (2016). Screening Iranian bread wheat lines under different water regimes using yield based drought tolerance indices. SABRAO Journal of Breeding and Genetics 48(4):491-503.

Dorostkar S, Dadkhodaie A, Heidari B (2015). Evaluation of grain yield indices in hexaploid wheat genotypes in response to drought stress. Archives of Agronomy and Soil Science 61(3):397-413.

Drikvand R, Doosty B, Hosseinpour T (2012). Response of rainfed wheat genotypes to drought stress using drought tolerance indices. Journal of Agricultural Science (Toronto) 4(7):126-131.

Eivazi AR, Mohammadi S, Rezaei M, Ashori S, Pour FH (2013). Effective selection criteria for assessing drought tolerance indices in barley (*Hordeumvulgare* L.) accessions. International Journal of

Agronomy and Plant Production 4(4):813-821.
Falconer DS (1989). Introduction to Quantitative Genetics. 3rd ed. Longman, London, England
Food and Agriculture Organization (FAO) (2017). Food and Agriculture Organization of the United Nations, Rome, Italy.
Farshadfar E, Moradi Z, Elyasi P, Jamshidi B, Chaghakabodi R (2012). Effective selection criteria for screening drought tolerant landraces of bread wheat (*Triticumaestivum* L.). Annals of Biological Research 3(5):2507-2516.
Farshadfar E, Poursiahbidi MM, Safavi SM (2013). Assessment of drought tolerance in land races of bread wheat based on resistance/tolerance indices. International Journal of Advanced Biological and Biomedical Research 1(2):143-58.
Farshadfar E, Zamani M, Motallebi M, Imamjomeh A (2001). Selection for drought resistance in chickpea lines. Iranian Journal of Agricultural Sciences 32(4):65-77.
Fernandez GCJ (1992). Effective selection criteria for assessing plant stress tolerance. pp. 257-270. In: Kuo CG (ed.) Adaptation of Food Crops to Temperature and Water Stress: Proceedings of an International Symposium, Tainan, Taiwan.
Fischer RA, Maurer R (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. Australian Journal of Agricultural Research 29(5):897-912.
Gavuzzi P, Rizza F, Palumbo M, Campanile RG, Ricciardi GL, Borghi B (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Canadian Journal of Plant Science 77(4):523-531.
Ghasemi M, Farshadfar E (2015). Screening drought tolerant genotypes in wheat using multivariate and stress tolerance score methods. International Journal of Biosciences 6:326-333.
Golabadi M, Arzani AS, Maibody SM (2006). Assessment of drought tolerance in segregating populations in durum wheat. African Journal of Agricultural Research 1(5):162-171.
Harris K, Subudhi PK, Borrell A, Jordan D, Rosenow D, Nguyen H, Klein P, Klein R, Mullet J (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. Journal of Experimental Botany 58(2):327-338.
Hossain AB, Sears RG, Cox TS, Paulsen GM (1990). Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. Crop Science 30(3):622-627.
Johari-Pireivatlou M (2014). Selection for drought tolerance in bread wheat genotypes with drought tolerance and susceptibility indices. Journal of Applied Environmental and Biological Sciences 4:128-133.
Kassahun B, Bidinger F, Hash C, Kuruvinashetti M (2010). Stay-green expression in early generation sorghum [*Sorghum bicolor* (L.) Moench] QTL introgression lines. Euphytica 172:351-362.
Kaya Y, Palta C, Taner S (2002). Additive main effects and multiplicative interactions analysis of yield performances in bread wheat genotypes across environments. Turkish Journal of Agriculture and Forestry 26(5):275-279.
Keneni G (2007). Concerns on mismatches between environments of selection and production of crop varieties in Ethiopia. East African Journal of Sciences 1(2):93-103.
Khakwani AA, Dennett MD, Munir M (2011). Drought tolerance screening of wheat varieties by inducing water stress conditions. Songklanakarin Journal of Science and Technology 33(2):135-142.
Khallili M, Kazemi A, Moghaddam A, Shakiba M (2004). Evaluation of drought toleranceindices at different growth stages of late-maturing corn genotypes. In: The 8th Iranian Congress of Crop Sciences and Breeding. 25-27 August 2004, Rasht, Iran. Rasht: University Press. pp. 298-298.
Kharrazi MA, Rad MR (2011). Evaluation of sorghum genotypes under drought stress conditions using some stress tolerance indices. African Journal of Biotechnology 10(61):13086-13089.
Kumar AA (2016). Botany, Taxonomy and Breeding. In: Rakshit S, Wang Y-H (eds.), The Sorghum Genome, Compendium of Plant Genomes, DOI 10.1007/978-3-319-47789-3_1. pp 1-25.
Madhusudhana R (2015). Application of DNA Markers for Genetic Improvement. In: Madhusudhana R, Rajendrakumar P, Patil JV (eds). Sorghum Molecular Breeding. Springer pp. 71-99.
Majidi MM, Tavakoli V, Mirlohi A, Sabzalian MR (2011). Wild safflower species (*'Carthamusoxyacanthus'*Bieb.): A possible source of drought tolerance for arid environments. Australian Journal of Crop Science 5(8):1055-1063.
Mardeh AS, Ahmadi A, Poustini K, Mohammadi V (2006). Evaluation of drought resistance indices under various environmental conditions. Field Crops Research 98(2-3):222-229.
Mera GA (2018). Drought and its impacts in Ethiopia. Weather and Climate Extremes 22: 24-35.
Ministry of Agriculture (MoA) (2018). Plant Variety release, protection and seed quality control directorate. Crop variety register, issue No. 21, Addis Ababa, Ethiopia pp. 68-77.
Mohammadi R (2016). Efficiency of yield-based drought tolerance indices to identify tolerant genotypes in durum wheat. Euphytica 211(1):71-89.
Nazari L, Pakniyat H (2010). Assessment of drought tolerance in barley genotypes. Journal of Applied Sciences 10(2):151-156.
Nouraein M, Mohammadi SA, Aharizad S, Moghaddam M, Sadeghzadeh B (2013). Evaluation of drought tolerance indices in wheat recombinant inbred line population. Annals of Biological Research 4(3):113-122.
Nouri A, Etminan A, Teixeira da Silva JA, Mohammadi R (2011). Assessment of yield, yield-related traits and drought tolerance of durum wheat genotypes (*Triticumturjidum* var. *durum*Desf.). Australian Journal of Crop Science 5(1):8-16.
Osmanzai M (1994). A screening method for productivity in moisture deficit environments.Sorghum Improvement Conference of North America, USA; University of Georgia, USA; International Crops Research Institute for the Semi-Arid Tropics, Patacheru 502:324, Andhra Pradesh, India.
Patterson HD, Thompson R (1971). Recovery of inter-block information when block sizes are unequal.Biometrika, 58:545-554.
R Core Team (2019). R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Raman A, Verulkar S, Mandal N, Variar M, Shukla V, Dwivedi J, Singh B, Singh O, Swain P, Mall A, Robin S (2012). Drought yield index to select high yielding rice lines under different drought stress severities. Rice 5:1-12.
Ramirez P, Kelly JD (1998). Traits related to drought resistance in common bean. Euphytica, 9: 127-136.
Reddy NR, Madhusudhana R, Murali MS, Seetharama N, Jagannatha VP (2014). Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. BMC Genomics 15(909):1471-2164.
Reddy PS (2017). Sorghum, *Sorghum bicolor* (L.) Moench. In: Patil JV (Ed.), Millets and Sorghum: Biology and Genetic Improvement. pp. 1-32.
Ribaut JM, Bänziger M, Betran J, Jiang C, Edmeades GO (2002). Breeding: Drought Tolerance Improvement in Tropical Maize. Quantitative Genetics, Genomics, and Plant Breeding pp. 85-99.
Rizza F, Badeck FW, Cattivelli L, Lidestri O, Di Fonzo N, Stanca AM (2004). Use of a water stress index to identify barley genotypes adapted to rainfed and irrigated conditions. Crop Science 44(6):2127-2137.
Rosenow DT, Clark LE, Dahlberg JA, Frederiksen RA, Odvody GN, Peterson GC, Miller FR, Woodfin CA, Schaefer K, Collins SD, Jones JW (2002). Release of four A/B sorghum parental lines ATx642 through ATx645. International Sorghum and Millets Newsletter 43:24-30.
Rosenow DT, Quisenberry JE, Wendt CW, Clark LE (1983). Drought tolerant sorghum and cotton germplasm. In: Developments in agricultural and managed forest ecology Elsevier 12: 207-222.
Rosielle AA, Hamblin J (1981). Theoretical aspects of selection for grain yield in stress and non-stress environments. Crop Science 21:943-946.
Sabadin PK, Malosetti M, Boer MP, Tardin FD, Santos FG, Guimaraes CT, Gomide RL, Andrade CL, Albuquerque PE, Caniato FF, Mollinari M (2012). Studying the genetic basis of drought tolerance in sorghum by managed stress trials and adjustments for phenological and plant height differences. Theoretical and Applied Genetics 124(8):1389-1402.
Sanchez AC, Subudhi PK, Rosenow DT, Nguyen HT (2002). Mapping QTLs associated with drought resistance in sorghum (*Sorghum*

*bicolor*L. Moench). Plant Molecular Biology 48(5-6):713-726.

Sardouie-Nasab S, Mohammadi-Nejad G, Nakhoda B (2015). Field screening of salinity tolerance in Iranian bread wheat lines. Crop Science 54(4):1489-1496.

Schneider KA, Rosales-Serna R, Ibarra-Perez F, Cazares-Enriquez B, Acosta-Gallegos JA, Ramirez-Vallejo P, Wassimi N, Kelly JD (1997). Improving common bean performance under drought stress. Crop Science 37(1):43-50.

Sharma KK, Lavanya M (2002). Recent developments in transgenics for abiotic stress in legumes of the semi-arid tropics. JIRCAS Working Report No. 23(23):61-73.

Singh BD (2002). Plant Breeding: Principles and Methods. Kalyani Publishers, New Delhi-Ludhiana.

Sory S, Gaoussou DA, Mory CM, Niaba T, Gracen V, Eric D (2017). Genetic analysis of various traits of hybrids sorghum (*Sorghum bicolor*(L) Moench), correlated with drought tolerance. Journal of Plant Biology and Soil Health 4(1):1-9.

Srinivas G, Satish K, Madhusudhana R, Reddy NR, Mohan M, Seetharama SN (2009). Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. Theoretical and Applied Genetics 118:1439-1454.

Subudhi PK, Rosenow DT, Nguyen HT (2000). Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. Theoretical and Applied Genetics 101(5-6):733-741.

Teshome A, Zhang J (2019). Increase of Extreme Drought over Ethiopia under Climate Warming. Advances in Meteorology 2019:1-18.

Thomas H, Dalton SJ, Evans C, Chorlton KH, Thomas ID (1995). Evaluation drought resistance in germplasm of meadow fescue. Euphytica 92:401-411.

Thomas H, Howarth CJ (2000). Five ways to stay green. Journal of Experimental Botany 51:29-337.

Thomas H, Smart CM (1993). Crops that stay green. Annals Applied Biology 123:193-233.

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G (1997). Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Molecular Breeding 3:439-448.

Visarada KBRS, Aruna C (2019). Sorghum: A Bundle of Opportunities in the 21st Century. In: Aruna C, Visarada KBRS, Venkatesh Bhat B, Tonapi VA (eds). Breeding Sorghum for diverse end uses pp. 1-14.

Wagaw K (2019). Review on Mechanisms of Drought Tolerance in Sorghum (*Sorghum bicolor* (L.) Moench) Basis and Breeding Methods. Academic Research Journal of Agricultural Science and Research 7(2):87-99.

Wassmann R, Jagadish SV, Sumfleth K, Pathak H, Howell G, Ismail A, Serraj R, Redona E, Singh RK, Heuer S (2009). Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. Advances in Agronomy 102:91-133.

Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT (2000). Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43(3):461-469.

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Full Length Research Paper

Influence of clam shells and *Tithonia diversifolia* powder on growth of plantain PIF seedlings (*var*. French) and their sensitivity to *Mycosphaerella fijiensis*

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Plantain prices in sub-Saharan markets are very high due to the fact that the supply does not cover the large demand. The main constraint of plantain cultivation is the seedlings unavailability in quantity and quality, which is essential to boost the creation of new plantations. The PIF technique could solve this problem if its substrate of production is amended with natural products for quality enhancement. This study aims to assess clam shells and *Tithonia diversifolia* effects on the growth of PIF plantain seedlings and their sensitivity to *Mycosphaerella fijiensis*. Plantain PIF seedlings were grown in an amended substrate. The treatment influences the seedlings germination rate, number of shoots, height, diameter, area of leaves and favours a less sensitivity to *M. fijiensis* compared to the controls. The presence of clam shells and *T. diversifolia* in the treatment especially enhanced the (1) vegetative growth and (2) less sensitivity as well as accumulation of proteins and polyphenols respectively. This combination shows a synergic action with dual role both as a biofertilizer and as a biopesticide. This work valorises the use of by-fishing products and bad herbs that are environmentally benign and affordable to poor smallholders' farmers, leading to a sustainable and responsible agriculture, as well as poor peasants' empowerment.

Key words: Plantain (*Musa* spp.), PIF seedlings, *Tithonia diversifolia*, clam shells, biofertilizer, biopesticide, *Mycosphaerella fijiensis*.

INTRODUCTION

Banana in the *Musaceae* family is a perennial monocotyledonous plant that originates from South East Asia and grows in tropical and subtropical regions. The *Musa* spp. is composed of many cultivars, notably need to be cooked before consumption as compared to dessert bananas. The contribution of plantain (Musa spp., genome AAB) cultivation for income generation is significant and vital for food security of the population in tropical and sub-tropical zones, especially in Central and West Africa.

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In Central Africa, Cameroon is the first in term of plantain production and ranks 9th in the world (4.94 million tons per year) (FAO, 2017). Plantain production is very low and inadequately covers the large demand, leading to very high prices for this commodity in local markets. Based on this, it is necessary to create new plantations to improve the performance of this crop and meet up with the large demand despite the unavailability of seedlings in quantity and quality (Ewané et al*.,* 2019).

Traditionally, a banana plant is obtained from suckers of another banana plant that is one plantain sucker for one plantain seedling, and is usually disseminated with farmlands soil which often contains pathogenic microorganisms. The vitroplants are ideal for new plantations creation since they are safe from any contamination, but are very expensive and not affordable to poor peasants. An innovative technique called PIF set up by the African Centre for Research on Banana-plantain (CARBAP) that is, plants issued from stem fragments is an alternative for smallholders' farmers owing to its many advantages (Kwa, 2002). When this technique is applied to one sucker, we can obtain 20 to 100 seedlings depending on the variety and the farmer's experience. This technique is essential for massive production of seedlings in quantity within a very short period of time (2 to 3 months) and at low cost. This innovative technique can help increase the number of plantain plantations in the subregion through an easy and cheap set up of plantain plantations, leading to an increase in the production as well as the purchasing power of the poor peasants. However, PIF seedlings are facing many problems during acclimatization like contamination of the seedlings on farmlands that could lead to plants mortality of about 60% during the establishment of new plantations and are now rejected by some farmers (Ewané et al., 2019).

Banana tree is permanently under the threat of many pathogens amongst a virulent, invasive and predominant pathogen called *M. fijiensis*, that causes severe reduction of the leaf area in all banana-growing countries. Moreover, *M. fijiensis* is responsible for black Sigatoka disease (BSD), the most economically destructive disease of bananas, that causes loses of about 50% of production (Onautshu, 2013). The use of synthetic products such as weed-killers, fertilizers, fungicides, pesticides in PIF seedlings production and on farmlands can be harmful to human and the environment, be responsible for the appearance of resistance in plant pathogens strains (Ewané et al*.,* 2013) and is not affordable to the smallholder farmers.

It was recently demonstrated in Cameroon that clam shells powder has a strong influence on PIF plantain seedlings growth and susceptibility to BSD in nurseries by its dual role as a biofertilizer and as a biofungicide (Ewané et al*.,* 2019). Therefore, regarding their properties, clam shells are a good candidate to improve the production in quality and quantity of PIF plantain seedlings. Another good candidate is *Tithonia diversifolia*, a woody herb of 2-3 m tall in the family *Asteraceae.* It is highly rich in nutrients, averaging about 3.5% nitrogen (N), 0.37% phosphorus (P) and 4.1% potassium (K) and decomposes rapidly after its application to the soil thereby enriching the soil with N, P and K for the growth of crops. With its antifungal properties, it plays an important role in diseases control and induces the crude synthesis of defense metabolites (flavonoids, tannins, alkaloids, pathogenesis related-proteins) for plants defense (Chagas-Paula et al*.,* 2012). Phytochemicals such as sesquiterpenoids, diterpenoids, alkaloids, flavonoids, chlorogenic acid derivatives, phenols, saponins, tannins, and terpenoids are present in the leaves, stems, and roots of *T. diversifolia* (Umar et al*.,* 2015; Kerebba et al., 2019).

Utilization of these two natural products (clam shells and *T. diversifolia*) could be a new approach to improve the quality and the quantity of plantain. Based on their cost benefit ratio, the association of the PIF technique and the powders of clam shells and *T. diversifolia* in the production of plantain seedlings could lead to the enhancement of the number of plantation and the productivity in the subregion, the less utilization of synthetic inputs in agriculture, the less production cost leading to the poor small holder farmer poverty alleviation. The aim of this study is to examine the effect of clam shells and *T. diversifolia* powder on the growth promotion of plantain PIF seedlings in nursery and on their protection against *M. fijiensis*.

MATERIALS AND METHODS

Plantain suckers of French variety were obtained from Lékié division (Obala) of Centre Region of Cameroon. The short cycle of production and the good productivity capacity were the selected criteria for the choice of this variety.

The clam shells (organic matter) came from the municipality of Mouanko, located in the Littoral region and more specifically in the Sanaga Maritime division, precisely on the North bank of the Sanaga River about twenty kilometers east of its mouth in the Gulf of Guinea. To obtain the organic matter powder, the fresh clams were washed, dried in the sun, broken into large pieces, then reduced to powder and finally sifted.

T. diversifolia tissues were obtained from farm lands around the Biotechnology Centre of University of Yaoundé 1 located at Nkolbisson (Yaoundé-Cameroon).

The strain of the causal agent of black Sigatoka disease (*Mycosphaerella fijiensis*) was provided by the African Centre for Research on Bananas and Plantains (CARBAP) of Njombé in the Littoral region of Cameroon.

The sawdust, sand and black soil were used as substrates and sterilized in an oven at different temperatures and time intervals as described by Ewané et al. (2019). The sawdust was used for growth of plantain PIF seedlings in the greenhouse while a mixture at a ratio of 2/3 of black soil and 1/3 of sand was used in the shade.

Experimental design

This research was conducted in Yaoundé (Centre Region,

Table 1. Experimental design for the study of the influence of clam shells and *Tithonia diversifolia* powder on vegetative growth of plantain PIF seedlings and their sensitivity to *Mycosphaerella fijiensis*.

Location	Completely Randomized Block Device	
	Greenhouse	**Shade**
Phase	Germination	Acclimatization
Purpose	Production of the PIF seedlings	Survey of the seedling's growth
Experimental Unit (EU)	Each treatment	Each treatment
Substrate to amend	Sawdust	Black soil and sand
Number of plants/EU	Three (03) Explants	At least three (3) plants
Container	Propagator	Plastic planter bags
Block	A sterilized substrate block (B1)	A non-sterilized substrate block (B2)
Condition	Controlled Condition Use of Sterile Substrate (SS)	Uncontrolled (Farmer) Condition Use of non-Sterile Substrate (nSS)
Number of Treatment	Four (04)	Four (04)
Treatment	1. Sterile Substrate + Clam shells (SS+CS) 2. Sterile Substrate + *T. diversifolia* (SS+Td) 3. Sterile Substrate + Clam shells + *T. diversifolia* (SS+CS+Td) 4. Sterile Substrate only as Control (SS)	1. Non-Sterile Substrate + Clam shells (nSS+CS) 2. Non-Sterile Substrate + *T. diversifolia* (nSS+Td) 3. Non-Sterile Substrate + Clam shells + *T. diversifolia* (nSS+CS+Td) 4. Non-Sterile Substrate only as Control (nSS)

Cameroon), from September 2015 to March 2016 under controlled conditions in the laboratory and in the greenhouse (Table 1). The PIF technique was done in two steps the germination of the explants in the greenhouse and 2) an acclimatization phase of the seedlings under shade. During this second step (November 2015 to January 2016), the average temperature and the mean monthly rainfall of the locality were respectively 28 °C and 53 mm. The suckers were prepared through trimming, shelling and the trauma of the shoot apical meristem following the method used by Ewané et al. (2019). The different experimental units were classified by block on the shelves in a greenhouse and covered with a white and transparent plastic. Explants tracking (watering) in the greenhouse allowed them to germinate and produce seedlings.

Evaluation of the vegetative growth in the greenhouse and in the shade

The germination rate and the number of PIF seedlings per experimental unit were evaluated after every seven days starting from the second week of introduction of explants in the greenhouse for a period of four successive weeks. This evaluation was done according to the method reported by Ewané et al., (2019). The seedlings with two to three small open leaves and three to four radicles were transferred after eight weeks in plastic planter bags in the shade for acclimatization.

The height and the diameter of the seedlings' pseudo-stems, and the total leaf area of the seedlings' leaves were evaluated for three plants selected per experimental unit in the shade. The total leaf surface (TLS) of each plantain seedling was determined using the method reported by Ewané et al., (2019). Every seven days starting from the day the seedlings entered the shade, the measurements were taken for each experimental unit for three successive weeks.

Evaluation of the sensitivity to black Sigatoka disease

M. fijiensis's strain was used for artificial inoculations of the leaves of plantains seedlings and was obtained according to the protocol of Ewané et al., (2019).

The leaves of the same age i.e. about 12 weeks from three plants per experimental unit were selected the day of the experimentation, detached and transported to the laboratory for inoculation. Before inoculation, a leaf of each plant was conserved at - 45°C in a plastic sachet for biochemical analysis of the before inoculation stage, while the ones to be inoculated were cleaned and kept for two hours at air temperature. A 100 µL droplet of *M. fijiensis* suspension (10^6zoospores/mL) was then deposited on the middle of leaf surface. The infected leaves were kept under controlled condition of relative humidity in the laboratory in a basin and covered with transparent film. The evaluation of necrosis's progression was done by measuring the length (L) and the width (W) of the necrotic surface after every two days for 12 days in order to visualize the rot spreading on the leaf's surface. The 'necrotic surface area' (NSA) in mm2 was calculated for each measurement by assuming a rectangular shape to the necrosis as in the formula of Ewané et al. (2019): NSA = L x W.

Biochemical analyses

The determination of the content of total native protein and total phenolic compounds were carried out in two stages (before and after inoculation) on the whole leaves. The leaves samples involved were cut at 1 cm beyond the necrotic point or beyond the marked scar (sections with no symptoms). For these analyses, each treatment was repeated thrice.

Extraction samples were carried out according to the method reported by Pivorani et al., (2008) with modification and by El Hadrami et Baaziz (1997) respectively for total native protein and phenolic compounds. 1 g of fresh leaf was used for each extraction followed by quantification as described by Ewané et al. (2019). The protein concentration was expressed in mg equivalent (Eq) of bovine serum albumin (BSA) per g of fresh weight (FW) while that of phenolic compounds was measured in mg equivalent of gallic

Table 2. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the percentage of germination and number of cumulative shoots of plantain seedlings in the greenhouse.

Source	Percentage of germination (R^2 = 100%)			Number of shoots (R^2 = 99%)		
	DF	*F*	*P*	DF	*F*	*P*
Condition	1	2190	< 0.0001	1	6	0
Treatment	3	1653	< 0.0001	3	590	< 0.0001
Day	3	9881	< 0.0001	3	769	< 0.0001
Condition×Treatment	3	3	0	3	1	0
Condition×Day	3	451	< 0.0001	3	2	0
Treatment×Day	9	277	< 0.0001	9	17	< 0.0001
Condition×Treatment×Day	9	78	< 0.0001	9	1	1

DF is the degree of freedom; *F* is the value of F test and *P* is the probability.

acid per g of fresh weight.

Statistical analyses

The effects of *T. diversifolia* and clam shells powders on plantain seedlings vegetative growth, sensitivity to BSD, and total proteins and polyphenols were analysed by subjection of the value (percentage of germination, number of shoots, height and diameter of seedlings, leaves surface area, necrotic surface area, total proteins and total polyphenolic) to mixed three-way ANOVA performed with XLSTAT software. Each plant being taken as experimental unit and condition, treatment and day as factors. Multiple comparisons of the means were done by applying Tukey's test at 5% probability level. Pearson correlation analysis between the different variables was also performed with XLSTAT software.

RESULTS

Effect of clam shells and T. diversifolia on the PIF seedlings vegetative growth

The germination rate and the number of shoots were found to be significantly influenced (*P*< 0.0001) by the three variables (condition, the treatment and the day) with respective R^2 values of 1 and 0.99 (Table 2). The most influential variable of the three was the day. The percentage of germination was consistently higher in the treated PIF substrates compared with the controls.

The number of shoots was consistently higher in the amended PIF substrates compared with the controls. The germination occurs fast in the controlled condition (SS) compared to the non-controlled condition (nSS), and the significant difference was very low between both conditions for the number of shoots (Figures 1 and 2).

Treatment effect was almost the same for all the amended substrate in the greenhouse with 100% of germination obtained 28 days after seeding (DAS) regardless of the condition. A significant interaction (*P*<0.0001) between the condition and the day, the treatment and the day, and the condition, the treatment and the day was observed (Table 2 and Figure 1). However, the total germination (100%) was obtained after 35 DAS in the control experimental unit. Showing thus, two statistically different groups between the amended and the control PIF seedlings regardless of the condition in term of germination percentage.

Treatment effect was especially marked for CS + Td amendment that generated more shoots in PIF substrate 35 DAS (average value: 17), followed by Td amendment (average value: 13) and CS amendment (average value: 11) compared to the control (average value: 8) as confirmed by the significant interaction (*P*< 0.0001) between the treatment and the day, although no significant interaction was observed between the condition and the day; the condition, the treatment and the day (Table 2 and Figure 2). Showing thus, four statistically different groups were distinguished between the amended and the control PIF seedlings regardless of the condition in terms of the number of shoots.

The PIF seedlings height and diameter of shoots, and the area of leaves were found to be significantly influenced (P< 0.0001) by the condition, the treatment and the day with respective R2 values of 0.97, 0.96 and 0.96 (Table 3). Between these three variables, the most influential variable was the treatment for the height of shoots, and the condition for the diameter of shoots and area of leaves. The height, the diameter and the leaves surface area were consistently higher in the amended PIF seedlings compared with the controls. The difference between the controlled condition (SS) and non-controlled condition (nSS) was significant for the height and the diameter of pseudo stems, and for the leaves area surface (Figures 3 to 6).

Treatment effect was especially marked in non-controlled condition (nSS) for the CS + Td amendment that had 21 days after weaning (daw), seedlings with the higher height (average value: 13.93 cm), the bigger diameter of pseudo-stems (average value: 2.25 mm), and the larger leaves area surface (average value: 73.52 cm^2), followed by the *T. diversifolia* amendment (average value: 12.01 cm; 1.81 mm and 68.68 cm^2 respectively) and the CS amendment (average value: 10.35 cm; 1.58 mm and 60.61 cm^2 respectively) compared to the control (average value: 8 cm; 1.22 mm and 48.7 cm^2 respectively). A significant interaction (*P*< 0.0001) was

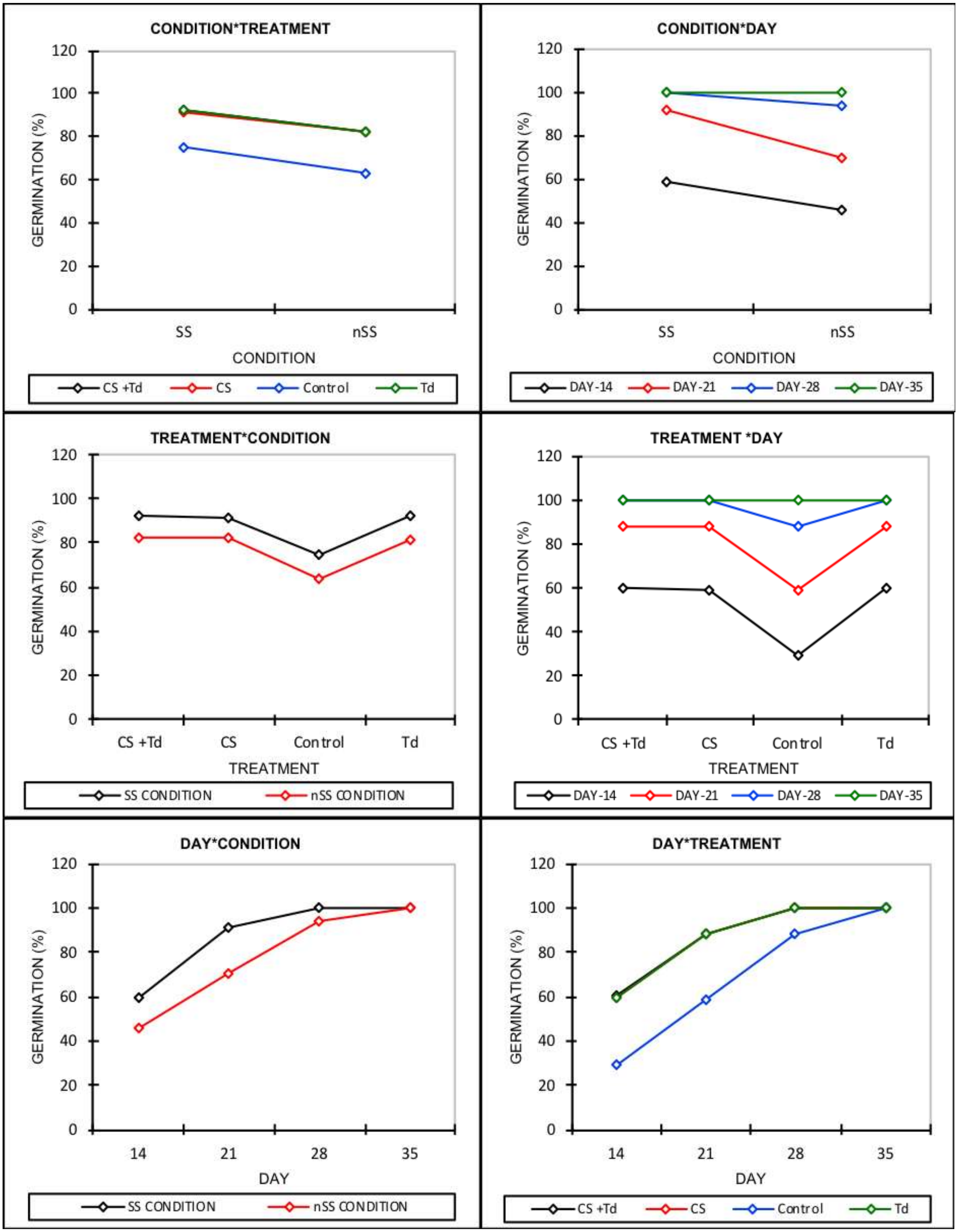


Figure 1. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the percentage of germination of PIF plantain seedlings in course of time. Each point represents the average mean of three replicates for each treatment.

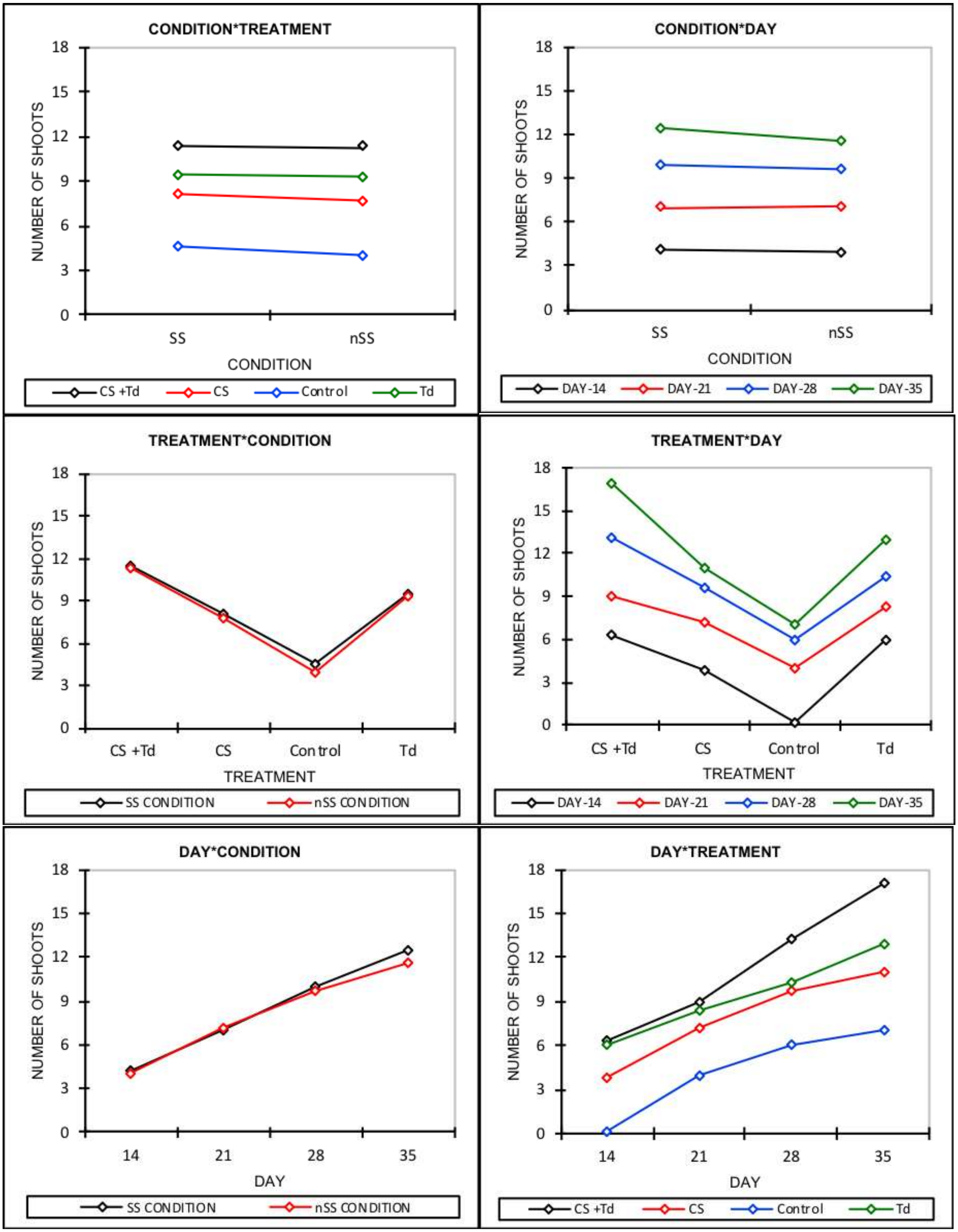


Figure 2. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the number of cumulative shoots of PIF plantain seedlings in course of time. Each point represents the average mean of three replicates for each treatment.

Table 3. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the height of shoots, the diameter of shoots, the foliar surface area of leaves of plantain seedlings in the shade.

Source	Height of shoots (cm) [R^2 = 97%]			Diameter of shoots (cm) [R^2 = 96%]			Area of leaves (mm^2) [R^2 = 96%]		
	DF	*F*	*P*	DF	*F*	*P*	DF	*F*	*P*
Condition	1	377	< 0.0001	1	865	< 0.0001	1	959	< 0.0001
Treatment	3	488	< 0.0001	3	93	< 0.0001	3	121	< 0.0001
Day	3	22	< 0.0001	3	57	< 0.0001	3	5	0
Condition×Treatment	3	63	< 0.0001	3	8	< 0.0001	3	11	< 0.0001
Condition×Day	3	0	1	3	6	0	3	0	1
Treatment×Day	9	1	1	9	3	0	9	0	1
Condition×Treatment×Day	9	1	1	1	1	0	9	0	1

DF is the degree of freedom; *F* is the value of F test and *P* is the probability.

found between the condition and the treatment, although no significant interaction was observed between the condition and the day, the treatment and the day for the height of pseudo-stems and the leaves surface area, and between the condition, the treatment and the day for all the three variables (Table 3, Figures 3 to 6). Showing thus, four statistically different groups between the amended and control PIF seedlings in terms of the height and diameter of shoots, and the area of leaves.

Effect of clam shells and T. diversifolia on the PIF seedlings sensitivity to BSD

The PIF seedlings sensitivity to black Sigatoka disease was found to be very significantly influenced (P< 0.0001) by the condition, the treatment and the day with R2 value of 0.97 (Table 4) and the most influential variable was the day. The black Sigatoka disease severity was consistently lower in the treated PIF substrates compared to the controls. The difference between the level of severity in the controlled condition (SS) and the non-controlled condition (nSS) was significant but very low (Figure 7).

Treatment effect was especially marked for the amendment containing clam shells (CS + Td and CS) that had seedlings with consistently lower necrotic surface area (average value: 1.46 cm^2 and 1.23 cm^2) 12 daw, followed by *T. diversifolia* amendment (average value: 3.39 cm^2) compared to the control (average value: 4.84 cm^2). A significant interaction (P < 0.0001) was found between the condition and the treatment, the condition and the day, the treatment, and the day and the condition, the treatment and the day (Table 3 and Figure 7). Showing thus, three statistically different groups between the treated and the control PIF seedlings in terms of sensitivity to *M. fijiensis*.

Effect of clam shells and T. diversifolia on proteins and polyphenols accumulation

The proteins accumulation (R^2 = 0.96) in PIF seedlings was found to be very significantly influenced (P< 0.0001) by the treatment and the stage while the variables influencing very significantly (P< 0.0001) the polyphenols accumulation (R^2 = 0.98) were the condition, the treatment and the stage (Table 5). The difference between the amount of proteins and polyphenols accumulated in the controlled condition (SS) and non-controlled condition (nSS) was significant only for polyphenol accumulation but very low for both variable (Figures 8 and 9). The most influential variable in the accumulation of proteins and polyphenols was respectively the treatment and the stage. The proteins and polyphenols amount were high in the amendment PIF substrates compared with the controls regardless of the condition.

The stage effect was especially marked for amended PIF seedlings that had consistent amount of proteins and polyphenols after inoculation compared to the amount before inoculation as confirmed by the significant interaction (P< 0.0001) of stage (Table 5, Figures 8 and 9). The treatment effect was especially marked for amended PIF seedlings before inoculation (BI) and after inoculation (AI) which had respective consistent average values of proteins and polyphenols especially for treatment CS + Td (BI 0.255 mg and 0.041 mg; AI: 0.526 mg and 0.089 mg), followed by treatment CS (BI: 0.247 mg and 0.038 mg; AI: 0.461 mg and 0.079 mg) and treatment Td (BI: 0.113 mg and 0.028 mg; AI: 0.257 mg and 0.050 mg) compared to the controls (BI: 0.082 mg and 0.020 mg; AI: 0.150 mg and 0.037 mg) as confirmed by the significant interaction (P< 0.0001) of treatments (Table 5, Figures 8 and 9). The amounts of total proteins and total polyphenols were expressed in mg equivalent of BSA per g of fresh weight and mg equivalent of gallic acid per g of fresh weight. Moreover, a significant interaction was found (P<0.0001) between the treatment and the stage, although no significant interaction was observed for the condition; between the condition and the treatment for total proteins, between the condition and the stage; the condition, the treatment and the stage for both variables (Table 5, Figures 8 and 9). Showing thus, four statistically different groups were distinguished

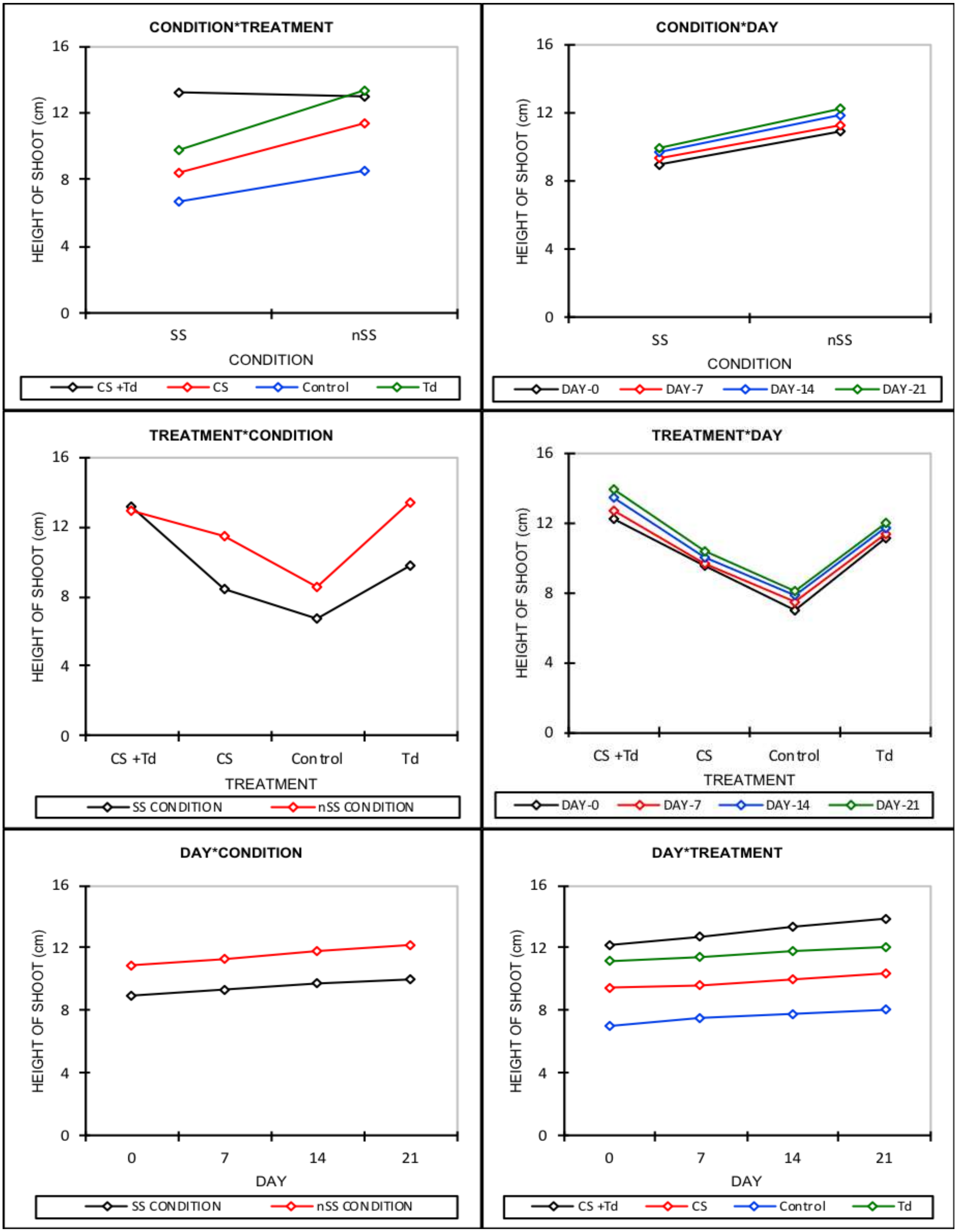


Figure 3. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the height of PIF plantain seedlings in course of time. Each point represents the average mean of three replicates for each treatment.

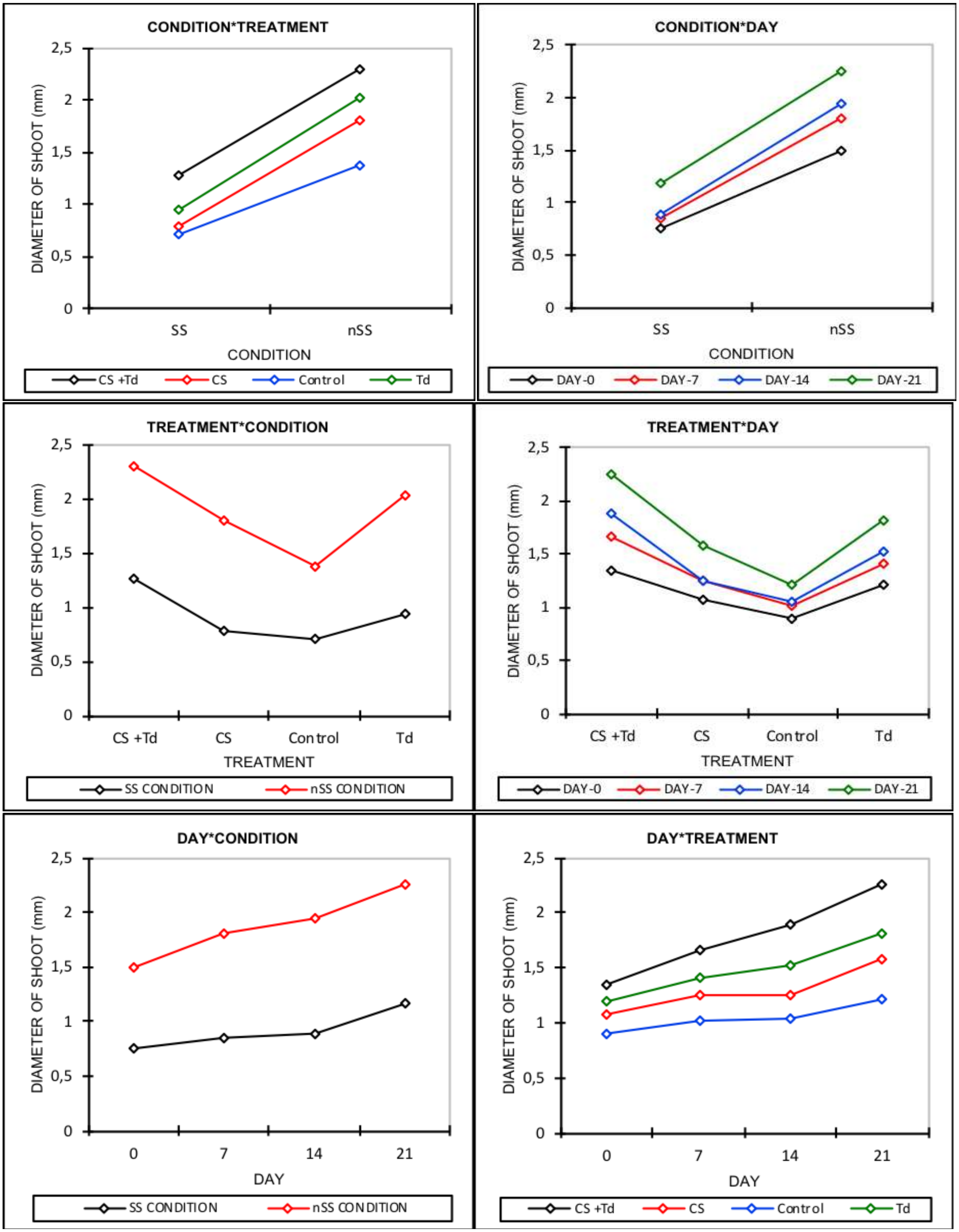


Figure 4. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the diameter of PIF plantain seedlings pseudo stem in course of time. Each point represents the average mean of three replicates for each treatment.

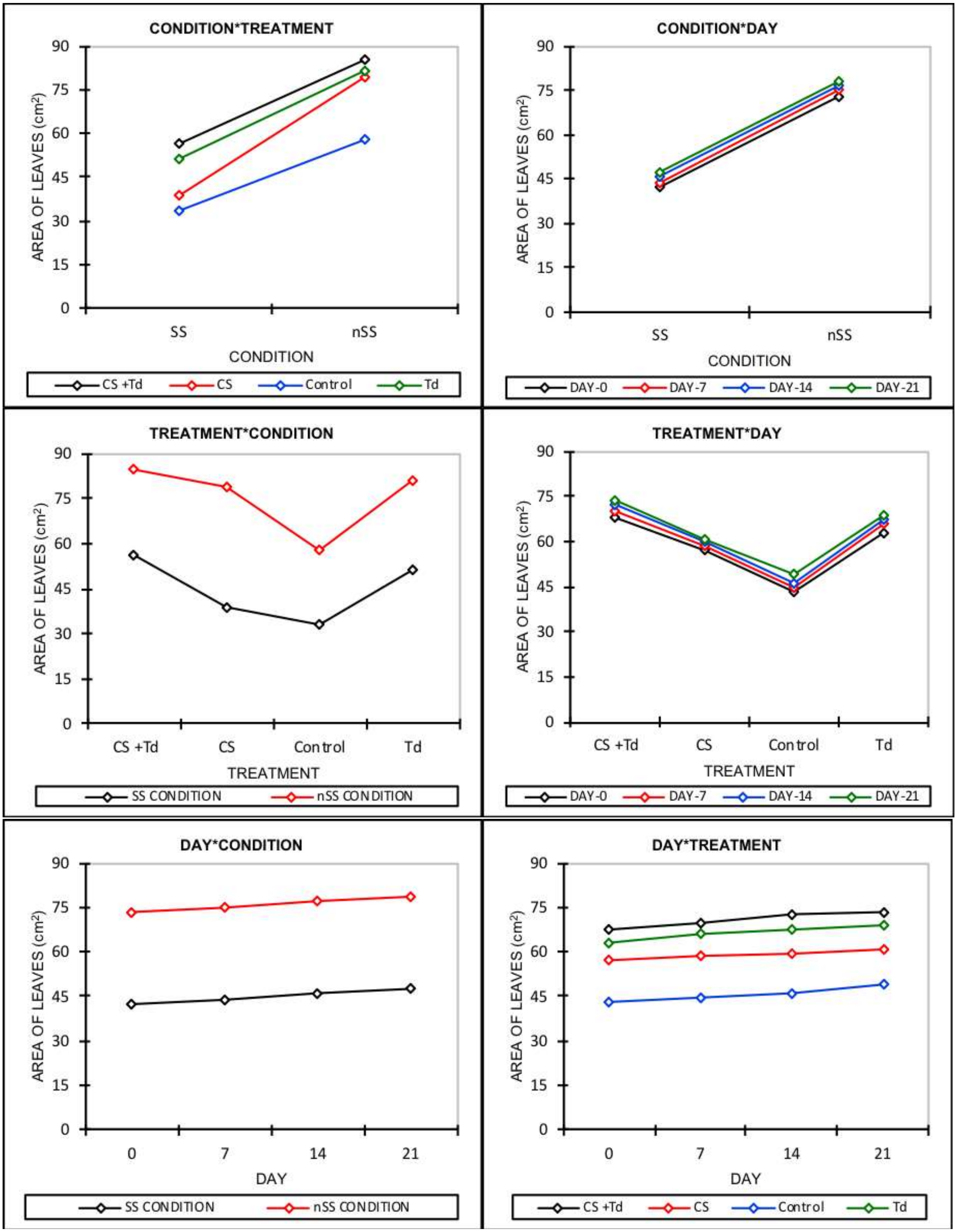


Figure 5. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings leaves surface area in course of time. Each point represents the average mean of three replicates for each treatment.

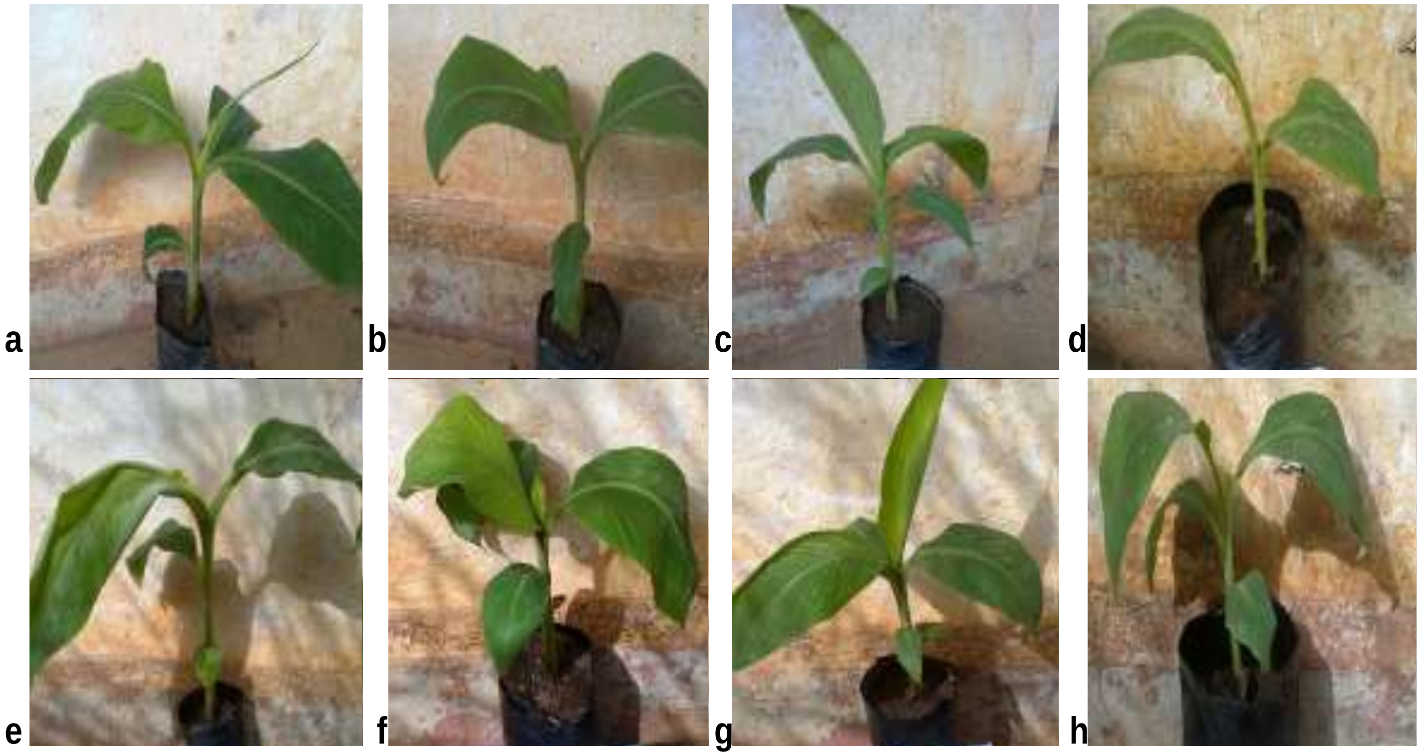


Figure 6. PIF seedlings 40 days old after weaning, grown on (**a**) sterile substrate, clam shells and *T. diversifolia* (SS+CS+Td), (**b**) sterile substrate and *T. diversifolia* (SS+Td), (**c**) sterile substrate and clam shells (SS+CS), (**d**) sterile substrate only as control (SS), (**e**) non-sterile substrate, clam shells and *T. diversifolia* (nSS+CS+Td), (**f**) non-sterile substrate and *T. diversifolia* (nSS+Td), (**g**) non-sterile substrate and clam shells (nSS+CS), (**h**) non-sterile substrate only as control (SS).

Table 4. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the plantain seedlings sensitivity to black Sigatoka disease.

Source	BSD sensitivity (cm^2) [R^2 = 97%]		
	DF	***F***	***P***
Condition	1	46	< 0.0001
Treatment	3	15857	< 0.0001
Day	6	17723	< 0.0001
Condition×Treatment	3	441	< 0.0001
Condition×Day	6	45	< 0.0001
Treatment×Day	18	1451	< 0.0001
Condition×Treatment×Day	18	50	< 0.0001

DF is the degree of freedom; *F* is the value of F test and *P* is the probability.

between the amended and the control PIF seedlings in terms of total proteins and polyphenols accumulation.

Pearson correlation analysis between the different variables

The amount of total proteins and total polyphenols were negatively correlated with the BSD severity in the French variety as confirmed by the scatter plots (Figure 10). Between most vegetative growth variables (germination percentage, height of shoots, diameter of pseudo stems and area of leaves), a strong positive correlation was found. It was evidenced that germination percentage, height of shoots, diameter of pseudo stems and leaves surface area were positively and strongly correlated to BSD severity, as well as being poorly linked to total proteins and total polyphenols content of PIF seedlings in nursery.

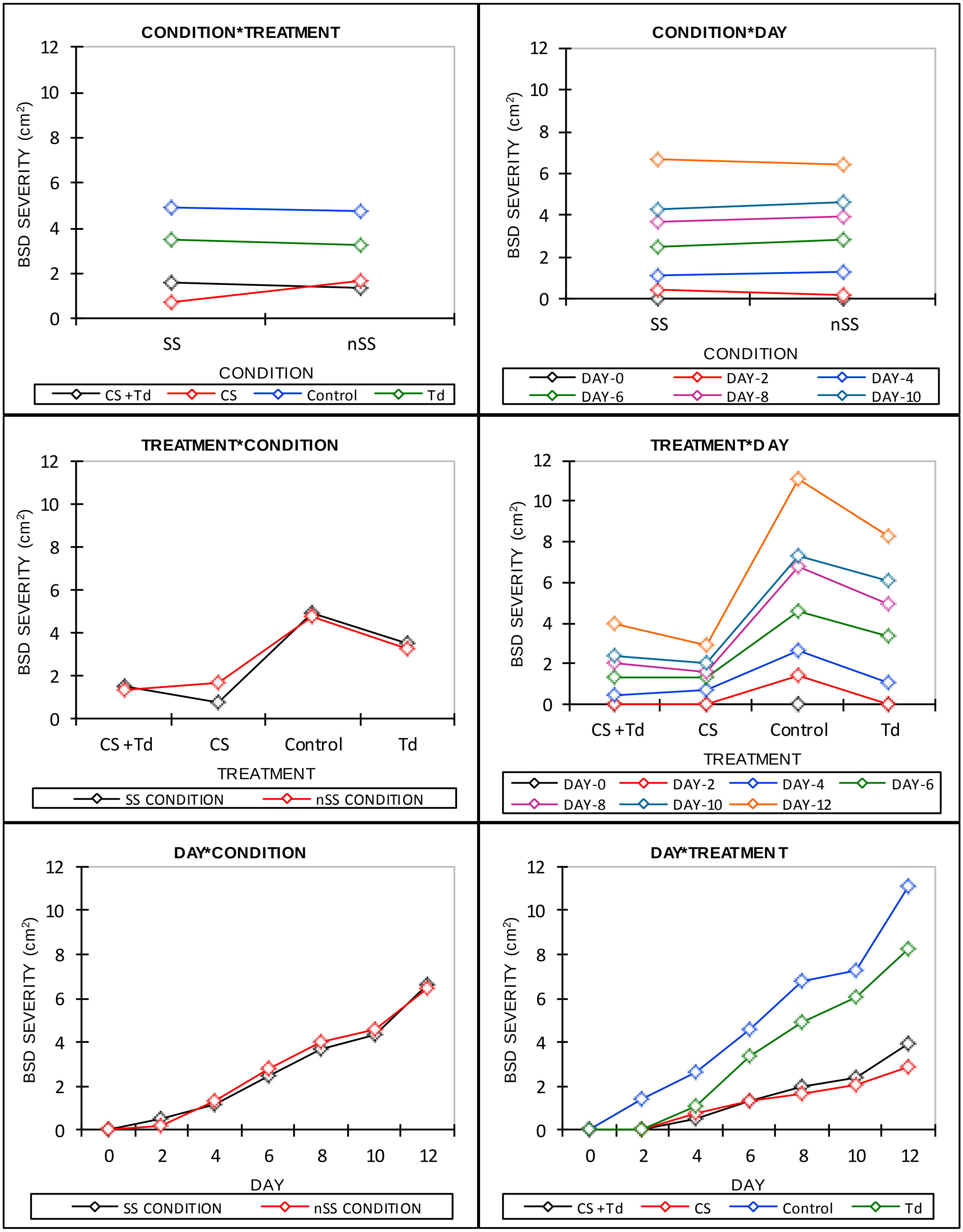


Figure 7. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings sensitivity to BSD in course of time. Each point represents the average mean of three replicates for each treatment.

Table 5. Variance analysis of clam shells and *Tithonia diversifolia* powder effects on the accumulation of proteins and polyphenols in plantain seedlings for both stages (before inoculation and after inoculation).

Source	Total proteins (mg Eq BSA/g FW) [R^2 = 96%]			Total polyphenols (mg Eq Cat/g FW) [R^2 = 98%]		
	DF	*F*	*P*	DF	*F*	*P*
Condition	1	0	1	1	5	0
Treatment	3	170	< 0.0001	3	267	< 0.0001
stage	1	297	< 0.0001	1	972	< 0.0001
Condition×Treatment	3	0	1	3	3	0
Condition×Stage	1	1	0	1	0	1
Treatment×Stage	3	19	< 0.0001	3	52	< 0.0001
Condition×Treatment*Stage	3	1	0	3	2	0

DF is the degree of freedom; *F* is the value of F test and *P* is the probability.

DISCUSSION

The aim of this work was to assess the effects of clam shells and *T. diversifolia* powders amendment on the growth promotion of plantain PIF seedlings and their sensitivity to *M. fijiensis*. The results of this study have provided evidence for wide variations in the germination rate, number of shoots, height of shoots, diameter of pseudo stems, area of leaves, the number and length of roots (data not shown), in the sensitivity to *M. fijiensis* of amended plantain PIF seedling and in the accumulation of total proteins and polyphenols before and after inoculation as recently shown on banana treated with shells in nursery (Ewané et al*.,* 2019), as well as on cocoa (Téné et al*.,* 2017, 2019). The clam shells and *T. diversifolia* powder treatment affects positively the generation of shoots, sensitivity to BSD and accumulation of proteins and polyphenols regardless of the condition as proven by less difference between both conditions. However, for the vegetative growth characters, the treatment effect is more important in the non-sterile condition (nSS) compare to the sterile condition (SS). The efficiency of the treatment in sterile condition as well as in the non-sterile condition, which is suitable for the poor peasant, seems to be proven through this result.

The clam shells treatments (Td+CS and CS) especially stimulated the PIF seedlings defense response with respective percentage of protection of 74.59 and 69.84% compared to the controls. These results are in accordance with previous study that have shown an increase in pre-existing (before inoculation) and *de novo* synthesized (after inoculation) proteins, polyphenols as well as some enzymes involved in plant tissues defense on plantain PIF seedlings (Ewané et al*.,* 2019), and on cocoa (Téné et al*.,* 2017, 2019). Indeed, plant antifungal metabolites are preformed inhibitors that are pre-existing in healthy plants (phytoanticipins), or they may be synthesized *de novo* in response to pathogen attack or various non-biological stress factors (Pusztahelyi, 2018; Pusztahelyi et al*.,* 2015). These compounds are considered as chemical or physical barriers, playing key roles in the defense against pathogens infection. This protective effect relies on (1) the improvement of the soil microbial communities in both the abundances and structures (Malerba and Cerana, 2019), (2) the interaction between the substrate, the plant and the plant microbiome leading to the recognition by specific receptors present on the plant cell plasma membrane, the triggering of biochemical pathways associated with defense responses and activated immunity through the systemic acquired defense (Pusztahelyi, 2018). It would be interesting to assess the defense enzymes involved in the protection of plantain PIF seedlings against diseases.

The *T. diversifolia* treatments (Td+CS and Td) especially enhance respectively the germination rate (25.97 and 25.84%), the number of shoots (58.82 and 46.15%), the height of shoots (74.13 and 50.13%), diameter of pseudo stems (84.43 and 48.36%) and leaves area (50.96 and 41.40%) of plantain PIF seedlings compared to the controls. The results of this study are in accordance with one from a recent study that has shown the increase of the growth and yield after the use of *T. diversifolia* green biomass alone in the culture of cassava (Bilong et al., 2017). *T. diversifolia* seems to act as an organic fertilizer that probably improve the quality of the soil physicochemical and biological properties through increases of the seedling's growth and sensitivity to pathogens. Moreover, the combined effect of *T. diversifolia* leaves with inorganic fertilizers on the yield of maize, tomato and cassava has also been demonstrated (Kaho et al., 2011; Ngosong et al*.,* 2016; Bilong et al*.,* 2017). *T. diversifolia* tissues decompose rapidly and are richer in excellent physicochemical properties which probably provide the PIF substrates with elements such as nitrogen, magnesium, potassium (Oyerinde et al*.,* 2009), coupled to the action of key enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease), as well as the amelioration of nitrogen transport in functional leaves (Kaho et al*.,* 2011), for the acceleration of germination and plant growth promotion. However, the inhibitory effects of *T. diversifolia* during this research (data not shown) was noticed for some

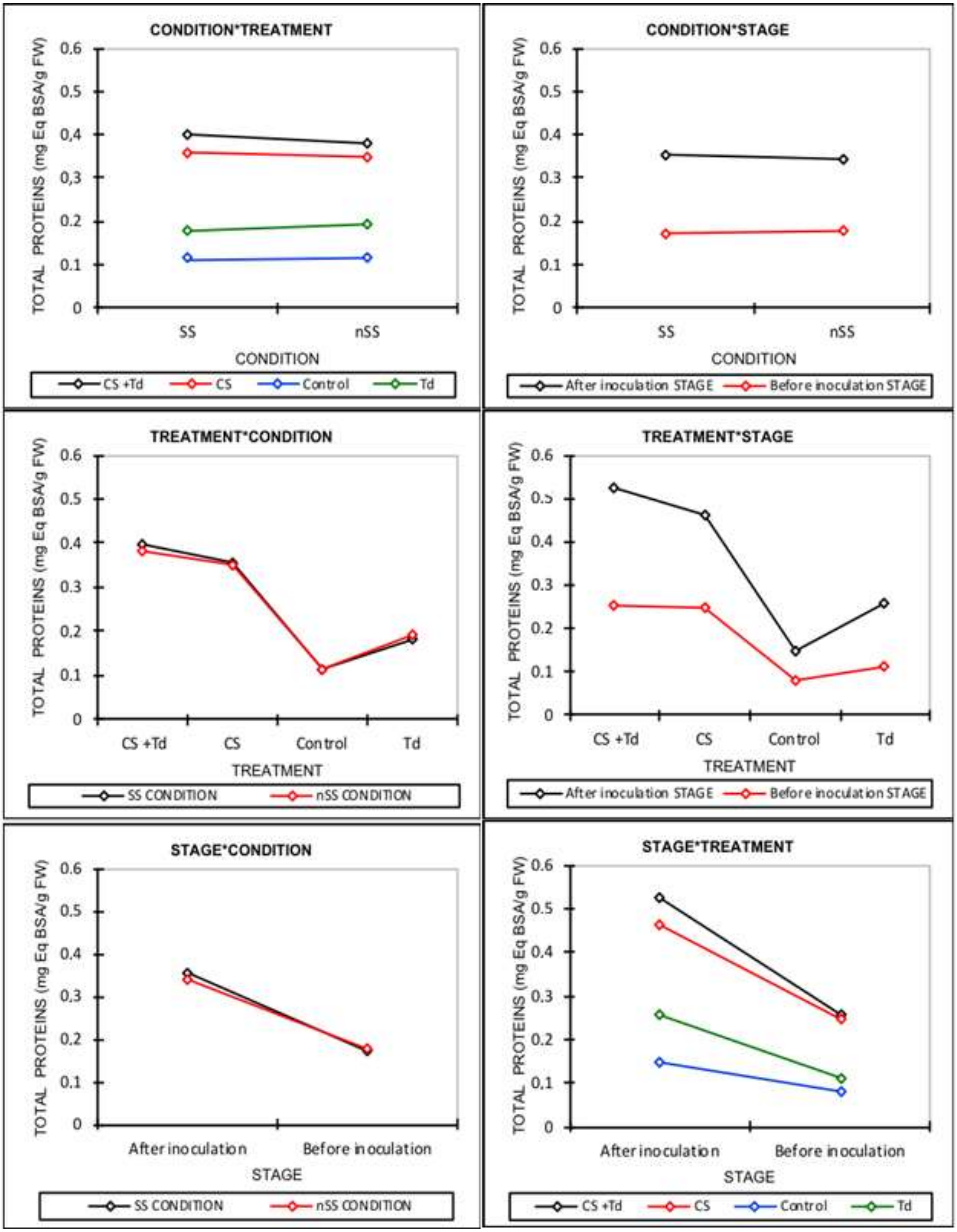


Figure 8. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings accumulation of total proteins at both stages (before inoculation and after inoculation). Each point represents the average mean of three replicates for each treatment.

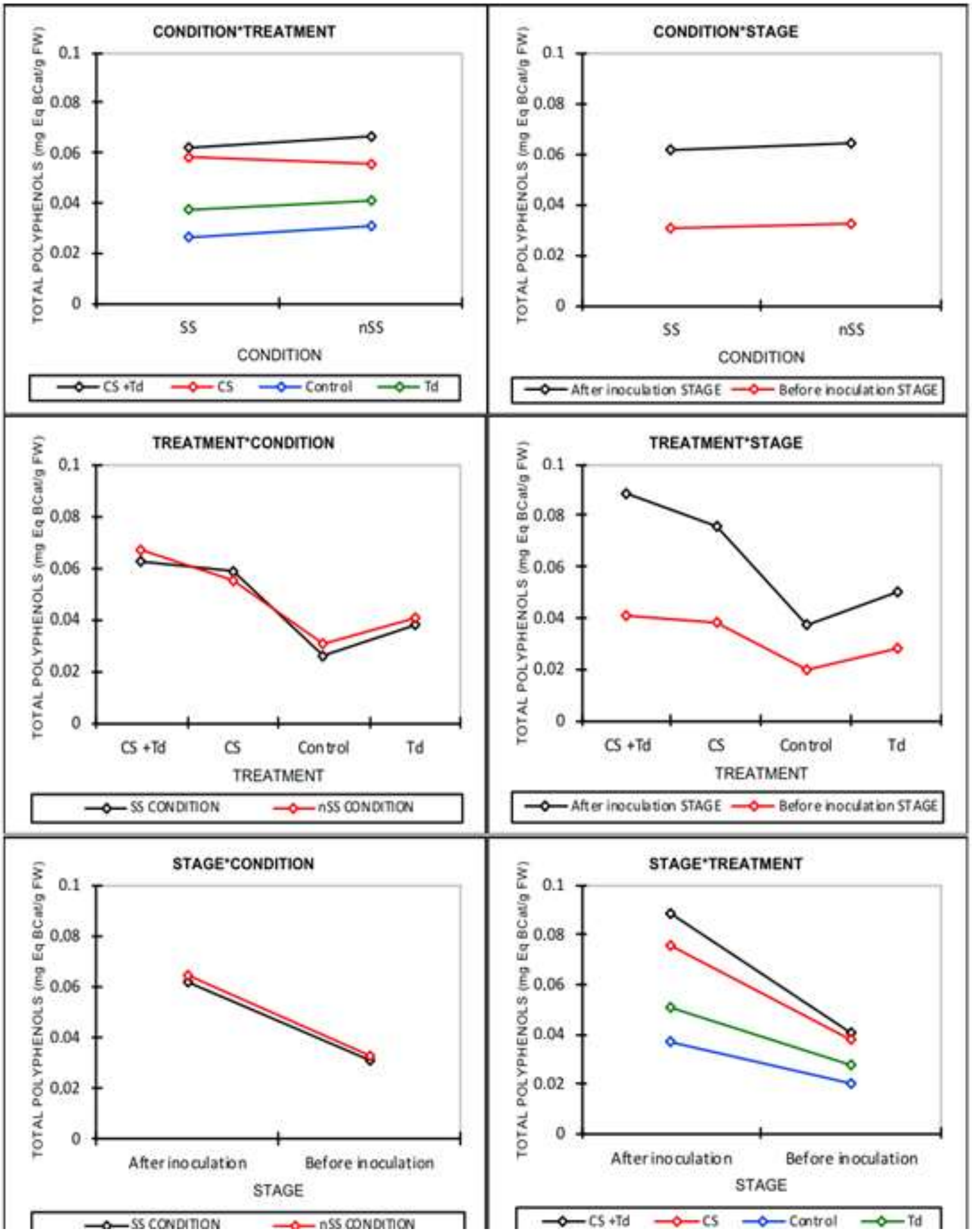
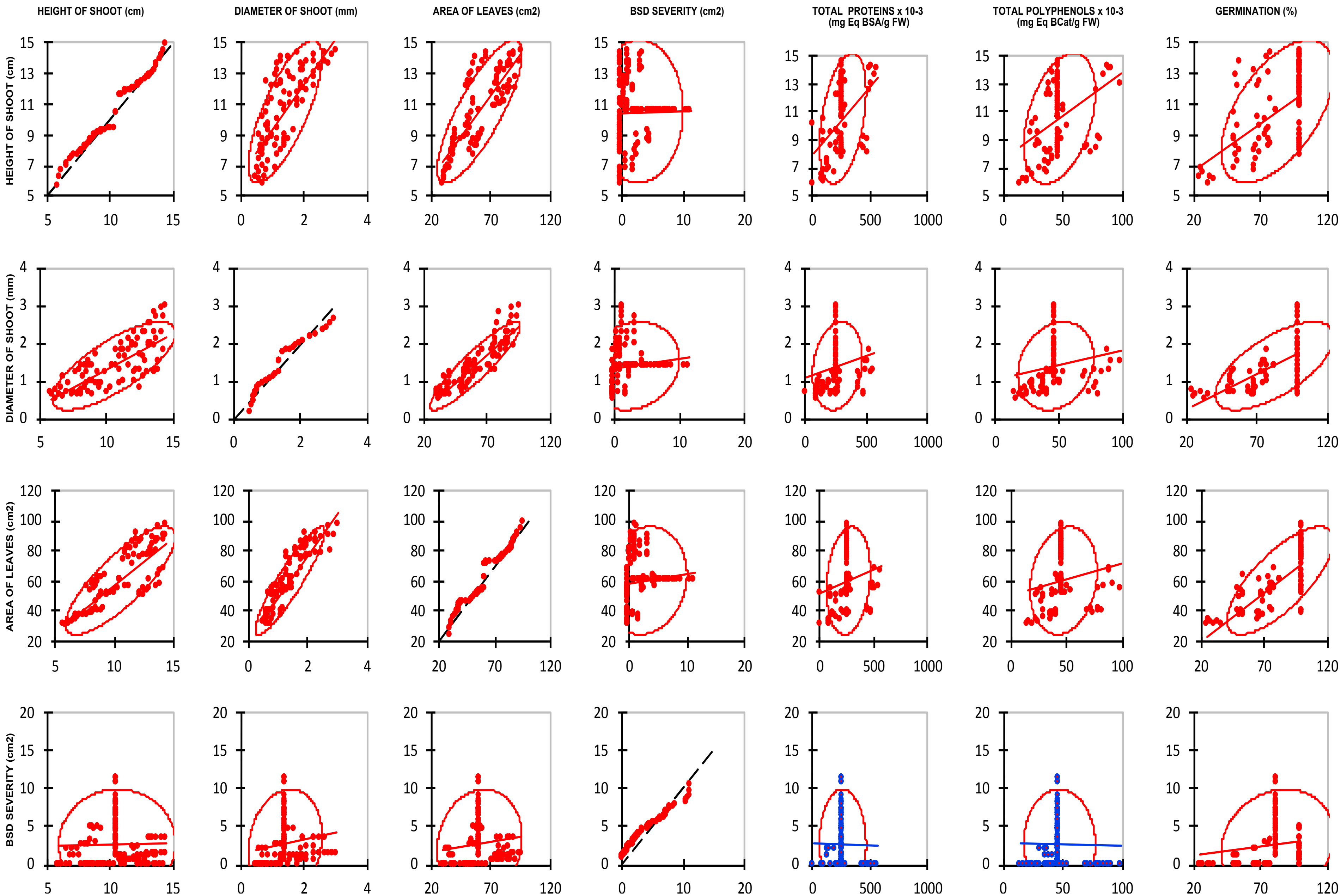


Figure 9. Interaction plots (condition, treatment and day) of the clam shells and *T. diversifolia* powder effects on the PIF plantain seedlings accumulation of total polyphenols at both stages (before inoculation and after inoculation). Each point represents the average mean of three replicates for each treatment.


HEIGHT OF SHOOT (cm)
DIAMETER OF SHOOT (mm)
AREA OF LEAVES (cm2)
BSD SEVERITY (cm2)
TOTAL PROTEINS x 10-3 (mg Eq BSA/g FW)
TOTAL POLYPHENOLS x 10-3 (mg Eq BCat/g FW)
GERMINATION (%)
HEIGHT OF SHOOT (cm)
DIAMETER OF SHOOT (mm)
AREA OF LEAVES (cm2)
BSD SEVERITY (cm2)

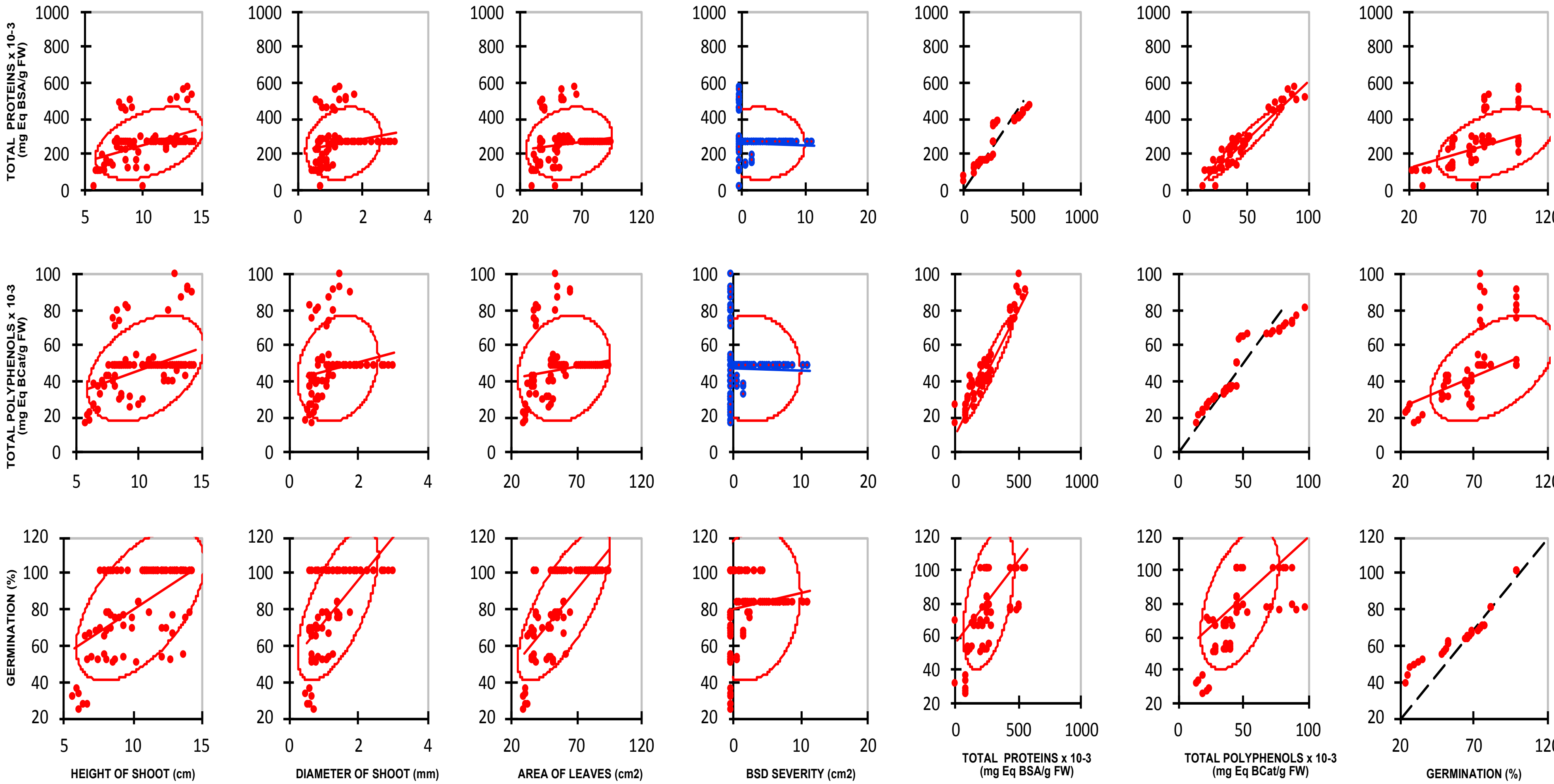


Figure 10. Relationship between the different variables of PIF plantain seedlings: germination, height of shoots, diameter of pseudo stems, area of leaves, BSD severity, total proteins and total polyphenols. The Scatter plots shows positive (red) or negative correlation (blue), but also the strength of the relationship.

concentrations. Indeed, the use of aqueous fresh shoot extract of *T. diversifolia* have shown *in vitro* both stimulatory and inhibitory effects on *Cleome gynandra* (spider plant) germination and growth (Hemsley and Gray, 2005).

The combination of clam shells and *T. diversifolia* powder show a synergic action with dual role both as a biofertilizer and a biopesticide since they affect the growth promotion of PIF plantain seedlings and their protection against *M. fijiensis* in nursery and could probably enhance agricultural yield in the field. Indeed, the major compounds present in *T. diversifolia* are nitrogen, magnesium, potassium, flavonoids, sesquiterpene lactone and alkaloids… (Oyerinde et al., 2009), while the ones in the clam shells are chitin, calcium and magnesium carbonate, proteins, (Khoushab and Yamabhai, 2010) and they all activated the growth promotion and natural defense systems through the increased synthesis of nutrients and defensive metabolites (Mondal et al*.,* 2012; Akter et al*.,* 2018; Malerba and Cerana, 2019). This association for plantain PIF seedlings treatment revealed significant increase of growth characters and less sensitivity to BSD compared to the controls, confirming a positive effect compared to the individual effects of *T. diversifolia* alone and clam shells alone. There is a need to assess this dual effect of the combined products at different ages and on other pathosystems.

The treatments containing clam shells (CS) especially improved significantly the total proteins and phenolics accumulation in plantain PIF seedlings. The amount of pre-existing proteins and polyphenols compounds is important in the treated seedlings and rises significantly after inoculation (*de novo* synthesized) compared to the controls. This amount double after infection for the treatment Td+CS compared to other treatments, especially that of *de novo* synthesis of Td treatment. Suggesting thus, different rates of accumulation depending probably on the level of sensitivity to diseases and the type of interaction (compatible or incompatible) establish between the plantain PIF seedlings and the *M. fijiensis* strain (Ewané et al*.,* 2012). The treatment confers an important pool of pre-existing and *de novo* synthesized proteins and polyphenols that seem to be enough to participate in defense reactions and to overcome infection.

A positive correlation was found between the total amount of proteins and polyphenols before and after inoculation, and all the agromorphological vegetative growth variables which are involved in their growth promotion, while it was negative for the BSD severity. A lack in this study lies in the fact that 12 days after inoculation (DAI) seem to be almost too late for the assessment of the biochemical events occurring in plantain PIF seedlings in the first hours and days after inoculation and the establishment of infections as previously suggested by Ewané et al*.* (2019). Therefore, there is a need to access the physiological mechanisms involved in the combination of clam shells and *T. diversifolia* powder effect on growth promotion and protection against diseases in plantain PIF seedling.

Conclusion

The clam shells and *T. diversifolia* treatment enhance efficiently plantain PIF seedlings quality in nursery and therefore behave as a seedling's vaccine against mortality in the fields. These results have shown that clam shells and *T. diversifolia* alone, or in association are able to play a dual role (biofertilizer and biopesticide) in PIF plantain seedlings growth positive regulation and improved defense responses against phytopathogens in terms of germination rate, number of shoots, length of shoots, diameter of pseudo stems, area of leaves, BSD severity, proteins accumulation and phenolic accumulation. However, the effect of the combination of both products was more efficient and has shown the best effects. There is a need to investigate the biochemical and molecular stimulation mechanisms involved in growth promotion and induced resistance against pathogens stimulation in the plantain PIF seedlings by clam shells and *T. diversifolia* powder treatments. Moreover, there is a need to continue this experimentation to the field in order to show the impact of this result compared to the conventional agriculture in terms of production costs, yield, productivity and the gains. Despite the fact that by-fishing products and bad herbs are environmentally benign compared to synthetic products, they are commonly neglected; hence, this study opens a way for their utilisation for an improved productivity, a sustainable and responsible agriculture, affordable for poor African small holders' farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Akter J, Jannat R, Hossain MM, Ahmed JU, Rubayet TM (2018). Chitosan for plant growth promotion and disease suppression against anthracnose in Chilli. International Journal of Environment, Agriculture and Biotechnology 3:3.

Bilong EG, Ngome AF, Abossolo-Angue M, Birang À Madong, Ndaka BSM, Bilong P (2017). Effets des biomasses vertes de *Tithonia diversifolia* et des engrais minéraux sur la croissance, le développement et le rendement du manioc (*Manihot esculenta* Crantz) en zone forestière du Cameroun. International Journal of Biological and Chemical Science 11(4):1716-1726.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Annals of Biochemistry 72:248-254.

Chagas-Paula DA, Oliveira RB, Rocha BA, da Costa FB (2012). Ethnobotany, chemistry and biological activities of the genus *Tithonia* (*Asteraceae*). Chemistry and Biodiversity 9:210-235.

El Hadrami I, Baaziz M (1997). Somatic embryogenesis and analysis of peroxidase in *Phoenix dactylifera*. Biologia Plantarum 37:197-203.

Ewané CA, Chillet M, Castelan F, Brostaux Y, Lassois L, Ngando EJ, Hubert O, Chilin-Charles Y, Lepoivre P, de Lapeyre de Bellaire L (2013). Impact of the extension of black leaf streak disease on banana sensitivity to post-harvest diseases. EDP Sciences. Fruits 68:351-365.

Ewané CA, Lepoivre P, de Lapeyre de Bellaire L, Lassois L (2012). Involvement of phenolic compounds in the sensitivity of bananas to crown rot. A review. Biotechnologie, Agronomie, Societé et Environnement 16(3):393-404.

Ewané CA, Ndongo F, Ngoula K, Tene Tayo PM, Opiyo SO, Boudjeko T (2019). Potential biostimulant effect of clam shells on growth promotion of plantain PIF seedlings (*var*. Big Ebanga & Batard) and relation to black Sigatoka disease susceptibility. American Journal of Plant Science 10:1763-1788.

Food and Agriculture Organization (FAO) (2017). Food and Agriculture Organization of the United Nations. FAO statistics: Bananas. Available at: http://faostat3.fao.org/home/index fr.htm

Hemsley A, Gray (2005). Allelopathic effects of Mexican sunflower *Tithonia diversifolia* on germination and growth of Spider plant (*Cleome gynandra* L.). Journal of Biodiversity and Environmental Sciences 2(8):26-35.

Kaho F, Yemefack M, Feudjio-Teguefouet P, Tchantchouang JC (2011). Effet combiné des feuilles de *Tithonya diversifolia* et des engrais inorganiques sur les rendements du maïs et les propriétés d'un sol ferralitique au Centre Cameroun. Tropicultura 29:39-45.

Khoushab F, Yamabhai M (2010). Chitin Research Revisited. Marine Drugs 8:1988-2012.

Kwa M (2002). Techniques horticoles de production de masse de plants de banane : la technique des plants issus de fragments de tige (PIF). Fiche technique CARBAP 4 p.

Lassoudière A (2007). Le bananier et sa culture. Editions Quae 384 p.

Malerba M, Cerana R (2019). Recent Applications of Chitin- and Chitosan-Based Polymers in Plants. Polymers 11:839.

Marigo G (1973). Méthode de fractionnement et d'estimation des composés phénoliques chez les végétaux. Analysis 12:106-110.

Mondal MMA, Malek MA, Puteh AB, Ismail MR, Ashrafuzzaman M, Naher L (2012). Effect of foliar application of chitosan on growth and yield in okra. Australian Journal of Crop Science 5:918-921.

Kerebba N, Oyedeji AO, Byamukama R, Kuria SK, Oyedeji OO (2019). Pesticidal activity of *Tithonia diversifolia* (Hemsl.) A. Gray and *Tephrosia vogelii* (Hook f.); phytochemical isolation and characterization: A review. South African Journal of Botany 121:366-376.

Ngosong C, Mfombep PM, Njume CA, Tening AS (2016). Comparative advantage of *Mucuna* and *Tithonia* residue mulches for improving tropical soil fertility and tomato productivity. International Journal of Plant Soil Science 12(3):1-13.

Onautshu OD (2013). Caractérisation des populations de *Mycosphaerella fijiensis* et épidémiologie de la cercosporiose noire du bananier (*Musa* spp.) dans la région de Kisangani-République Démocratique du Congo. Thèse de doctorat ès science. Université Catholique de Louvain 309 p.

Oyerinde RO, Otusanya OO, Akpor OB (2009). Allelopathic effect of *Tithonya diversifolia* on the germination, growth and cholorophyl of maize (*Zea mays* L.). Scientific Research and Essay 4(12):879-888.

Pirovani PC, Heliana ASC, Regina CR, Dayane SG, Fatima CA, Fabienne M (2008). Protein extraction for proteome analysis from cacao leaves and meristems, organs infected by *Moniliophthora perniciosa*, the causal agent for the witches' broom diseases. Electrophoresis Journal 29:2391-2401.

Pusztahelyi T, Holb IJ, Pócsi I (2015). Secondary metabolites in fungus-plant interactions. Frontiers in Plant Science 6:1-23.

Pusztahelyi T (2018). Chitin and chitin-related compounds in plant-fungal interactions. Mycology 9:189-201.

Téné TPM, Dzelamonyuy A, Omokolo ND, Boudjeko T (2019). Enhancement of *Theobroma cacao* seedling growth and tolerance to *Phytophthora megakarya* by heat-treated oyster shell powder. American Journal of Plant Sciences 10:578-594.

Téné TPM, Ewane CA, Effa OP, Boudjeko T (2017). Effects of chitosan and snail shell powder on cocoa (*Theobroma cacao* L.) growth and resistance against black pod disease caused by *Phytophthora megakarya.* African Journal of Plant Science 11:331-340.

Umar OB, Alex RD, Obukohwo EE (2015). Phytochemical and proximate composition of *Tithonia diversifolia* (Hemsl.) A. Gray. Annals Food Science and Technology 16:195-200.

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Full Length Research Paper

Response of leaf epidermal cells under ozone stress and ascorbic acid treatment in Pepper plant

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The present investigation studied the effectiveness of ozone (O_3) concentrations on epidermal cells of pepper (*Capsicum frutescens* L.) leaves and its response to ascorbic acid (AS). The plants were grown at two sites in Riyadh, King Saud University (KSU) Campus, and the industrial city (IC) under an average of 42.33 and 138.66 ppb of O_3. Two groups grown at KSU site as a control; one of them was treated with tap water (TW) and the other was treated with TW+AS, while the remaining two groups were transferred to IC site, treated as described previously. Treatment with 300 mg/L AS was performed once every 15 days until the end of the experiment. The plants grown under separately high concentrations of O_3 and AS increased the stomatal numbers, dimensions and cell dimensions in both upper and lower epidermises of leaves in comparison to control plant leaves. Treatment with O_3+AS significantly increased the length of the upper and lower epidermal cells, while it decreased the cell widths in comparison to plants grown under only O_3. The AS might have a mitigating effect on the impacts of O_3 on leaf epidermal cells of the pepper plant particularly, with respect to cell width.

Key words: Ozone, epidermal cell traits, pepper, ascorbic acid.

INTRODUCTION

Pepper (*Capsicum frutescens* L.) is an annual herb or shrub, and belongs to the Solanaceae family. It is one of the most important vegetables grown in parts of the humid and semi-arid tropics (Aliyu, 2000). The fruits are extensively used as a cooking condiment (Alabi, 2006). Pepper contains an excellent source of vitamins A and C as well as phenolic compounds, which are important antioxidant (Shotorbani et al., 2013). Pepper is also used for the prevention and treatment of cold and fever (Udoh et al., 2005), as it contains vitamin C (Osuna-García et al., 1998). In addition, capsaicin has been shown to have great potential as a chemotherapeutic agent against several cancers (Oyagbemi et al., 2010; Clark and Lee, 2016). The leaf surface of the plant is the major part that receives, absorbs, and accumulates air pollutants (Chauhan and Joshi, 2010). The gaseous pollutants enter the leaf through the stomata, which have the potential to alter the metabolic processes of the plant and react with the intercellular water to form reactive oxygen species (ROS) that act on the plasma membrane and cause oxidative stress in the mesophyll cells of the leaf (Bray, 2000; Roshchina and Roshchina, 2013; Iriti and Faoro,

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2008), cellular damage in the leaves, reduce photosynthesis, decrease carbon allocation to sink tissues, and affect plant biomass and radial growth (Wittig et al., 2009).

O_3 is one of the gaseous pollutants that have impact on many aspects of the plants, such as the morphological, physiological, and anatomical characteristics. These effects vary with the intensity and duration of O_3 exposure (Pasqualini, 2003). Exposure of alfalfa to high ozone concentrations (85-120 ppb) causes obvious effects on cell organelles such as chloroplasts, plastoglobules, nuclei, vacuoles and chromatin in leaf mesophyll tissue and stem cortex, and no clear effects of ozone were noted on starch grain shapes and the mitochondria in the leaf mesophyll and stem cortex cells (El-Zaidy et al., 2019). The stomatal density in the leaf epidermis of different plants was affected by elevated O_3 concentration (Evans et al., 1996; Paakkonen et al., 1998; Frey et al., 1996; Lawson et al., 2002; Zouzoulas et al., 2009; Gostin, 2009; Wahid and Ahmad, 2003; Pedroso and Alves, 2008), and caused sluggishness stomata efficiency, and gradual loss in stomatal control over transpiration (Feng et al., 2018). However, few works indicated lack of significant impact on the stomatal density of certain plants when exposed to O_3 stress (Giacomo et al., 2010; Riikonen et al., 2010; Dumont et al., 2014). Elevated O_3 concentration was reported to affect the epidermal cell density of the plant leaves (Lawson et al., 2002; Wahid and Ahmad, 2003; Pedroso and Alves, 2008; Zouzoulas et al., 2009; Riikonen et al., 2010). Moreover, Riikonen et al. (2010) reported that high concentration of O_3 increased the epidermal cell size with no obvious effect on cuticular striations and epicuticular wax crystallites. Meanwhile, ascorbic acid (AS) is a growth regulator that plays important roles in many physiological processes (Ejaz et al., 2012; Kim et al., 2008; Hathout, 1995; Mukherjee and Choudhuri, 1985). González-Reyes et al. (1998) reported the effective role of AS in stress resistance, whereas Veljovic-Jovanovic et al. (2001) found that AS concentration is low in the O_3-sensitive plant tissues, which confirms its role in oxidative stress. AS increased the thickness of both the midrib and lamina of leaf blades, the size of the main vascular bundle of the midrib, and also increased the average diameter of the vessel in the leaves of tomato plants, *Zea mays*, and *Mentha arvensis* (Ali, 2001; Ali et al., 2015; Hendi and Boghdady, 2016). Leaf seedlings of pre-soaked seeds with AS increased the stomatal length and decreased the epidermis cell length on both the surfaces. Although AS application reduced the epidermis cell number on the upper surface, it had no effect on this feature on the lower surface (Cavusoglu and Bilir, 2015). Treatment with AS increased the mitotic divisions and cellular dimensions in the cell elongation region of the plant root (Kaviani, 2014). Despite the vast amount of data on the effects of O_3 and AS on the physiological, biochemical, and molecular characteristics of plants; the effects of O_3 and AS on the epidermal cell traits of *C. frutescens* L. have not been studied in detail. Because the epidermis is the protective layer and acts as a barrier between the outer environment and the internal structures of the plant body, this research was an initiative to study the impact of high concentration of O_3 on the leaf epidermal cell traits, and its response to treatment by AS, which might assist in better understanding of the phenomena occurring in the leaves.

MATERIALS AND METHODS

Two sites were selected for this research in Riyadh city with different pollution levels; the first site was King Saud University campus for the control group (Cont.), and the second site was the industrial city (IC). Pepper seeds were obtained from a local market in Riyadh, Saudi Arabia. Seeds were sterilized with 1% sodium hypochlorite for 7 min, and then rinsed with sterilized double distilled water. Seeds were planted under natural environmental conditions in used plastic pots containing sterile sandy and alluvial soil (ratio 1:1). A fungicide was added to prevent the fungal growth; plants were left to grow until the generation of initial leaves, and then transferred to the study sites. The plants were divided into four groups: two control groups that were left to grow in King Saud University site; one of them was treated with tap water, and the other was treated with tap water and AS; while the remaining two groups were transferred to IC site upon exposure with O_3, where one of them was treated with tap water, and the other was treated with tap water and AS. Irrigation was performed once every 15 days using 300 mg/L AS until the end of the experiment. The leaf surfaces were cleaned with distilled water, followed by silicon rubber imprinting for studying the epidermal characteristics according to Lloyd (1908). The slides were then examined and photographed using Zeiss Photomicroscope III. Epidermal cell dimensions and the stomatal number and dimensions were captured at 40X. Twenty-five measurements were recorded for each parameter and the number of stomata was counted in a microscopic field area of 0.25 mm^2. All measurements and descriptions were recorded at the vegetative growth end before flowering (after 90 days of sowing). Measurement of O_3 concentrations was performed daily for three months at each of the study sites using a measuring device (AEROQUAL Series 200 with Monitor), average readings of ozone (O_3) levels in control plants site (KSU Campus) was 42.33 ppm, while in polluted plant site second site (IC) was 138.66 ppm. The data obtained were statistically analyzed using SAS version 8.2 (SAS 2002) in a completely randomized design (CRD) to test the differences among the treatment levels.

RESULTS AND DISCUSSION

The data indicate the differences in the O_3 levels at the study sites, where O_3 level at the first site (KSU Campus) was 42.33 ppb, which was within the global limits for air pollution by ozone in accordance to McCarthy and Lattanzio (2015); while it was 138.66 ppb in the second site (IC) indicated higher pollution levels than the global limits for air pollution by ozone. Hence, we expected to detect some effects on the epidermal traits, such as stomatal number and its dimensions, and epidermal cell size.

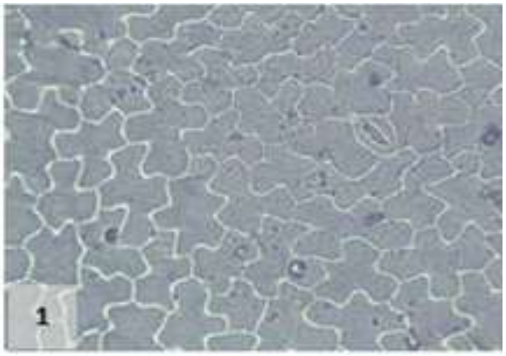

Figure 1. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

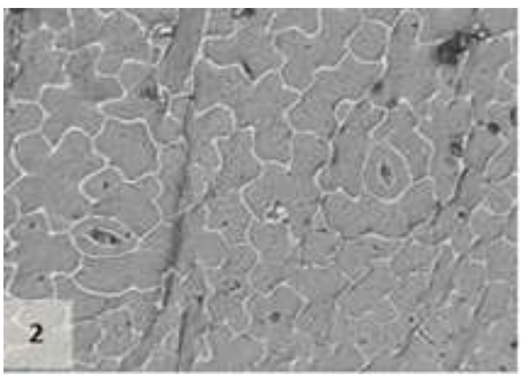

Figure 2. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

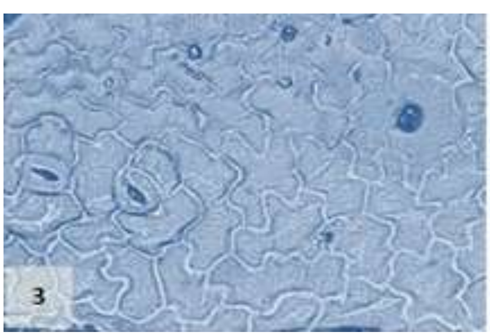

Figure 3. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

The results shown in Figures 1 to 11 indicate the differences in the epidermis traits of the studied pepper leaves. The results revealed that there were variations in the stomatal densities (Figures 1 to 8) between the upper and lower epidermal cell of the pepper leaves at King Saud University Campus site (control plants); this trait differed from that of the other plant species (AbdulRahaman and Oladele, 2003). The stomatal responses to the environmental changes are important to maintain the movement of gases and water in and out of the leaves (Hetherington and Woodward, 2003). As O_3 enters the plant leaves through the open stomata, the plant controls this process via stomatal closure or decreases stomatal conductance. Hence, the closure of stomata is a mechanism for controlling O_3 diffusion into the stomatal chamber for decreasing the O_3 concentrations in the mesophyll cells of the leaves (Madkour and Laurence, 2002). The results showed that the stomata of the plants that grew under high concentration of O_3 were affected in comparison to control plants (Figures 5 and 6), where the stomatal number increased significantly in both the upper and lower epidermis of the leaf, which indicated that O_3 might induce the increase in the stomatal numbers in both the epidermises. This result was consistent with the findings of the previous studies (Frey et al., 1996; Paakkonen et al., 1998; Zouzoulas et al., 2009). The stomatal dimensions decreased significantly in the lower epidermis of the leaf, while the stomatal length increased in the upper epidermis of the leaf of the plant grown under O_3. This was in line with the results of different studies (Zouzoulas et al., 2009; Gostin, 2009; Dumont et al., 2014).

The cell dimensions of the upper and lower epidermises of the leaves of the plants grown under O_3 were significantly increased (Figures 5 and 6) in comparison to control plant leaves; the mentioned changes may be due to the exposure to ozone. These observations were consistent with the results of some researchers, who revealed that high concentrations of O_3 affected the epidermal cell dimensions (Lawson et al., 2002; Wahid and Ahmad, 2003; Riikonen et al., 2010). The results also showed that AS application increased the number of stomata on both the upper and lower epidermis of the leaves in comparison to the control plant leaves (Figures 3 and 4). However, the increase in the stomatal number was significant only in the lower epidermis; this result was in line with that obtained by Arafa et al. (2014) and Cavusoglu and Bilir (2015). The AS application may have a role in increasing the stomatal connectivity (Hinckley and Braatne, 1994; Dieter et al., 1995). Application of AS also caused an increase in the stomatal width and length in the upper and lower epidermis of the leaf, which was significant only in the upper epidermis. The increase in the stomatal width indicates that AS might have an impact on the guard cells by increasing in its size, thereby increasing the

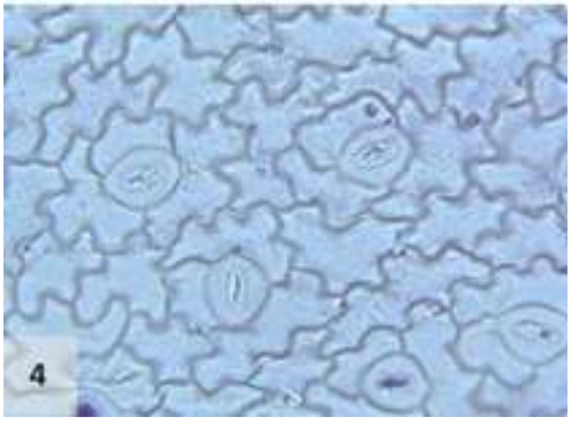

Figure 4. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

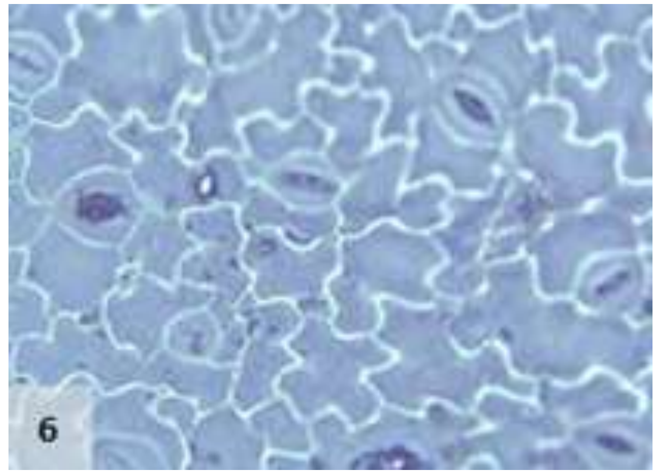

Figure 6. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

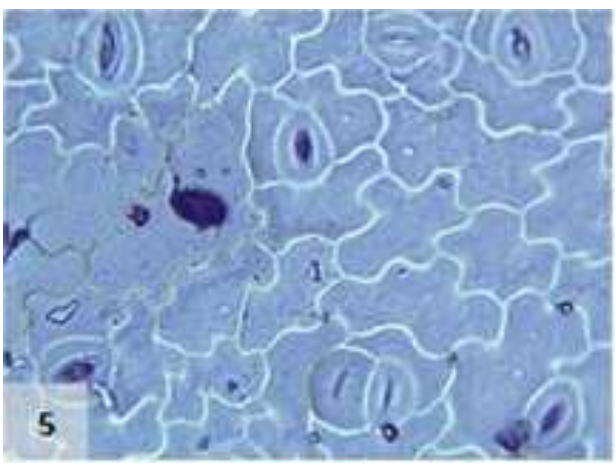

Figure 5. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

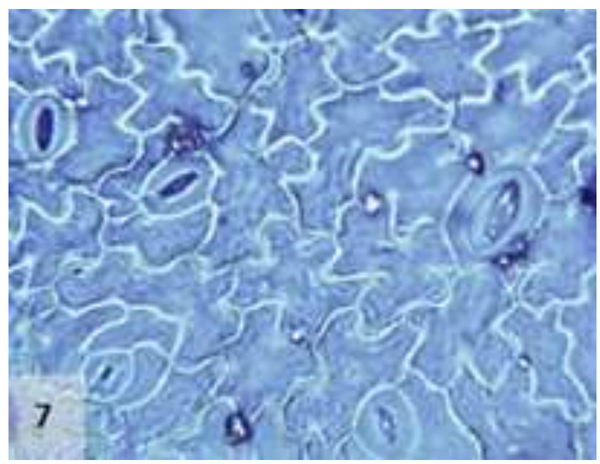

Figure 7. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

dimensions of the stomata. This finding is consistent with the results of previous research that indicated that AS influences the cell elongation in different parts of the plant body (De Gara et al., 1996; Tommasi et al., 1999; Horemans et al., 2000; Kaviani, 2014; Cavusoglu and Bilir, 2015). This could be due to the effect of AS on the crosslinking between the protein and polysaccharide in the cell wall that leads to loosening of the cell wall. Therefore, cell expansion and elongation (Padh, 1990; Smirnoff, 1996). Figures 1 and 3 show that the epidermal cell shapes were irregular, and the anticlinal cell walls were sinuous/undulate and appeared to be slightly changed, maybe because of the change in the dimensions of the cell.

There was an increase in the dimensions of the upper and lower epidermal cells of the leaves of the plants treated with AS (Figures 3 and 4); however, this increase was not significant in comparison to the leaves of control plant. This result agrees to a certain extent with the findings of Ali (2001), Ali et al. (2015), and Hendi and Boghdady (2016), where they reported that AS induces some anatomical changes in the plants. In addition, the present result was in conformity with the results of previous studies, which reported that AS plays a role in

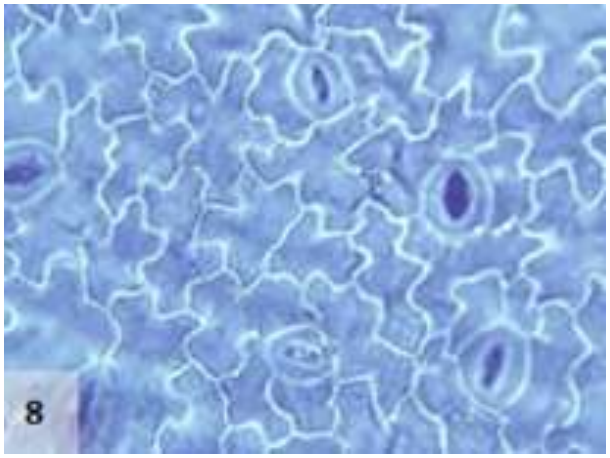

Figure 8. Surface view of the upper and lower epidermal cells of pepper leaves plant under study. (1, 2) cont.1: Upper Epidermis Cells (UE), 2: lower epidermis cells (LE). (3, 4) SA. 3: (UE), 4: (LE). (5, 6) O_3. 5: (UE), 6: (LE). (7, 8) O_3 +As. 7: (UE), 8: (LE). Ascorbic acid (AS), Ozone (O_3).

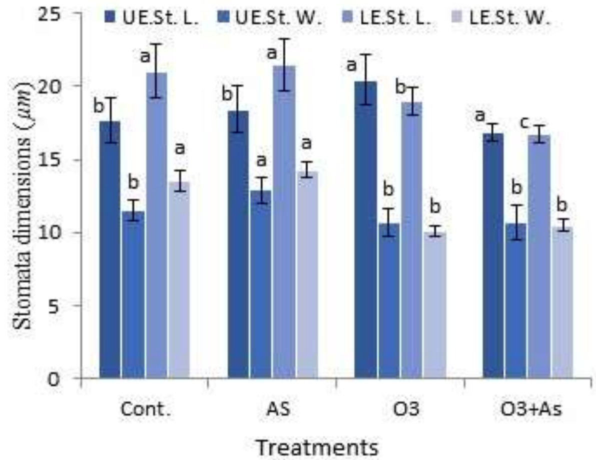

Figure 10. Average of Stomata dimensions in Pepper (*C. frutescens* L.) Leaves under Ozone (O_3), Ascorbic acid (AS) and O_3 +AS in the study sites.

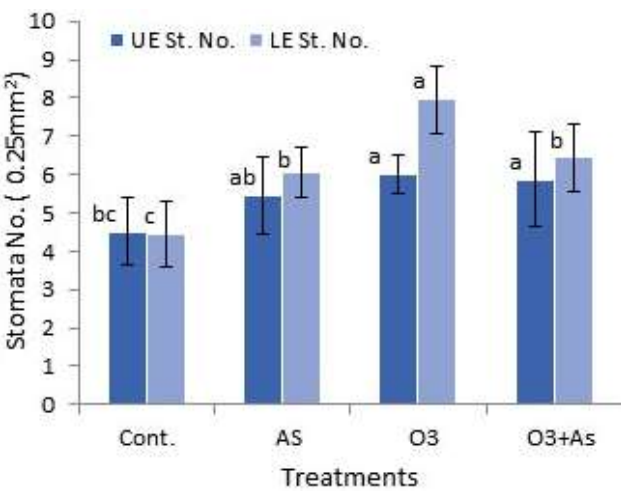

Figure 9. Average of Stomata numbers in Pepper (*C. frutescens* L.) Leaves under Ozone (O_3), Ascorbic acid (AS) and O_3 +AS in the study sites.

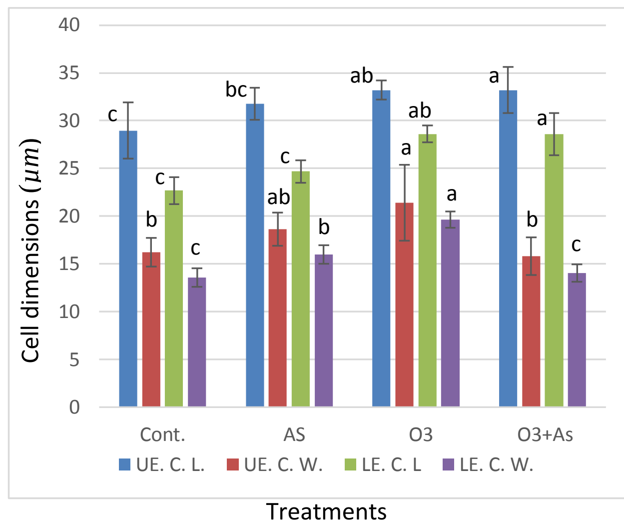

Figure 11. Average of cell dimensions in Pepper (*C. frutescens* L.) Leaves under Ozone (O_3), Ascorbic acid (AS) and O_3+AS in the study sites.

the expansion and elongation of the cells (Padh, 1990; Wang and Faust, 1992; De Gara et al., 1996; Smirnoff and Pallanca, 1996; Tommasi et al., 1999; Horemans et al., 2000; Kaviani, 2014; Cavusoglu and Bilir, 2015).

The results obtained also showed that AS application to the plants treated with O_3 decreased the stomatal number and their dimensions in both the upper and lower epidermis in comparison to control plant leaves (Figures 7 and 8), which was significant in the lower epidermis. These results may indicate that the effect of AS contradicts with the effect of O_3 in reducing the number of stomata in the leaves of the pepper plants. Moreover, the results were inagreement with the findings of previous studies, which reported that AS is a growth regulator and plays important roles in many physiological processes (Kim et al., 2008; Veljovic-Jovanovic et al., 2001; Ejaz et al., 2012). Additionally, AS improves plant tolerance and reduces the harmful effects of stress on plant growth (González-Reyes et al., 1998; Gadalla, 2009; Elwan and El-Hamahmy, 2009). AS also protects the plants from ROS, which are formed during periods of environmental stress associated with O_3 exposure (Runeckles and Chevone, 1992; Smirnoff and Pallanca, 1996; Conklin and Barth, 2004; Burkey et al., 2006).

The present results further show that there was a significant increase in the length of the upper and lower epidermis cells in the plants grown under O_3 and AS together (O_3 + AS) (Figures 7 and 8). On the other hand, there was no significant decrease in the width of the upper and lower epidermis cells of the treated leaves in comparison to control leaves. Plants grown under O_3 + AS had significantly increased length of the upper and lower epidermis cells, and significantly decreased width of the cells in comparison to the plants grown under only O_3. It appeared that the width of epidermal cells was more responsive to AS in comparison to their length.

Conclusion

The results of the present study revealed that high concentration of O_3 or AS increased the stomatal numbers and their dimensions, and the cell dimensions in both the upper and lower epidermises of the leaves of the pepper plant (*C. frutescens* L.) in comparison to the leaves of the control plant. Plants exposed to high concentration of O_3 and treated with AS had significantly increased length of the upper and lower epidermal cells. Therefore, we can hypothesize that ascorbic acid may have a mitigating effect on the impact of O_3 on the epidermal cell elongation of pepper leaves.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

AbdulRahaman A, Oladele F (2003). Stomatal complex types, size, density and index in some vegetable species in Nigeria. Nigerian Journal of Botany16:144-150.

Alabi D (2006). Effects of fertilizer phosphorus and poultry droppings treatments on growth and nutrient components of pepper (Capsicum annuum L). African Journal of Biotechnology 5(8):671-677.

Ali Z (2001) Ascorbic acid induced anatomical changes in the leaves and stems of tomato plants. Bulletin of the National Research Centre 26(3):371-382.

Ali Z, Hussein M, El-Taher A (2015). Effect of antioxidants on some morphological and anatomical features of maize grown under salinity conditions. International Journal of ChemTech Research 8(6):389-400.

Aliyu L (2000). Effect of Organic and Mineral Fertilizers on Growth, Yield and Composition of Pepper (*Capsicum annuum* L.). Biological Agriculture and Horticulture 18(1):29-36.

Arafa A, Khafagy M, Abo- El Kheer A, Fouda R, El-Banna M (2014). Stomatal Density in the Leaves of Sweet Pepper Plant as Affected by Certain Bio-Stimulants Under Salt Stress Conditions. Journal of Plant Production 5(4):649-662.

Bray EA (2000) Responses to abiotic stresses. Biochemistry and Molecular Biology of Plants pp. 1158-1203.

Burkey KO, Neufeld HS, Souza L, Chappelka AH, Davison AW (2006). Seasonal profiles of leaf ascorbic acid content and redox state in ozone-sensitive wildflowers. Environmental Pollution 143(3):427-434.

Cavusoglu K, Bilir G (2015). Effects of ascorbic acid on the seed germination, seedling growth and leaf anatomy of barley under salt stress. Journal of Agricultural and Biological Science 10:124-129.

Chauhan A, Joshi P (2010). Effect of ambient air pollutants on wheat and mustard crops growing in the vicinity of urban and industrial areas. New York Science Journal 3(2):52-60.

Clark R, Lee SH (2016). Anticancer properties of capsaicin against human cancer. Anticancer Research 36(3):837-843.

Conklin PL, Barth C (2004). Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. Plant, Cell and Environment 27(8):959-970.

De Gara L, De Pinto M, Paciolla C, Cappetti V, Arrigoni O (1996). Is ascorbate peroxidase only a scavenger of hydrogen peroxide. Plant peroxidases: biochemistry and physiology University of Geneva, Geneva pp. 157-162.

Dieter V, Matyssek R, Herppich W (1995).Experimentielle Pflanzenoekologie. Grundlagen und Anwendungen. https://www.amazon.de/Experimentelle-Pflanzen%C3%B6kologie-Dieter-von-Willert/dp/3131344016

Dumont J, Cohen D, GÉRard J, Jolivet Y, Dizengremel P, Le Thiec D (2014). Distinct responses to ozone of abaxial and adaxial stomata in three Euramerican poplar genotypes. Plant, Cell and Environment 37(9):2064-2076.

Ejaz B, Sajid ZA, Aftab F (2012). Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth parameters of Saccharum spp. hybrid cv. HSF-240 under salt stress. Turkish Journal of Biology 36(6):630-640.

El-Zaidy M, Al Musalim A, Al Sahli A, Doaigey A, Yakout SM, Arif IA, Saleh IA (2019). Effects of ozone on cell organelles of alfalfa (Medicago sativa L.) seedlings. Saudi Journal of Biological Sciences. In press.

Elwan MWM, El-Hamahmy MAM (2009). Improved productivity and quality associated with salicylic acid application in greenhouse pepper. Scientia Horticulturae 122(4):521-526.

Evans LS, Albury K, Jennings N (1996). Relationships between anatomical characteristics and ozone sensitivity of leaves of several herbaceous dicotyledonous plant species at Great Smoky Mountains National Park. Environmental and Experimental Botany 36(4):413-420.

Feng Z, Li P, Xu Y, Bo S (2018). The response of water use efficiency in poplar to elevated ozone In: International Conference on Ozone and Plant Ecosystems, 21-25 May, 2018. Florence, Italy.

Frey B, Scheidegger C, Gunthardt-Goerg MS, Matyssek R (1996). The effects of ozone and nutrient supply on stomatal response in birch (Betula pendula) leaves as determined by digital image-analysis and X-ray microanalysis. New Phytologist 132(1):135-143.

Gadalla S (2009). The role of antioxidants in inducing wheat flag leaf osmotic adjustment under salinity stress. Journal of Agricultural Science - Mansoura University 34(11):10663-10685.

Giacomo B, Forino LMC, Tagliasacchi AM, Bernardi R, Durante M (2010) Ozone damage and tolerance in leaves of two poplar genotypes. Caryologia 63(4):422-434.

González-Reyes JA, Córdoba F, Navas P (1998) Involvement of Plasma Membrane Redox Systems in Growth Control of Animal and Plant Cells. Plasma Membrane Redox Systems and their Role in Biological Stress and Disease. Springer Netherlands. doi:10.1007/978-94-017-2695-5_8

Gostin IN (2009) Air pollution effects on the leaf structure of some Fabaceae species. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37(2):57.

Hathout T (1995) Diverse effects of uniconazole and nicotinamide on germination, growth, endogenous hormones and some enzymic activities of peas. Egyptian Journal of Physiological Sciences (Egypt). 19(1-2):77-95.

Hendi D, Boghdady M (2016). Response of Spearmint Plants Grown under Sandy Soils Condition to some Growth Stimulators. International Journal of PharmTech Research 9(4):92-101.

Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. Nature 424(6951):901-908.

Hinckley T, Braatne J (1994) Stomata. Plant-environment Interactions pp. 323-355.

Horemans N, Foyer HC, Potters G, Asard H (2000). Ascorbate function and associated transport systems in plants. Plant Physiology and Biochemistry 38(7-8):531-540.

Iriti M, Faoro F (2008) Oxidative stress, the paradigm of ozone toxicity in plants and animals. Water, Air, and Soil Pollution 187(1-4):285-301

Kaviani B (2014). Effect of ascorbic acid concentration on structural characteristics of apical meristems on in vitro Aloe barbadensis Mill. Acta Scientiarum Polonorum Hortorum Cultus 13:3.

Kim JE, Jin DH, Lee SD, Hong SW, Shin JS, Lee SK, Jung DJ, Kang JS, Lee WJ (2008) Vitamin C inhibits p53-induced replicative senescence through suppression of ROS production and p38 MAPK activity. International journal of molecular medicine 22(5):651-655

Lawson T, Craigon J, Black CR, Colls JJ, Landon G, Weyers JDB (2002) Impact of elevated CO2 and O3 on gas exchange parameters and epidermal characteristics in potato (*Solanum tuberosum* L.). Journal of Experimental Botany 53(369):737-746.

Lloyd F (1908). The physiology of stomata, vol . 82. Carnegie Institution of Washington, Washington.

Madkour SA, Laurence JA (2002). Egyptian plant species as new ozone indicators. Environmental Pollution 120(2):339-353.

McCarthy JE, Lattanzio RK (2015) Ozone Air Quality Standards: EPA’s 2015 Revision. Congressional Research Service 29.

Mukherjee SP, Choudhuri MA (1985). Implication of hydrogen peroxide - ascorbate system on membrane permeability of water stressed vigna seedlings. New Phytologist 99(3):355-360.

Osuna-García JA, Wall MM, Waddell CA (1998). Endogenous Levels of Tocopherols and Ascorbic Acid during Fruit Ripening of New Mexican-Type Chile (*Capsicum annuum* L.) Cultivars. Journal of Agricultural and Food Chemistry 46(12):5093-5096.

Oyagbemi AA, Saba AB, Azeez OI (2010). Capsaicin: A novel chemopreventive molecule and its underlying molecular mechanisms of action. Indian Journal of Cancer 47(1):53.

Paakkonen E, Vahala J, Pohjola M, Holopainen T, Karenlampi L (1998). Physiological, stomatal and ultrastructural ozone responses in birch (Betula pendula Roth.) are modified by water stress. Plant, Cell and Environment 21(7):671-684.

Padh H (1990). Cellular functions of ascorbic acid. Biochemistry and Cell Biology 68(10):1166-1173.

Pasqualini S (2003). Ozone-Induced Cell Death in Tobacco Cultivar Bel W3 Plants. The Role of Programmed Cell Death in Lesion Formation. Plant Physiology 133 (3):1122-1134.

Pedroso ANV, Alves ES (2008). Anatomia foliar comparativa das cultivares de Nicotiana tabacum L. (Solanaceae) sensível e tolerante ao ozônio. Acta Botanica Brasilica 22(1):21-28.

Riikonen J, Percy KE, Kivimäenpää M, Kubiske ME, Nelson ND, Vapaavuori E, Karnosky DF (2010). Leaf size and surface characteristics of Betula papyrifera exposed to elevated CO2 and O3. Environmental Pollution 158(4):1029-1035.

Roshchina VV, Roshchina VD (2013). Ozone and plant cell. Springer Science and Business Media 267 p.

Runeckles V, Chevone B (1992). Crop responses to ozone. Surface-level ozone exposures and their effects on vegetation. Lefohn, A.S. (Ed.) edn. Lewis Publishers, Chelsea, Michigan

SAS (2002) User’s Guide, Statistics (Release 8.02). SAS Inst. Inc, Cary, NC, USA.

Shotorbani NY, Jamei R, Heidari R (2013). Antioxidant activities of two sweet pepper Capsicum annuum L. varieties phenolic extracts and the effects of thermal treatment. Avicenna Journal of Phytomedicine 3(1):25.

Smirnoff N (1996). Botanical briefing: The function and metabolism of ascorbic acid in plants. Annals of Botany 78(6):661-669.

Smirnoff N, Pallanca JE (1996) Ascorbate metabolism in relation to oxidative stress. Biochemical Society Transactions 24(2):472-478.

Tommasi F, Paciolla C, Arrigoni O (1999). The ascorbate system in recalcitrant and orthodox seeds. Physiologia Plantarum 105(2):193-198.

Udoh DJ, Ndon BA, Asuquo PE, Ndaeyo N (2005). Crop production techniques for the tropics. Ibadan: Concept Publications Limited.

Veljovic-Jovanovic SD, Pignocchi C, Noctor G, Foyer CH (2001). Low Ascorbic Acid in the vtc-1 Mutant of Arabidopsis Is Associated with Decreased Growth and Intracellular Redistribution of the Antioxidant System. Plant Physiology 127(2):426-435.

Wahid A, Ahmad S (2003). Environmental pollution and its effects on chilli (*Capsicum annuum* L.) grown in fields of Lahore [Pakistan]. Biologia (Pakistan).

Wang SY, Faust M (1992). Ascorbic acid oxidase activity in apple buds: relation to thidiazuron-induced lateral budbreak. HortScience 27(10):1102-1105.

Wittig VE, Ainsworth EA, Naidu SL, Karnosky DF, Long SP (2009). Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. Global Change Biology 15(2):396-424.

Zouzoulas D, Koutroubas SD, Vassiliou G, Vardavakis E (2009). Effects of ozone fumigation on cotton (*Gossypium hirsutum* L.) morphology, anatomy, physiology, yield and qualitative characteristics of fibers. Environmental and Experimental Botany 67(1):293-303.

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Genetic gain of maize (*Zea mays* L.) varieties in Ethiopia over 42 years (1973 - 2015)

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Currently under production, thirty-eight Ethiopian maize varieties released majorly for three agro-climatic zones over the past thirty-nine, twenty-nine and twelve years for the high altitudes, mid–altitudes and low altitudes, respectively, were conducted at three different research center's field trials, using randomized complete block design with three replications in 2015 main cropping season to estimate the genetic gains made on yield and yield related traits. The regression analysis indicated average annual and annual relative genetic gains of 62.3 (0.19%), 59.0 (0.57%) and –2.64 (–0.16%) in kg ha^{-1} yr^{-1} for grain yields, respectively, at Ambo Plant Protection Research Center (APPRC), Bako National Maize Research Center (BNMRC) and Melkassa Agricultural Research Center (MARC). Correlational analysis on the field studied traits indicated positively significant associations of grain yields with grain filling rate, ear length, number of kernels per row, number of ears per plant, biomass production rate, biomass yield and harvest index; also, negatively significant associations were shown for days to anthesis and days to silking at APPRC. Grain yield showed positively significant associations with ear length, plant height, grain filling rate, thousand kernel weight, biomass production rate and harvest index at BNMRC, while those only with harvest index were shown at MARC. Relatively considerable genetic gains and inconsiderable genetic reductions due to grain yields, grain yield related traits and grain yields associations with the other studied maize breeding traits had been observed across the released maize varieties from the three agro–ecological zones of Ethiopia.

Key words: Annual genetic gains, annual relative genetic gains, correlational analysis, highland maize, lowland maize, mid–altitude maize, regression analysis.

INTRODUCTION

Maize (*Zea mays* L.) arrived in Africa through various introductions as long ago as 500 years (McCann, 2005). Since its introduction to Africa, maize has thus become the number one crop in Africa both in cultivated area and

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total grain production (FAOSTAT, 2015). It is believed that maize was first introduced to Ethiopia in the 16th or 17th century (Haffnagel, 1961). Since its introduction, it has gained importance as a food and feed crop in the country, which has remained being considered as one of the priority crops in an effort to meet the food demand of the country's increasing population.

In Ethiopia, maize grows from moisture stress areas to high rainfall areas and from lowlands to the highlands (Kelemu and Mamo, 2002). Amongst the cultivated major cereal crops of Ethiopia, maize ranks second to *teff* [*Eragrostis tef* (Zucc.)] in area and first in production. Maize remained to be the largest and most productive crop, leading the major cereal crops in Ethiopia since the mid–1990s in terms of both crop yield and production. Over the last decades, maize coverage has reached 2.4 million ha from being a mere garden crop to an economical cereal crop in Ethiopia. The trends in national maize productivity levels show a small but consistent increase from about 1.5 t ha^{-1} in the early 1990s to 2.3 t ha^{-1} in the late 2000s (CSA, 2015).

Maize research in Ethiopia started in the early 1950s and passed through distinct stages of research and development (Kebede et al., 1993). Since 1973, the maize research program of Ethiopia has been receiving International Maize and Wheat Improvement Center (CIMMYT) germplasms (Mosisa et al., 2001). In the late 1990s, the breeders began to develop inbred lines from different source materials using the pedigree breeding method. Currently, the maize breeding program introduces fixed or intermediate (semi–processed) inbred lines from international research institutes such as the CIMMYT and International Institute of Tropical Agriculture (IITA) (Legesse et al., 2012).

In Ethiopia, right from the beginning of the comprehensive maize breeding program in the early 1980s, the maize breeding program has passed through many distinct stages of research and development (Degene and Habtamu, 1993). Progressively in the 1990s, the multidisciplinary approach was consolidated. Currently, the strategic focus of Ethiopia's public sector maize breeding programs are to develop improved maize varieties and hybrids for three specific types of agro–ecological zones (low, medium and high altitude maize growing areas of the country); four types of varieties (extra–early, early, intermediate, and late maturing varieties); and four types of attributes (yield improvement, drought tolerance, earliness and disease resistance) (Ethiopian Agricultural Research Organization (EARO), 2000; Ministry of Agriculture and Rural Development (MoARD), 2004–2016; Dawit et al., 2010).

In the last four decades, more than 40 improved varieties of maize including hybrids and Open Pollinated Varieties (OPVs) have been developed and released by the Ethiopian Institute of Agricultural Research (EIAR) in collaboration with the CIMMYT (Zeng et al., 2014). Despite maize suited diverse agro–climate subsists, and strategic maize breeding efforts were made, the production of maize in the country remained low; with the estimated national average yield of 3.25 t ha^{-1} (CSA, 2014), which is low in light of the potential productivity of the world average of 5.64 t ha^{-1} with a productivity record of 10.73 t ha^{-1} by the US for the year 2014/15 (United States Agency for International Development–Foreign Agricultural Service (USDA–FAS), 2016).

Quantifying breeding achievements in yield and associated traits and understanding the crucial characteristics of the crop associated with the genetic gains achieved through breeding is an essential step for improving the current knowledge of yield limiting factors and the design for the future breeding strategies. Historical series of maize varieties have been deployed and used to assess the genetic gains achieved during a period of time through breeding in several countries: in Argentina by Echarte et al. (2013), USA by Russell (1985), Duvick (2005a), Jason et al. (2013) and Chen et al. (2016), and Africa by Badu–Apraku et al. (2013, 2014) and Omolaran et al. (2014). However, in Ethiopia there are few and scanty information that exist on the genetic gains in grain yield and other agronomic traits during the maize breeding eras for the released and registered highland, mid–altitude and lowland maize varieties in Ethiopia.

Periodic evaluation of genetic improvement of improved maize varieties will help identify traits of potential value for future breeding enhancement and target them for higher productivity for the majority of subsistence farmers engaged in the production of the maize crop. With these, the objectives of this study were to estimate the genetic gain made over decades and to identify changes in morpho–physiological characters associated with genetic improvements in grain yield potential of maize varieties.

MATERIALS AND METHODS

Description of experimental sites and materials

The experiment was conducted on three sets of seven (7) highland, twenty (20) mid–altitude and eleven (11) lowland maize varieties that have been released in Ethiopia and currently under production over the past forty–two (42) years; they were grown at APPRC (08°57'N, 38°07'E, altitude 2225 m), BNMRC (09°06'N, 37°09'E, altitude 1650 m) and MARC (08°25'N, 39°20'E, altitude 1550 m) respectively. A total of thirty–eight (38) maize varieties used in the experiment are summarized in Table 1.

Owing to the suited diverse agro–climatic conditions in Ethiopia, maize growing areas are broadly classified into four ecological zones: high altitude moist (1800–2400 m), mid–altitude moist (1000–1800 m), low altitude moist (< 1000 m) and moisture stress (500–1800 m) (EARO, 2000; Mandefro et al., 2001). The strategic focus of Ethiopia's public sector maize breeding programs is highlighted by efforts to develop improved maize varieties and hybrids for three specific types of zones categorized as highland (1800–2400 m), mid–altitude (1000–1800 m) and lowland (500–1800 m) (MoARD, 2004–2016; Dawit et al., 2010). Accordingly, the experiment was done on the three different agro–ecological maize–growing zones of the country.

Table 1. Descriptions of Ethiopian highland, mid–altitude and lowland maize varieties used for the experiments.

Variety name	Variety type	Year of release	Breeder (Maintainer)	Altitude (m)	Seed color
Highland maize varieties					
Alemaya Composite	OPV	1973	Haramaya University	1600–2200	White
Kuleni	OPV	1995	EIAR/BNMRC	1700–2200	White
Rare–1	OPV	1997	Haramaya University	1600–2200	White
AMH800	Hybrid	2005	EIAR/APPRC	1800–2500	White
AMH850	Hybrid	2008	EIAR/APPRC	1800–2600	White
AMH851	Hybrid	2009	EIAR/APPRC	1800–2600	White
AMH760Q	Hybrid	2012	EIAR/APPRC	1600–2400	White
Mid–altitude maize varieties					
Abobako	OPV	1986	EIAR/BNMRC	500–1000	White
BH140	Hybrid	1988	EIAR/BNMRC	1000–1800	White
Guto–LMS	OPV	1988	EIAR/BNMRC	1000–1700	White
BH660	Hybrid	1993	EIAR/BNMRC	1600–2200	White
BH540	Hybrid	1995	EIAR/BNMRC	1000–2000	White
PHB3253	Hybrid	1995	Pioneer Hi–Bred	1000–2000	White
Gibe–1	OPV	2001	EIAR/BNMRC	1000–1700	White
BH670	Hybrid	2002	EIAR/BNMRC	1700–2400	White
Gambela Composite	OPV	2002	EIAR/BNMRC	300–1000	White
BH543	Hybrid	2005	EIAR/BNMRC	1000–2000	White
HB30G19	Hybrid	2006	Pioneer Hi–Bred	1000–2000	White
SC627	Hybrid	2006	Syngenta	1000–2000	White
HQPY545	Hybrid	2008	EIAR/BNMRC	1000–1800	Yellow
BH661	Hybrid	2011	EIAR/BNMRC	1600–2400	White
P2859W	Hybrid	2011	Pioneer Hi–Bred	1000–2000	White
Gibe–2	OPV	2011	EIAR/BNMRC	1600–1800	White
P3812W	Hybrid	2012	Pioneer Hi–Bred	1000–2000	White
BH546	Hybrid	2013	EIAR/BNMRC	1000–1800	White
BH547	Hybrid	2013	EIAR/BNMRC	1000–1800	White
P3506W	Hybrid	2015	Pioneer Hi–Bred	800–1800	White
Lowland maize varieties					
Melkasa1	OPV	2001	EIAR/MARC	1000–1750	Yellow
Melkasa2	OPV	2004	EIAR/MARC	1200–1700	White
Melkasa3	OPV	2004	EIAR/MARC	1200–1700	White
Melkasa4	OPV	2006	EIAR/MARC	1000–1600	White
Melkasa5	OPV	2008	EIAR/MARC	1000–1700	White
Melkasa6Q	OPV	2008	EIAR/MARC	1000–1750	White
Melkasa7	OPV	2008	EIAR/MARC	1000–1750	Yellow
MHQ138	Hybrid	2012	EIAR/MARC	1000–1800	White
MH130	Hybrid	2012	EIAR/MARC	1000–1800	White
MH140	Hybrid	2013	EIAR/MARC	1000–1800	White
Melkasa1Q	OPV	2013	EIAR/MARC	1000–1750	Yellow

Source: MoARD (2004–2016).

Experimental design and field management

All the experiments were laid out in a Randomized Complete Block Design (RCBD) with three replications. The three sets of experimental units consisted of four (4) rows of 5.25 m long (with spacing of 0.75 m between rows × 0.25 m between plants), 5.1 m (0.75 m between rows × 0.30 m between plants) and 5 m (0.75 m between rows × 0.25 m between plants), respectively, at APPRC, BNMRC and MARC.

Planting for the three sets of experiments were undertaken on June 05 and 08, 2015 respectively at BNMRC for the mid–altitude maize varieties and at APPRC for the highland maize varieties;

while on July 09, 2015 for the lowland maize varieties at MARC by hand sowings two seeds per hill, which were later thinned to one plant per hill. The same field managements were used for the three sets of experiments, on which pre–emergence herbicides (Atrazine at the rate of 4 L ha^{-1} for broad leaved weeds and Primagram at the rate of 4 L ha^{-1} for grass weeds), nitrogen fertilizer in the form of Urea and phosphorus fertilizer in the form of Diammonium Phosphate were applied as per the specific recommendations for the areas. Similarly, hand weeding was done twice at 25 and 45 days after emergence; and weed slashing was done once at the flowering stages.

Statistical analysis

All measured parameter's field data were subjected to an Analysis of Variance (ANOVA) using SAS statistical software version 9.00 (SAS, 2002) to estimate the prevalent variation among the test varieties. Treatments and replications were the class variables, while the response variables were the traits considered for the data collected. The ANOVA Model:

$$Y_{ij} = m + G_i + B_j + e_{ij}$$

Where:
Y_{ij} = Observed value of genotype i in block j
m = Grand mean of the experiment
G_i = Effect of genotype i
B_j = Effect of block j
e_{ij} = Random error effect of genotype i in block j

The test of mean separation was employed depending on the significance of ANOVA. Mean separation was undertaken using Duncan's Multiple Range Test (DMRT) at the 5% level of significance. Correlation among all the traits was calculated using the means of each variety applying the PROC CORR procedure in SAS. Linear regression analysis was used to calculate the genetic gain for each trait considered in the study. The breeding effects were estimated as a genetic gain for grain yield and associated agronomic traits in maize improvement by regressing mean of each character for each variety against the year of release of the variety using the PROC REG procedure in SAS. The relative gain achieved over the year of release periods for each traits under consideration were determined as a ratio of genetic gain to the corresponding mean value of old variety and was expressed as a percentage using software program Microsoft Office (Excel 2010).

The annual rate of gain was calculated as:

$$\text{Annual rate of gain (b)} = \frac{\text{Cov XY}}{\text{Var X}}$$

Where: Cov = Covariance
Var = Variance
X = the year of variety release
Y = the mean value of each character for each variety

The correlation between traits using means of each variety was calculated as:

$$\text{Correlation coefficient between X and Y (rxy)} = \frac{\text{Cov (X, Y)}}{\sqrt{\text{Var(X)Var(Y)}}}$$

Where:
rxy = Correlation coefficient between X and Y
Cov (X, Y) = Covariance between X and Y
Var (X) = Variance of X
Var (Y) = Variance of Y

RESULTS

Analysis of variance of grain yield and other agronomic traits of Ethiopian highland, mid–altitude and lowland maize varieties

Analysis of variance for grain yield traits indicated significant (P≤ 0.05 and P≤ 0.01) differences for number of ears per plant, ear length, number of kernels per row, grain yield, biomass yield, biomass production rate and harvest index. In contrast, non–significant (P> 0.05) differences were observed among the seven highland maize varieties for ear diameter, number of kernel rows per ear and thousand kernel weight at APPRC, while highly significant (P≤ 0.01) differences were observed for all studied yield and productivity traits of the twenty mid–altitude maize varieties at BNMRC (Table 2). Results of the analysis of variance for the studied grain yield traits of the eleven lowland maize varieties at MARC indicated highly significant (P≤ 0.01) differences among varieties for the number of ears per plant, ear length, grain yield, biomass yield, biomass production rate and harvest index while significant (P≤ 0.05) differences among varieties were shown in number of kernels per row. Ear diameter and number of kernel rows per ear showed non–significant (P> 0.05) difference amongst the studied grain yield traits (Table 2).

The analysis of variance for the growth and phenological traits of the seven highland maize varieties studied at APPRC showed highly significant (P≤ 0.01) differences that were observed for days to anthesis, days to silking, grain filling rate and ear height; whereas non–significant (P> 0.05) differences were observed for days to maturity, grain filling period and plant height. Further, the results of the analysis of variance for all growth and phenological traits of the twenty mid–altitude maize varieties and the eleven lowland maize varieties studied, respectively at BNMRC and MARC, showed highly significant (P≤ 0.01) differences (Table 3).

Genetic gains in grain yield and other agronomic traits of Ethiopian highland, mid–altitude and lowland maize varieties

Regression of the mean values of the highland maize varieties correspondingly with the year of releases over the past 39 years demonstrated positive and non–significant (P> 0.05) annual predictive and average relative genetic gain of 62.26 (1.24%) kg ha^{-1} yr^{-1} for grain yield and 76.37 (0.37%) kg ha^{-1} yr^{-1} for biomass yield at APPRC (Figure 1A and B).

Positively significant (P≤0.05) annual and relative annual genetic improvement trend was made over the highland maize varieties for number of ears per plant by 0.0081 (0.90%) ear $plant^{-1}$ yr^{-1} while, negatively non-significant (P>0.05) genetic reductions of thousand kernel weight by -0.43 (-0.14%) g. yr^{-1} and ear diameter by

Table 2. Mean squares for the studied grain yield traits of Ethiopian highland, mid–altitude and lowland maize varieties evaluated at APPRC, BNMRC and MARC (2015).

Source	NEP	Ear Length	Ear Diameter	NKE	NKR	TKW	Grain Yield	Biomass Yield	BPR	Harvest Index
Highland maize varieties										
Variety (6)[a]	0.061**	1.858*	0.041^{ns}	0.608^{ns}	7.169*	2103.385^{ns}	5871455**	15843554**	542.514**	48.353**
Error (12)	0.007	0.565	0.026	0.283	1.698	836.164	437775	2442467	69.809	3.246
Mean	1.122	19.938	4.623	13.286	38.31	303.629	6691.179	22918.44	132.125	28.958
CV (%)	7.332	3.769	3.513	4.003	3.401	9.524	9.888	6.819	6.324	6.222
R^2	0.824	0.64	0.46	0.565	0.683	0.607	0.89	0.829	0.829	0.886
Mid–altitude maize varieties										
Variety (6)[a]	0.177**	7.562**	0.307**	2.814**	16.793**	6307.135**	5627111**	28456387**	1288.482**	22.779**
Error (12)	0.056	0.718	0.095	0.349	6.52	338.264	1026574	7100042	344.206	3.285
Mean	1.312	19.151	4.928	15.393	41.637	326.97	8544.83	22840.95	158.006	37.372
CV (%)	18.033	4.424	6.261	3.84	6.133	5.625	11.857	11.666	11.742	4.85
R^2	0.636	0.853	0.625	0.802	0.565	0.903	0.735	0.670	0.654	0.779
Lowland maize varieties										
Variety (6)[a]	0.168**	2.441**	0.105^{ns}	0.563^{ns}	9.372*	704.267*	989364**	5140983**	175.528**	220.758**
Error (12)	0.005	0.662	0.048	0.294	2.965	174.238	122137	266761	19.477	26.681
Mean	0.855	13.882	3.674	12.87	27.585	181.206	1610.233	5973.738	56.665	27.568
CV (%)	8.602	5.86	5.947	4.214	6.243	7.284	21.704	8.646	7.788	18.737
R^2	0.942	0.656	0.549	0.492	0.632	0.671	0.817	0.909	0.824	0.819

[a] **–** Degrees of freedom. R^2 **–** Coefficient of determination. ***,** –** Significant at 0.05 and 0.01 level of probability, respectively. ^{ns} **–** non–significant. NEP **–** Number of Ears per Plant, NKE **–** Number of Kernel Rows per Ear, NKR **–** Number of Kernels per Row, TKW **–** Thousand Kernel Weight and BPR **–** Biomass Production Rate.

Table 3. Mean squares for the studied growth and phenological traits of Ethiopian highland, mid–altitude and lowland maize varieties evaluated at APPRC, BNMRC and MARC (2015).

Source	Days to Anthesis	Days to Silking	Days to Maturity	GFP	GFR	Plant Height	Ear Height
Highland maize varieties							
Variety (6)[a]	14.825**	24.079**	26.873^{ns}	10.968^{ns}	906.221**	309.464^{ns}	719.052**
Error (12)	2.516	2.341	33.611	32.825	75.211	115.708	37.778
Mean	93.619	95.571	173.524	79.905	83.75	259.952	142.929
CV (%)	1.694	1.601	3.341	7.17	10.355	4.138	4.3
R^2	0.783	0.856	0.435	0.364	0.868	0.746	0.918
Mid–altitude maize varieties							
Variety (19)[a]	30.126**	36.852**	6.74**	23.891**	1034.726**	1153.942**	1289.366**
Error (38)	2.239	2.646	1.348	4.089	205.054	75.963	64.795
Mean	74.933	75.95	144.567	69.633	122.656	297.84	154.253
CV (%)	1.997	2.142	0.803	2.904	11.675	2.926	5.218
R^2	0.872	0.88	0.726	0.757	0.717	0.895	0.913
Lowland maize varieties							
Variety (10)[a]	106.339**	133.358**	835.024**	445.424**	1406.018**	107.697**	272.564**
Error (20)	1.803	2.885	12.579	13.57	64.856	27.352	13.027
Mean	62.576	64.485	105.182	42.848	41.928	121.303	58.97
CV (%)	2.146	2.634	3.372	8.597	19.208	4.311	6.121

Table 3. Contd.

R^2	0.968	0.959	0.971	0.943	0.918	0.671	0.914

[a] – Degrees of freedom; R^2 – Coefficient of determination; [**] – Significant at 0.01 level of probability; [ns] – non–significant; GFP – Grain Filling Period and GFR – Grain Filling Rate.

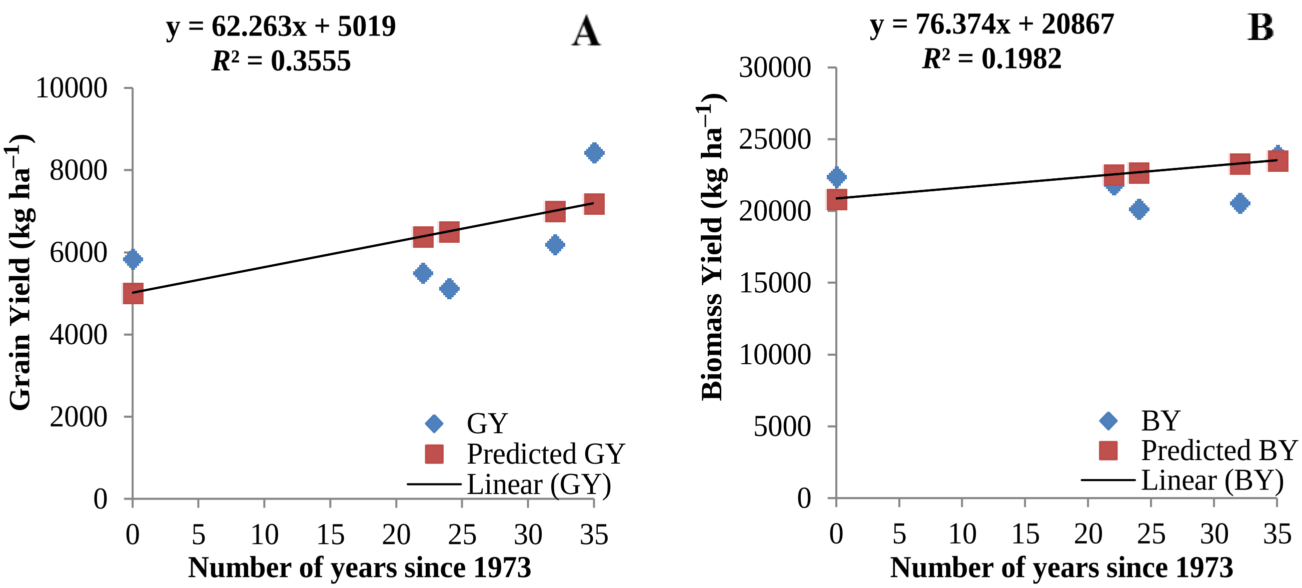

Figure 1. Genetic gain in grain yield (A) and biomass yield (B) of the highland maize varieties released from 1973 to 2012.

-0.0088 (-0.18%) cm yr^{-1} were shown in Table 4. Grain filling rate indicated positively non–significant (P> 0.05) annual and relative genetic gain of 0.76 (1.19%) kg ha^{-1} day^{-1} yr^{-1}. Similarly, days to anthesis and silking indicated negatively non–significant (P> 0.05) annual and relative genetic gain of –0.10 (–0.11%) days yr^{-1} and –0.13 (–0.13%) days yr^{-1}, respectively for the highland maize varieties at APPRC (Table 4).

The regression of the mean values of the mid–altitude maize varieties correspondingly with the year of releases over the past 29 years demonstrated positively non–significant (P> 0.05) annual predictive and average relative genetic gain of 58.97 (0.78%) kg ha^{-1} yr^{-1} for grain yield and 95.63 (0.45%) kg ha^{-1} yr^{-1} for biomass yield at BNMRC (Figure 2A and B).

Positively non–significant (P> 0.05) annual genetic improvement trends were also made over the mid–altitude maize varieties for thousand kernel weight by 1.12 (0.36%) gm. yr^{-1}, ear length by 0.03 (0.17%) cm yr^{-1} and ear diameter by 0.0076 (0.16%) cm yr^{-1} (Table 4). Negatively significant (P≤ 0.05) genetic annual predictive and average relative genetic improvements on shortening the durations by –0.18 (–0.24%) days yr^{-1} for days to anthesis and –0.19 (–0.24%) days yr^{-1} for days to silking were made; while positive and highly significant (P≤ 0.01) genetic improvement was made over the mid–altitude maize varieties upon prolonging the duration for grain filling period by 0.20 (0.30%) days yr^{-1} at BNMRC (Table 4).

Regression of the mean values of the lowland maize varieties correspondingly with the year of releases over the past 12 years demonstrated positive and non–significant (P>0.05) annual predictive and average relative genetic gain of 32.64 (0.57%) kg ha^{-1} yr^{-1} for biomass yield. Differently, demonstrated negative and non–significant predictive average annual rate of decrease was shown by –2.64 (–0.16 %) kg ha^{-1} yr^{-1} for grain yield at MARC (Figure 3A and B).

Positively significant (P≤ 0.05) annual and relative annual genetic improvement trends were made over the lowland maize varieties by 0.07 (0.53%) rows–ear yr^{-1} for number of kernel rows per ear while positively non–significant (P> 0.05), annual and relative annual genetic improvement trends were made by 0.02 (0.53%) cm yr^{-1} for ear diameter, 0.04 (0.14%) kernels–row yr^{-1} for number of kernels per row, and 0.03 (0.05%) kg ha^{-1} day^{-1} for biomass production rate. Exceptionally compared to the other studied yield traits, negatively non–significant (P> 0.05) annual and relative annual genetic gain reductions were shown over the lowland maize varieties by –1.25 (–0.66 %) gm. yr^{-1} for thousand kernel weight, –0.02 (–1.97%) ear $plant^{-1}$ yr^{-1} for number of ears

Table 4. Relative genetic gains of grain yield and other agronomic traits of Ethiopian highland, mid–altitude and lowland maize varieties evaluated at APPRC, BNMRC and MARC (2015).

Trait	*Highland Maize Varieties*				*Mid–altitude Maize Varieties*				*Lowland Maize Varieties*			
	b	RGG (% yr^{-1})	R^2	Intercept	*b*	RGG (% yr^{-1})	R^2	Intercept	*b*	RGG (% yr^{-1})	R^2	Intercept
DA	–0.10	–0.11	0.40	96.44	–0.18*	–0.24	0.29	78.06	0.44	0.74	0.09	59.44
DS	–0.13	–0.13	0.38	99.06	–0.19*	–0.24	0.26	79.24	0.47	0.77	0.08	61.17
DM	–0.09	–0.05	0.17	175.99	0.01	0.01	0.01	144.31	0.69	0.69	0.03	100.29
GFP	0.02	0.02	0.01	79.49	0.20**	0.30	0.42	66.25	0.27	0.66	0.01	40.94
GFR	0.76	1.19	0.34	63.41	0.54	0.48	0.07	113.38	–0.68	–1.46	0.02	46.78
PH	0.20	0.08	0.08	254.16	0.24	0.08	0.01	293.73	–0.06	–0.05	0.00	121.76
EH	0.43	0.33	0.14	131.24	–0.43	–0.26	0.04	161.55	0.50	0.89	0.05	55.46
NEP	0.0081*	0.90	0.59	0.90	0.0007	0.05	0.00	1.30	–0.02	–1.97	0.11	0.99
EL	0.02	0.09	0.09	19.49	0.03	0.17	0.03	18.6	–0.0022	–0.02	0.00	13.90
ED	–0.0088	–0.18	0.20	4.79	0.0076	0.16	0.05	4.80	0.02	0.53	0.17	3.54
NKE	–0.0124	–0.09	0.13	13.62	–0.0061	–0.04	0.00	15.51	0.07*	0.53	0.39	12.40
NKR	0.07	0.02	0.40	36.33	0.02	0.06	0.01	41.22	0.04	0.14	0.01	27.32
TKW	–0.43	–0.14	0.05	315.22	1.12	0.36	0.05	307.89	–1.25	–0.66	0.11	190.05
GY	62.26	1.24	0.36	5018.97	58.97	0.78	0.16	7539.35	–2.64	–0.16	0.00	1628.93
BY	76.37	0.37	0.20	20867.25	95.63	0.45	0.08	21210.44	32.64	0.57	0.01	5742.26
BPR	0.51	0.43	0.26	118.36	0.70	0.48	0.10	145.59	0.03	0.05	0.00	56.48
HI	0.17	0.71	0.33	24.31	0.11	0.32	0.14	35.46	–0.37	–1.24	0.03	30.23

b – Regression coefficient. R^2 – Coefficient of determination. RGG – Annual relative genetic gains. *,** – Significant at 0.05 and 0.01 level of probability, respectively. DA – Days to Anthesis, DS – Days to Silking, DM – Days to Maturity, GFP – Grain Filling Period, GFR – Grain Filling Rate, PH – Plant Height, EH – Ear Height, NEP – Number of Ears per Plant, EL – Ear Length, ED – Ear Diameter, NKE – Number of Kernel Rows per Ear, NKR – Number of Kernels per Row, TKW – Thousand Kernel Weight, GY – Grain Yield, BY – Biomass Yield, BPR – Biomass Production Rate and HI – Harvest Index.

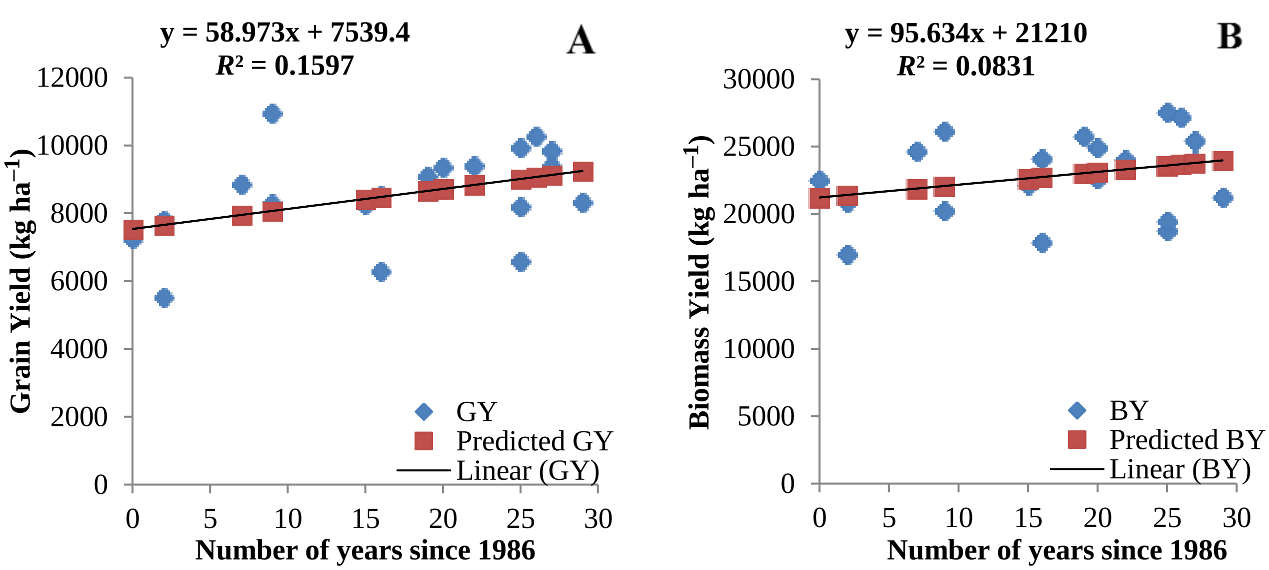

Figure 2. Genetic gain in grain yield (A) and biomass yield (B) of the mid–altitude maize varieties released from 1986 to 2015.

per plant, –0.0022 (–0.02%) cm yr^{-1} for ear length and –0.37 (–1.24%) kg ha^{-1} yr^{-1} for harvest index (Table 4). Positively non–significant (P>0.05) annual genetic gain reductions were made over the lowland maize varieties by 0.44 (0.74%) days yr^{-1} for days to anthesis and 0.47 (0.77%) days yr^{-1} for days to silking while non–significant

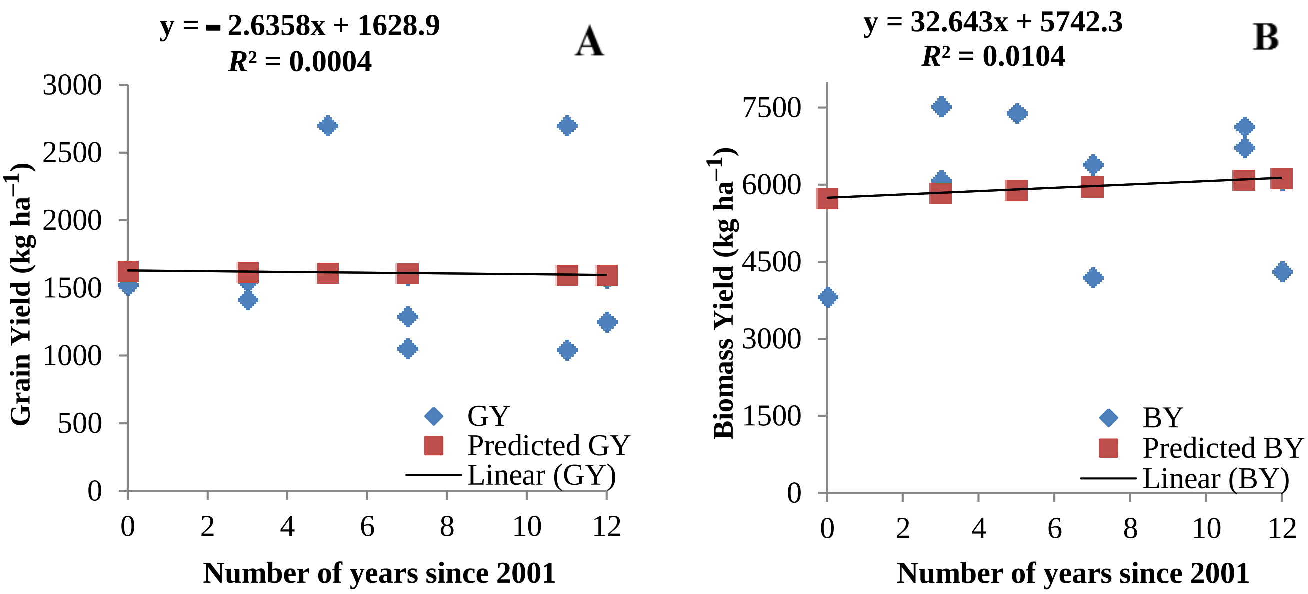

Figure 3. Genetic gain in grain yield (A) and biomass yield (B) of the lowland maize varieties released from 2001 to 2013.

Table 5. Correlation of agronomic parameters with grain yield of Ethiopian highland, mid–altitude and lowland maize varieties evaluated at APPRC, BNMRC and MARC MARC (2015).

Trait	Highland Maize Varieties	Mid–altitude Maize Varieties	Lowland Maize Varieties
DA (P value)	–0.82* (0.0233)	–0.20 (0.4009)	–0.20 (0.5638)
DS (P value)	–0.91** (0.0041)	–0.20 (0.3957)	–0.24 (0.4752)
DM (P value)	–0.55 (0.1986)	0.29 (0.2208)	0.10 (0.7805)
GFP (P value)	0.09 (0.8400)	0.38 (0.1017)	0.26 (0.4319)
GFR (P value)	0.99** (<0.0001)	0.97** (<0.0001)	0.40 (0.2229)
PH (P value)	0.17 (0.7157)	0.52* (0.0181)	0.22 (0.5163)
EH (P value)	–0.11 (0.8198)	0.28 (0.2347)	0.05 (0.8805)
NEP (P value)	0.85* (0.0161)	0.01 (0.9651)	0.32 (0.3382)
EL (P value)	0.89** (0.0072)	0.56* (0.0110)	0.49 (0.1304)
ED (P value)	–0.23 (0.6140)	0.08 (0.7389)	–0.07 (0.8354)
NKE (P value)	–0.30 (0.5120)	0.09 (0.7069)	–0.21 (0.5408)
NKR (P value)	0.94** (0.0018)	0.41 (0.0690)	0.39 (0.2336)
TKW (P value)	0.36 (0.4287)	0.48* (0.0311)	0.10 (0.7805)
BY (P value)	0.82* (0.0234)	0.90** (<0.0001)	0.42 (0.2006)
BPR (P value)	0.90** (0.0055)	0.91** (<0.0001)	0.54 (0.0841)
HI (P value)	0.91** (0.0043)	0.59** (0.0064)	0.69* (0.0194)

(P> 0.05) negative annual genetic and relative genetic gain reduction of –0.68 (–1.46%) kg ha^{-1} day^{-1} yr^{-1} was made for grain filling rate at MARC (Table 4).

Correlation of grain yield and other agronomic traits of Ethiopian highland, mid–altitude and lowland maize varieties

Correlation coefficients for the grain yield among the seven highland maize varieties released over the past 39 years had shown a positive and highly significant (P≤0.01) associations with grain filling rate (r= 0.99**), ear length (r= 0.89**), number of kernels per row (r= 0.94**), biomass production rate (r= 0.90**) and harvest index (r= 0.91**); while grain yield was positive and significantly (P≤0.05) associated with number of ears per plant (r= 0.85*) and biomass yield (r= 0.82*). Differently, highly significant (P≤ 0.01) and negative association for days to silking (r= –0.91**); and significant (P≤0.05) and negative association for days to anthesis (r= –0.82*) were shown with the grain yield at APPRC (Table 5).

Correlation coefficients for the grain yield among the twenty mid–altitude maize varieties released over the past 29 years had shown a positive and highly significant (P≤0.01) associations with grain filling rate (r= 0.97**), biomass yield (r= 0.90**), biomass production rate (r= 0.91**) and harvest index (r= 0.59**), while grain yield was positive and significantly (P≤0.05) associated with plant height (r= 0.52*), ear length (r= 0.56*) and thousand kernel weight (r= 0.48*) at BNMRC (Table 5).

Correlation coefficients for the grain yield among the eleven lowland maize varieties released over the past 12 years had shown a positive and significant (P≤ 0.05) association only with harvest index (r= 0.69*) at MARC (Table 5).

DISCUSSION

Analysis of variance of grain yield and other agronomic traits of maize varieties

The highly significant mean squares observed for grain yield and other measured traits over the breeding period indicate that genetic differences exist among cultivars within each breeding period over Ethiopian released highland, mid–altitude and lowland maize varieties. The analysis of variance for grain yield traits indicated significant (P≤0.01) differences on the number of ears per plant and grain yield among the varieties released in Ethiopia over highland, mid–altitude and lowland maize varieties. These findings were in agreement with the genetic gain study findings of highly significant (P≤ 0.01) differences on the number of ears per plant and grain yield which were indicated both under multiple stress and non–stress environments at Nigeria, Ghana and Benin by Badu–Apraku et al*.* (2014); and both under Striga–*infested*, Striga–*free* and across different research environments in Nigeria and Benin by Badu–Apraku et al*.* (2013). While Omolaran et al*.* (2014) on another finding from Nigeria reported significant (P≤0.05) differences on the number of ears per plants and grain yields both under different levels of nitrogen and maize hybrids, other grain yield traits of Ethiopian released highland and lowland maize varieties that showed non–significant (P> 0.05) differences over the number of kernel rows per ears. Contrariwise Omolaran et al*.* (2014) reported highly significant (P≤0.01) differences over the number of kernel rows per ear both under different levels of nitrogen and maize hybrids.

Genetic gains in grain yield and other agronomic traits of maize varieties

Maize genetic gains in grain yield and other measured traits for Ethiopian released maize varieties currently under production within breeding periods in the present studies prompted the examination of the archived and predicted genetic gains that the Ethiopian released highland and mid–altitude maize varieties over the past 39 and 29 years demonstrated positive genetic gains for the grain and biomass yields. Comparably numerous estimates of genetic yield gain of maize hybrids have been shown, without exception, that genetic yield gains during the past 70 years have been positive and linear. Estimates of the average annual gain vary but tend to fall in the range of 65–75 kg ha^{-1} according to Duvick (2005a). This agrees with a recent result from USA by Chen et al*.* (2016) who evaluated commercial maize hybrids released over 38 years that reported increased breeding progress over the grain yield by an average of 66 kg ha^{-1} yr^{-1}. However, the present studies for the Ethiopian released lowland maize varieties during the past 12 years differently demonstrated genetic reduction for grain yield, while only minimal genetic gain for biomass yield were shown.

The highland and mid–altitude maize varieties demonstrated that non–significant and significant genetic gain improvements on duration reductions had been possible for days to anthesis and silking, while non–significant genetic gain decrease was made upon duration reduction for days to anthesis and silking for the lowland maize varieties. In the history of the maize breeding programs of some countries, there have been consistent as well as inconsistent trends made possible on reducing the durations of days to anthesis and silking. Many researchers agree for growth and flowering traits that days to silking and anthesis have not significantly changed over time respectively according to (Russell, 1985; Duvick, 1997, 2005a). On the contrary, Omolaran et al*.* (2014) and Badu–Apraku et al*.* (2014) reported over the three different breeding eras, that days to anthesis were significantly and consistently lowered over the newly released ones than the oldest released ones. This clearly indicates that throughout the history of the maize breeding program there has been a continual trend made possible on reducing the durations of days to anthesis and silking in many countries.

Highly significant genetic improvement was made upon prolonging grain filling period for the mid–altitude maize varieties, while non–significant genetic improvements were made for the highland and lowland maize varieties. The first two shown findings agreed with Campos et al*.* (2006) who reported for maize that the grain filling period has been non–significantly improved over the past fifty years of breeding in the U.S. corn–belt. Non–significant genetic reduction of grain filling rate was made for the lowland maize varieties, while genetic increases of grain filling rate were made for the highland and mid–altitude maize varieties. The shown genetic gain increases and reduction of grain filling rates were the ones that have played the role for the realized grain yield as well as thousand kernel weight potentials over the Ethiopian released maize varieties. It was obvious that kernel set must be followed by kernel filling to ensure that yield potential is realized. Kernels near the tip of the ear will often abort after several weeks of growth if drought–affected. Remobilized assimilate stored in the stem prior to and during the flowering period normally plays a role in buffering filling rate only in the last half of filling (Edmeades, 2013).

Non–significant genetic improvements for number of ears per plant over the mid–altitude maize varieties and reduction for number of ears per plant over the lowland maize varieties were shown, while significant genetic improvement was shown for number of ears per plant over the highland maize varieties. Unlike the lowland maize varieties, comparable results on different hybrids maize varieties grown in USA reported that number of ears per plant was found to increase over the decades (Crosbie, 1982; Russell, 1985; Duvick et al., 2004). Similarly, in Nigeria and Benin significant improvement was observed in number of ears per plant for the different maize cultivars of the three breeding periods when grown in Striga–*infested* and Striga–*free*. That the genetic gains increase made in the number of ears per plant were 0.006 and 0.002 ear plant^{-1} yr^{-1} over the evaluated different maize cultivars respectively, under Striga–*infested* and Striga–*free* conditions. Nevertheless, the different maize cultivars evaluated under Striga–*free* condition, the number of ears per plant were ranged equally from 0.9 ear plant^{-1} yr^{-1} for cultivars during the breeding period 1 (1988–2000) to 0.9 ear plant^{-1} yr^{-1} for cultivars during the breeding period 3 (2007–2010), while under Striga–*infested* conditions the number of ears per plant was ranged from 0.8 to 0.9 ear plant^{-1} yr^{-1} over the two similar breeding periods (Badu–Apraku et al., 2013). Badu–Apraku et al. (2014) also reported the number of ears per plant on maize, that the genetic gains were changed significantly by 0.52 and 0.70 ear plant^{-1} yr^{-1} during the three breeding eras respectively; under multiple stress and non–stress environments evaluated at 16 and 35 different sites.

Positively non–significant genetic improvements respectively, over the highland and mid–altitude maize varieties, and negatively non–significant genetic reductions over lowland maize varieties were shown for harvest index. The demonstrated research findings for the genetic improvements for harvest index towards the released maize varieties agreed that the harvest index did consistently change over time; and that in Argentina the harvest index over the evaluated Argentinean maize hybrids, have increased from 0.41 to 0.52 kg ha^{-1} yr^{-1} on those maize hybrids varieties grown under the optimal conditions over those past 30 year period of 1960–1990 (Echarte and Andrade, 2003; Echarte et al., 2004). From another study, particularly at higher plant densities in Iowa–USA, the harvest index showed a significant relative improvement of 0.1 kg ha^{-1} yr^{-1} over the maize varieties released for the past 61 year period of 1930–1991 (Duvick, 1997). On the contrary in Iowa–USA, for the long–term genetic gain in maize yield for the conditions of the U.S. corn–belt, the harvest index have remained constant over maize hybrids released between the 1930s–2000s for the past 70–80 years (Duvick, 2005b; Tollenaar and Lee, 2006). Another recent study on the commercial hybrid maize varieties released in the USA over the eight commercial DeKalb hybrid maize varieties released over 38 year period from 1967–2005 compared at 2 locations, 2 nitrogen fertilizer rates and 3 plant densities, showed that the harvest indices were similar across hybrid maize varieties except for low values with the 1967 and 1975 released hybrid maize varieties at West Lafayette, USA; and with the 1975 and 1982 released hybrid maize varieties at Wanatah, USA (Chen et al., 2016).

Relationship of grain yield and other agronomic traits of maize varieties

Genetic improvements of grain yield in the Ethiopian released highland and mid–altitude maize varieties over the past 39 and 29 years; grain filling rate, ear length, biomass production rate, biomass yield and harvest index were equally amongst the possible contributors oneness associated positively and significantly with the grain yields. Days to anthesis and days to silking were also amongst the possible contributors oneness associated negatively and significantly with the grain yields while, number of ears per plant and number of kernels per row were amongst the possible contributors oneness being positively and significantly associated with the grain yields over the Ethiopian released highland maize varieties. Equally, thousand kernel weights were the other ones amongst the possible contributors being associated positively and significantly with the grain yields over the Ethiopian released mid–altitude maize varieties. While only the harvest index were the ones among the possible contributors being associated positively and significantly with the grain yields over the Ethiopian released lowland maize varieties for the past 12 years.

For maize, grain yield is a function of number of plants per area, the proportion of these plants that produce a harvestable ear, kernel number per ear, and the weight of each individual kernel. Similar findings to the Ethiopian released highland and mid–altitude maize varieties were reported from Nigeria by Omolaran et al. (2014) on maize genetic gains studies under different nitrogen regimes, for highly significant and positive associations of grain yield with the number of kernels per row and thousand kernel weight; while highly significant and negative associations of grain yield with the days to anthesis, days to silking and plant height were identified. Other similar findings from Canada, for a significant and positive association of grain yield with thousand kernel weight (Lee and Tollenaar, 2007), and grain yield with number of kernels (Tollenaar et al., 1992); and from Nigeria and Benin by

Badu–Apraku et al. (2013) grain yield with plant height were also reported. Meanwhile, other considered and analyzed success result studies on conventional maize crop improvements over the past 50 years for drought tolerance also indicated the negative association between grain yield and reduced interval between anthesis and silking (Campos et al., 2004).

As regards the Ethiopian released lowland maize varieties, harvest index was shown to be associated positively and significantly with the grain yields, and harvest index trait was also considered as being the ones among the possible contributors towards the grain yield genetic declinations. However, unlike the Ethiopian released lowland maize varieties, comparable results on grain yield improvement in Argentina has been associated with an increase in harvest index trait (Echarte and Andrade, 2003). In contrast to yield improvement in Argentina, previous studies (Crosbie, 1982; Duvick, 1997, 2005b; Tollenaar et al., 1994) have also shown that increase in ERA–hybrid grain yield in the USA can be attributable to changes in light interception due to increased leaf area index and changes in light utilization due to more erect upper leaves, maintenance of green leaf area and leaf photosynthesis during the grain filling period rather than yield per plant and harvest index. Similarly, Tollenaar and Lee (2006) reported from the USA that the yield increase was not associated with a change in maximum harvest index.

Conclusions

We studied the changes in yield gains on a morpho–physiological basis with respect to yield and yield component traits for 38 Ethiopian released maize varieties over the past 42–year periods which is currently under production in Ethiopia in the highland, mid–altitude and lowland Ethiopian major maize growing agro–ecology zones of the regions. The average rate of increase in grain yield corresponding to annual genetic gain was 62.26 (1.24%) kg ha^{-1} yr^{-1} over the tested 7 released highland maize varieties and 58.97 (0.78%) kg ha^{-1} yr^{-1} over the tested 20 released mid–altitude maize varieties. Differently, the other tested 11 released lowland maize varieties indicated average rate of decreases in grain yield was by **–**2.64 (**–**0.16) kg ha^{-1} yr^{-1} corresponding to annual genetic gain. Other tested phenological traits, and yield and yield components indicated a significant and positive annual genetic gain increase for number of ears per plant over the released highland maize varieties and grain filling period over the released mid–altitude maize varieties; while significant and negative annual genetic improvement were also observed in shortening the days to anthesis and days to silking over the released mid–altitude maize varieties. However, an average rate of decreases had been indicated in grain yield; a significant and positive annual genetic gain increase was indicated for number of kernel rows per ear over the released lowland maize varieties. Generally, the results of the present studies indicated that considerable genetic gains over the phenological traits, and inconsiderable genetic reductions over the yield and yield components have been made across the released highland, mid–altitude and lowland maize varieties for the three agro–ecological zones of Ethiopia. Typically, targeting one or few of those identified maize breeding traits relatively contributed to considerable genetic gains and reductions could be used for further improvements in the breeding program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Badu-Apraku B, Yallou CG, Oyekunle M (2013). Genetic gains from selection for high grain yield and Striga resistance in early maturing maize cultivars of three breeding periods under Striga–infested and Striga-free environments. Field Crops Research 147:54-67.

Badu–Apraku B, Morakinyo AB, Muhyideen O (2014). Agronomic traits associated with genetic gains in maize yield during three breeding eras in West Africa. Maydica 59:49-57.

Campos H, Cooper M, Habben JE, Edmeades GO, Schussler JR (2004). Improving drought tolerance in maize: A view from industry. Field Crops Research 90:19-34.

Campos H, Cooper M, Edmeades GO, Löffler C, Schussler JR, Ibañez M (2006). Changes in drought tolerance in maize associated with fifty years of breeding for yield in the United States Corn Belt. Maydica 51:369-381.

Chen K, Camberato J, Tuinstra M, Kumudini S, Tollenaar M, Vyn T (2016). Genetic improvement in density and nitrogen stress tolerance traits over 38 years of commercial maize hybrid release. Field Crops Research 196:438-451.

Crosbie TM (1982). Changes in physiological traits associated with long–term breeding efforts to improve grain yield of maize. In: Loden HD, Wilkinson D (Eds.) (1982). Proceedings of the 37Th annual research conference on corn and sorghum. American Seed Trade Association, Washington, DC, Chicago, IL pp. 206-223.

Central Statistical Agency (CSA) (2014). The Federal Democratic Republic of Ethiopia: Report on area and production of major crops, Central statistical agency agricultural sample survey. Addis Ababa, Ethiopia, May, 2014. Statistical Bulletin-532(1):1-121. Available at: http://www.csa.gov.et

Central Statistical Agency (CSA) (2015). Agricultural sample survey compiled time series data for national and regional level (From 1995/96 to 2014/15), Report on area and production of crops (Private Peasant Holdings, Meher Season). Addis Ababa, Ethiopia, March, 2015. Available at: http://www.csa.gov.et

Dawit A, Rashid S, Tripp R (2010). Seed system potential in Ethiopia: Constraints and opportunities for enhancing production. IFPRI (International Food Policy Research Institute) Working Paper. Washington, DC. July, 2010. Available at: http://www.ifpri.org/sites/default/files/publications/ethiopianagsectorwpseeds.pdf

Degene M, Habtamu Z (1993). Maize breeding and improvement for the eastern highlands of Ethiopia. In: Benti T, Ransom JK (Eds.) (1993). Proceedings of the 1St national workshop of Ethiopia. IAR/CIMMYT, Addis Ababa, Ethiopia, pp. 22-24.

Duvick DN, Smith JS, Cooper M (2004). Long-term selection in a commercial hybrid maize breeding program. In: Janick J (Ed.) (2004). Plant breeding reviews. Part 2. Long term selection: Crops, Animals, and Bacteria 24:109-151.

Duvick DN (1997). What is yield? In: Edmeades GO, Banziger M, Mickelson HR, Pena-Valdivia CB (Eds.) (1997). Developing drought and low-N tolerant maize. Proceedings of a symposium, CIMMYT, El Batan, Mexico 332-335.

Duvick DN (2005a). Genetic progress in yield of United States maize (*Zea mays* L.). Maydica 50:193-202.

Duvick DN (2005b). The contribution of breeding to yield advances in maize (*Zea mays* L.). Advanced Agronomy 86:83-145.

Ethiopian Agricultural Research Organization (EARO) (2000). Research strategy for maize. EARO, Addis Ababa, Ethiopia.

Echarte L, Andrade FH (2003). Harvest index stability of Argentinean maize hybrids released between 1965 and 1993. Field Crops Research 82:1-12.

Echarte L, Andrade FH, Vega CR, Tollenaar M (2004). Kernel number determination in Argentinean maize hybrids released between 1965 and 1993. Crop Science 44:1654-1661.

Echarte L, Nagore L, Di Matteo J, Cambareri M, Robles M, Maggiora AD (2013). Grain yield determination and resource use efficiency in maize hybrids released in different decades. *In: Agricultural chemistry* (2013). In Tech Open Science 2:19-36.

Edmeades GO (2013). Progress in achieving and delivering drought tolerance in maize-An update from the global status of commercialized biotech/GM crops: 2012, International Service for the Acquisition of Agri-Biotech Applications (ISAAA), Brief 44Ithaca, New York.

Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) (2015). Available at: http://faostat.fao.org/faostat/

Haffnagel HP (1961). Agriculture in Ethiopia. FAO, Rome, Italy.

Jason WH, Kevin AC, Devin MN, Frederick EB (2013). Changes in nitrogen use traits associated with genetic improvement for grain yield of maize hybrids released in different decades. Crop Science 53:1256-1268.

Kebede M, Gezahegne B, Benti T, Mosisa W, Yigzew D, Asefa A (1993). Maize production trends and research in Ethiopia. *In*: Benti T, Ransom JK (Eds.) (1993). Proceedings of the 1St national maize workshop of Ethiopia. IAR/CIMMYT, Addis Ababa, Ethiopia 4-12.

Kelemu F, Mamo G (2002). Suitable zones for growing maize in Ethiopia. In: Mandefro N, Tanner D, Afriyie ST (Eds.) (2001). Enhancing the contribution of maize to food security in Ethiopia. Proceedings of the 2Nd national maize workshop of Ethiopia. EARO and CIMMYT, Addis Ababa, Ethiopia, 27-30 ISBN: 92-9146-100-8

Lee EA, Tollenaar M (2007). Physiological basis of successful breeding strategies for maize grain yield. Crop Science 47(S3):S202-S215.

Legesse W, Mosisa W, Berhanu T, Girum A, Wende A, Solomon A, Tolera K, Dagne W, Girma, D, Temesgen C, Leta T, Habtamu Z, Habte J, Alemu T, Fitsum S, Andualem W, Belayneh A (2012). Genetic improvement of maize for mid-altitude and lowland sub-humid agro–ecologies of Ethiopia. In: Mosisa W, Twumasi-Afriyie S, Legesse W, Berhanu T, Girma, D, Gezahagn B, Dagne W, Prasanna BM (Eds.) (2012). Meeting the challenges of global climate change and food security through innovative maize research. Proceedings of the 3Rd national maize workshop of Ethiopia. Addis Ababa, Ethiopia, 24-34. ISBN: 978-970-648-184-9

Mandefro N, Tanner D, Afriyie ST (2001). Enhancing the contribution of maize to food security in Ethiopia. Proceedings of the 2Nd national maize workshop of Ethiopia. EARO and CIMMYT, Addis Ababa, Ethiopia 232 ISBN: 92-9146-100-8

McCann JC (2005). Maize and Grace: Africa's encounter with a new world crop, 1500-2000. Cambridge, MA: Harvard University Press.

Ministry of Agriculture and Rural Development (MoARD), Federal Democratic Republic of Ethiopia (2004), (2007), (2014) and (2016). Plant Variety Release, Protection and Seed Quality Control Directorate, Crop development department, Crop variety register, Issue Numbers': 7, 10, 17 and 18. Addis Ababa, Ethiopia.

Mosisa W, Jemal A, Leta T, Haji T, Legesse W, Kassa Y, Wonde A, Aschalew G, Sewagegne T, Teshale A, Tamirat B, Yoseph B, Habtamu Z (2001). Development of improved maize germplasm for the mid and low altitude sub–humid agro–ecologies of Ethiopia. In: Mandefro N, Tanner D, Afriyie ST (Eds.) (2001). Enhancing the contribution of maize to food security in Ethiopia. Proceedings of the 2Nd national maize workshop of Ethiopia. EARO and CIMMYT, Addis Ababa, Ethiopia, pp. 27-30. ISBN: 92–9146–100–8

Omolaran BB, Odunayo JO, Mohammed L, Sunday AI, Jimoh M, Micheal SA, Musibau AA, Suleiman YA (2014). Genetic gains in three breeding eras of maize hybrids under low and optimum nitrogen fertilization. Journal Agricultural Science 59(3):227-242. doi:10.2298/JAS1403227B

Russell WA (1985). Evaluations for plant, ear, and grain traits of maize cultivars representing seven eras of breeding. Maydica 30:85-96.

Tollenaar M, Dwyer LM, Stewart DW (1992). Ear and kernel formation in maize hybrids representing three decades of grain yield improvement in Ontario. Crop Science 32:432-438.

Tollenaar M, McCullough DE, Dwyer LM (1994). Physiological basis of the genetic improvement of corn. In: Slafer GA (Ed.) (1994). Genetic improvement of field crops. Marcel Dekker, Inc. New York 183-236.

Tollenaar M, Lee EA (2006). Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. *Maydica* 51:399-408.

United States Agency for International Development-Foreign Agricultural Service (USDA–FAS) (2016). Office of global analysis, World Agricultural Production (WAP), Circular series, 5-16, Washington, DC, May 2016. Available at: http://gain.fas.usda.gov

Zeng D, Alwang J, Norton GW, Shiferaw B, Jaleta M (2013). Ex-post impacts of improved maize varieties on poverty in rural Ethiopia: Diffusion and Impact of Improved Varieties in Africa (DIIVA), Consultative Group on International Agricultural Research (CGIAR) Standing Panel on Impact Assessment (SPIA). Brief Number 45, Rome, Italy. December, 2014. pp. 1-4. Available at: http://ispc.cgiar.org & http://impact.cgiar.org

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Agricultural technology adoption and its impact on smallholder farmer's welfare in Ethiopia

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Agricultural production and productivity play a paramount role in the livelihood of rural farm households. Agricultural technology affects agricultural productivity and the welfare of rural farm households. However, there is a gap in knowledge on the effect of different technology adoptions on farm household's welfare. This study examined the effect of improved wheat variety adoption on household's welfare in Ethiopia. The study was based on cross-sectional data collected through a semi-structured questionnaire from 150 sample farm households. Double hurdle and Endogenous Switching Regression model were employed. The result indicates that the improved wheat variety adoption decision and intensity of adoption of farm households have determined by credit access, extension visits, soil fertility, plot size, off-farm employment, age of household head, distance from input market, and farm experience. The estimated model also revealed that adoption of improved wheat varieties has a positive and significant effect in enhancing farm household's welfare. Therefore, adoption of yield-enhancing agricultural technologies should be more intensified to improve smallholder farmers' welfare.

Key words: Adoption, double hurdle, endogenous switching regression, impact, technology.

INTRODUCTION

The agricultural sector continues to play a dominant and strategic role in the development and growth of most developing nations of the world. Most importantly, its role as a source of food, raw material and employment cannot be overemphasized. In Sub-Saharan Africa (SSA), Asia and the Pacific, the agriculture-dependent population is over 60%, while in Latin America and high-income economies the proportions are estimated to be around 18 and 4%, respectively (World Bank, 2006). Therefore, the agricultural sector brings about economic growth and development, overcome poverty and enhance food security, through an increase in productivity of smallholder farmers. To this end, increasing agricultural productivity has been an issue that development institutions and governments in the world give attention to. However, achieving agricultural productivity and growth will not be possible without developing and disseminating yield-increasing technologies. Particularly, recently it is no longer possible to meet the needs of increasing numbers of people by expanding the area under cultivation (Asfaw

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et al., 2012). Improved technology use has paramount importance on rural household's crop productivity and welfare (Mekonen and Karelplein, 2014). Agricultural productivity can be enhanced through the use of improved agricultural technologies (Maertens and Barrett, 2013). It plays a significant role in fighting poverty, lowering per-unit costs of production, boosting rural incomes and reducing hunger (Kassie et al., 2011). Poor farmers could benefit from technology adoption by increasing production for home consumption and increasing gross revenue from crop sale (De Janvry and Sadoulet, 2002). In the same vein, improved agricultural technology adoption has the potential to deepen the market share of agricultural output through which the smallholder farmers' resource use and output diversification. Increasing productivity in agriculture depends on adopting production enhancing technologies and the innovativeness of farmers (Awotide et al., 2016).

Existing literature evidenced the positive impact of technology adoption on productivity, poverty reduction and welfare across the world (Awotide et al., 2016; Nyangena and Maurice, 2014). Similarly, in Ethiopia studies revealed the positive productivity and welfare implication of improved agricultural technologies (Asfaw et al., 2012; Mekonen and Karelplein, 2014) and improve the food security of smallholder farmers (Shiferaw et al., 2014). According to Mekonen and Karelplein (2014) adoption of improved seeds and chemical fertilizer alone will increase crop productivity by 7.38 and 6.32% per year of each in Ethiopia. Despite this in Ethiopia regardless of the increasing rate of adoption and its positive impact on production and productivity, a large extent of rural farm households are under deplorable living conditions.

Recently wheat production accounted for not less than 16% of the total cereal crop area in Ethiopia. About 36% of cereal farm households are directly dependent on wheat farming in Ethiopia. However, the national average productivity of wheat is 1.83 tons/ha (CSA, 2011), and 2.7 tons/ha in 2018. Wheat production is also projected to be 2.77 tons per hectare and the total area cultivated increased to 1.66 million hectares in 2019/2020 cropping season. Despite this Ethiopia didn't meet its domestic wheat demand. While it produces about 4.6 million metric tons every year, its consumption is beyond its production level (that is, 6.3 million metric tons per year) (Bickford, 2019). Besides the low level of productivity, there has been a growing tendency of demand for wheat both in rural and urban Ethiopia which leaves the people unable to afford for the growing demand and will aggravate the existing poverty situation in the country.

Although a number of studies revealed that extensive efforts have been taken to develop and disseminate several modern agricultural technologies, the systematic analysis of the adoption and livelihood impacts of these technologies have been scarce. Most studies in the literature have looked at the impact of cereal crops (maize, teff and sorghum) and other crops (groundnuts, pigeon peas, rice) on agricultural productivity and household welfare (Asfaw et al., 2012; Mekonen, and Karelplein, 2014; Jaleta et al., 2015; Awotide et al., 2016). Shiferaw et al. (2014) and Tesfaye et al. (2016) have tried to look at the welfare effect of improved wheat varieties in Ethiopia. Wheat is the fourth major staple food crop that the government and agricultural development institutions targeted the development and dissemination of improved wheat verities and provision of adequate seed timely and at affordable prices to farmers. Despite these efforts of the government and policymakers, much less is known about the welfare impact of wheat technology at the farm household level and the rate of adoption in Hadya Zone particularly in Misha district is very low where its welfare impact is unexplored, while the area is a wheat potential area. Therefore, the study aims to analyze the determinants of agricultural technology adoption decision, intensity and the impact of adoption on rural farm household's welfare in Hadya Zone.

METHODOLOGY

Sampling and methods of data collection

This study was held in Misha district of Hadya zone, based on information from the Hadya zone Agriculture office. Multi-stage sampling technique was used for the representative sample selection. First, the major wheat-growing district (Misha district) was selected purposively; second, we select five representative kebeles, out of 29 kebeles of the district where kebeles were purposively selected based on their wheat potential taken from the respective district agriculture office and finally, a representative sample of farm households was selected using simple random sampling technique. In the study, 30 households were randomly drawn from each kebele hence, a total of 150 farm households were drawn from five representative kebeles. The study used a structured questionnaire as the main data collection instrument. For data reliability and accuracy of the data collection instrument, we pre-test the questionnaire using a test-retest data reliability method and we found the coefficient of reliability 0.75, which implies the data is reliable. Alongside, the data collection was supplemented by an interview, focus group discussion and secondary data.

Analytical framework and estimation techniques

Decision and intensity of adoption of improved wheat variety

Rogers and Shoemaker (1971) defined adoption as the decision to apply innovation and to continue using it. Differences in adoption decisions are often due to the fact that farmers have different adaptive capacity, different objectives, preferences, and different socio-economic and biophysical characteristics (Shiferaw et al., 2008). In such a context, farmers' decisions regarding the adoption of innovations can be explained using the theory which guides maximization of expected utility. Following this theory, a farmer will adopt a given new technology if the expected utility obtained from the technology exceeds that of the indigenous one.

Different researchers used different models for analyzing the determinant of technology adoption. In principle, the decisions on whether to adopt and how much to adopt can be made jointly or separately (Gebremedhi and Swinton, 2003). The Tobit model was used to analyze under the assumption that the two decisions are affected by the same set of factors (Greene, 2000). However, the decision to adopt may well precede the decision about the intensity of use and hence the explaining variables in the two stages may differ. The underlying assumption of the Tobit model is farmers demanding modern inputs have unconstrained access to the technology (Bingxin and Alejandro, 2014). Therefore, the Tobit model is inappropriate in situations where some portion of farmers are constrained to access new technology and other portions of farmers are not considering the new technology. The Heckman selection model is also another alternative model used to analyze the intensity of technology adoption. In the Heckman model, the non-adopters are considered as they will never adopt under any circumstances (Jose, 1989). Hence, Heckman selection model is restrictive in the sense that non-adopters to adopt might be encouraged to adopt for various reasons like access and improvements in extension programs and changes in input prices. On the other hand, a double hurdle model which was first proposed by Cragg (1971), assumes that non-adopters are considered as a corner solution in a utility-maximizing model (Tafesse and Sodo, 2016). DH model is the modification of the Tobit model and the Heckman model because it is more flexible. In this model, households make two separate decisions. First households decide whether to participate or not. Secondly, they decide how much they adopt. Hence, this model gives a room for factors affecting the two decisions to differ as it model the decision process in two separate steps. It also considers the possibility of zero observation in the second stage of decision which may arise from an individual's choice or random circumstances.

Due to the above-mentioned reasons, this study adopts the double hurdle model. The first stage of this model is a probit model to analyze determinants of adoption, and the second stage is a truncated model for determinants of the level of adoption. Use of Cragg's model for analyzing adoption and intensity of adoption is common in agricultural economic literature); (Teklewold et al., 2006; Shiferaw et al., 2008; Gebregziabher and Holden, 2011; Tsehaye, 2016; Tafesse and Sodo, 2016).

The double-hurdle model is a parametric generalization of the Tobit model, in which two separate stochastic processes determine the decision to adopt and the level of adoption of technology. The two-stage questions in a typical DH model are: i) Have you adopted improved wheat varieties Adoption decision (yes/no)? and ii) If the decision is to adopt, how many improved varieties in kg you applied given different constraints-Intensity Decision (kg/ha)? Therefore, the double-hurdle model has an adoption (D) decision with an equation:

$$\begin{aligned} &Di = 1 \text{ if } D_i^* > 0 \text{ and } \quad Di = 0 \text{ if } D_i^* \leq 0 \\ &D_i^* = \acute{\alpha}Zi + Ui \qquad Ui \sim N(0,1) \\ &Y_i = Y_i^* \text{ if } Y_i^* > 0 \text{ and } D_i^* > 0 \\ &Y_i = 0, \text{ otherwise} \end{aligned} \tag{1}$$

Being $D^*{}_i$ a latent variable that takes the value 1 if a farmer use improved varieties technology and zero otherwise, Z_i is a vector of household characteristics and α is a vector of parameters. This function is the probit model estimation for the adoption decision of households.

$$Y_i^* = \beta' X_i + V_i, \quad V_i \sim N(0, 1) \tag{2}$$

Where Y^*_i is the observed proportion of agricultural technologies and X_i is a vector of household socioeconomic characteristics and β is a vector of parameters. Equation 2 is estimated using truncated regression. From Equation 1 and 2, Ui and Vi are stochastic error terms, which represents omitted, yet relevant but difficult to capture variables and measurement errors. It is assumed both to be normally, identically and independently distributed. There are two thresholds that should be passed in order to observe a positive level of improved wheat varieties application. First is the adoption threshold (if the farmer has adopted improved wheat varieties), and second is a level threshold (farmer has applied a non-zero improved wheat variety). The log-likelihood function for the double-hurdle model that nests the bivariate probit model and a truncated regression model is given following Cragg (1971) by:

$$\text{LogL} = \sum_{0} \ln\left[1 - \Phi(X^*{}_{1i}\alpha_1 \ )\left(\frac{X2i^*\,\alpha_2}{\sigma}\right)\right] + \sum \ln\left[\emptyset(X^*{}_{1i}\,\alpha_1)\frac{1}{\sigma}\emptyset\left(\frac{Yi^* - X2i^*\alpha2}{\sigma}\right)\right] \tag{3}$$

Where Φ and ∅ refer to the standard normal probability and density functions respectively, X_{1i} and X_{2i} independent variables for probit and truncated model, respectively, $\alpha 1$ and $\alpha 2$ are parameters to be estimated for the two models. Assuming the independence of the error terms in the probit and truncated model, the log-likelihood function of the double hurdle model can be maximized, without loss of information, by maximizing the two components separately: the probit model (overall observations) followed by a truncated regression on the non-zero observations.

A hypothesis test for the double hurdle model against the Tobit model will be checked using the log-likelihood ratio test statistics. The likelihood ratio test statistics Γ can be computed (Greene, 2000) as $\Gamma = -2[\ln LT-(\ln LP+\ln LTR)] \sim 2k$, where LT is the likelihood for the Tobit model; LP is the likelihood for the probit model; LTR is the likelihood for the truncated regressions model; and k is the number of independent variables in the equations. If the test hypothesis is written as: $\lambda\frac{\beta}{\acute{\alpha}}$ *and* $H_1 : \lambda \neq \frac{\beta}{\alpha}$ and H0 is rejected on a pre-specified significance level, provided $\Gamma > 2k$, it is a confirmation to the superiority of the double-hurdle specification over the Tobit model. It is in such a case, the decision for improved varieties adoption and the decision on how much to adopt is treated differently.

The independent variables and their definitions

A multitude of factors is found in the literature that affects the decision of farmers to adopt new agricultural technology and the level of adoption of these technologies. The set of explaining variables are household characteristics, physical, socio-institutional and plot-level characteristics included in the empirical models are selected following a review of many literature on farm level investment theory (Gebremedhin and Swinton, 2003; Tafesse and Sodo, 2016; Tsehay, 2016). These are explained in Table 1.

Adoption decision and its impact on household welfare

The empirical challenge in impact assessment using observational studies is establishing a suitable counterfactual against which the impact can be measured because of self-selection problems (Shiferaw et al., 2014). To accurately measure the impact of technology adoption on the welfare of farm households, the exposure to the technology should be randomly assigned so that the effect of observable and unobservable characteristics between the treatment and comparison groups is the same, and the effect is attributable entirely to the treatment. However, when the treatment groups are not randomly assigned, adoption decisions are likely to be influenced both by unobservable (e.g., managerial skills, motivation, and land quality) and observable heterogeneity that may be correlated to the outcome of interest. In developing countries particularly in rural areas, labour markets, credit markets and input

Table 1. Summary of definitions, measurements and expected signs of variables.

Definition of variables	Measurement of variables	The expected sign of variables
Dependent variables		
Adoption of improved varieties	Yes/No	
Amount of improved seed variety used	Continuous (Kg/ha)	
Independent variable		
Age of household head (age)	years	±
Level of Education of the household head (Educ)	Number of years of formal education	+
Household size (hhsize)	Adult equivalent	±
Sex of household head (sex)	1 if male, 0 otherwise	+
Farm size (land)	Total area cultivated in hectare	+
Plot area (plot size)	Total wheat plot area cultivated in a hectare	+
Livestock ownership(TLU)	Tropical livestock unit	+
Fertilizer (front)	Amount of fertilizer input used (kg/ha)	+
Off-farm income (off-farm)	1 if access to off-farm, 0 otherwise	±
Access to extension service(exten)	Number of extension visit by extension experts	+
Access to credit (Credit)	1 if access to credit, 0 otherwise	+
Distance from the plot area (Dplot)	Walking minutes (one way)	-
Distance from nearest input market (Mkt)	Walking minutes (one way)	-
Farm experience (Farmexpe)	Number of years of farming	±
Soil fertility (Soil)	1 if fertile, 0 otherwise	-

markets are either missing or imperfect (Asfaw et al., 2012). This imperfection might be associated with poverty, underdeveloped non-farm sector, asymmetric information and high transaction costs, mainly in credit and input markets. In such situations, the relevance of a separable household model where consumption and production decisions are made independently is questionable. According to Asfaw et al. (2012), a suitable framework for analyzing household microeconomic behaviour under market imperfections is a non-separable model. This is because non- separable models can take into account the problem of selectivity bias and endogeneity.

In the literature, various econometric approaches exist to deal with selection bias such as instrumental variable (IV) approaches, propensity score matching (PSM), generalized propensity score (GPS) matching in a continuous treatment framework, and Heckman selection model. However, while PSM only controls for observed heterogeneity, instrumental variable (IV) control for unobserved heterogeneity. The Heckman selection model also considers those who do not adopt technology will never adopt under any circumstances. Therefore, a recently more applicable model for impact assessment in the literature i.e. endogenously switching regression model is more appropriate for various reasons. Recent studies in impact assessment are shifting to endogenously switching regression (Asfaw et al., 2012; Shiferaw et al., 2014; Mekonen and Karelplein, 2014; Kassie et al., 2014).

The assumption behind using endogenously switching treatment effect regression is that, in addition to the observed variables, there might be an unobservable farm and/or household characteristics that could potentially influence both the adoption of improved wheat varieties and household welfare. A farm household self-selects into adopting agricultural technologies due to observable and unobservable variables. Estimating the impact of technology adoption on household welfare without accounting for this problem might suffer from potential endogeneity bias and thus the estimated results may over or under-estimate impacts compared to the actual impact. It will also result in inconsistent estimates of the effect of the adoption of agricultural technology on household welfare. Simultaneous equation model can explicitly account for such endogeneity (Hausman, 1978).

This problem of endogeneity can be addressed by randomly assigning improved variety to treatment and control households, which assure that using improved variety is the only differentiating factor between treated households and those excluded from it, so that the control group can be used to assess the counterfactual (what would have happened to adopters in the absence of the intervention) (de Janvery et al., 2010). However, households per se decide to adopt or not to adopt based on the available information at hand. Therefore, adopters and non-adopters may be systematically different, which necessitates specification of separate welfare outcome functions for adopters and non-adopters, while at the same time accounting for endogeneity. The econometric problem will thus involve both endogeneity (Hausman, 1978) and sample selection (Heckman, 1979). This motivates the use of an endogenous switching regression model that accounts for both endogeneity and sample selection (Alene and Manyong, 2007; Di Falco et al., 2011; Asfaw et al., 2012; Shiferaw et al., 2014; Mekonen and Karelplein, 2014; Kassie et al., 2014).

Endogenous switching regression model

In this study, adoption is defined if farmers used any of the improved wheat varieties, either freshly purchased, and/or recycled improved varieties. A farmer adopts improved varieties if the expected utility from adoption (Ua) is higher than the corresponding utility obtained from non-adoption (Una), that is, Ua- Una > 0. The benefit from adopting improved wheat varieties by the i^{th} farmer can be modelled as:

$$A_i^* = Z_i \alpha + \varepsilon i \quad \text{where } A_i^* = \begin{cases} 1 \; if \; Zi\,\alpha + \varepsilon i > 0 \\ 0, otherwise \end{cases} \quad (4)$$

Where Zi is a vector of household, farm and institutional variables that affect the decision to adopt and/or not to adopt improved wheat varieties and εi is an error term. For households growing improved wheat varieties and for those who didn't grow during the 2016/2017 production season, the outcome equation (welfare) corrected for endogenous adoption is given as:

Regime 1:

$$Y1i = X1i\beta1 + \delta1i\lambda1i + \eta1i \quad if\ Ai = 1\ (for\ IWV\ adopters) \quad (5)$$

Regime 2:

$$Y2i = X2i\beta2 + \delta2i\lambda2i + \eta2i \quad if\ Ai = 0\ (for\ IWV\ non\ adopters) \quad (6)$$

Where Yi per capita consumption expenditure of household I under regime 1 (adopter of IWV) and regime 2 (local indigenous variety), Xi is a vector of the plot, household, farm, other explanatory variables, and $\lambda_{1i} = \frac{\phi(Z_i\ \alpha)}{\varphi(Z_i\ \alpha)}\ and \lambda_{2i} = \frac{\phi(Z_i\ \alpha)}{1-\varphi(Z_i\ \alpha)}$ are the inverse Mill's ratios (IMR) computed from the selection equation and are included in Equations 5 and 6 to correct for selection bias in a two-step estimation procedure, that is, endogenous switching regression. β and σ are parameters to be estimated, and η is an independently and identically distributed error term.

Conditional Expectations and Treatment Effects

The structure of the expected conditional and average treatment effects under actual and counterfactual scenario is specified as:

a) $$[Y_{1i}|X, A_i = 1] = X_{1i}\beta_1 + \delta_{1\varepsilon}\lambda_{1i} \quad (Adopters\ with\ Adoption\ of\ Improved\ Wheat\ Variety \quad (7)$$

b) $$E[Y_{2i}|X, A_i = 0] = X_{2i}\beta_2 + \delta_{2\varepsilon}\lambda_{2i} \quad (Non-adopters\ without\ adoption) \quad (8)$$

$$E[Y_{2i}|X, A_i = 1] = X_{1i}\beta_2 + \delta_{2\varepsilon}\lambda_{1i} \quad (Adopters\ had\ they\ decided\ not\ adopt\ IWV \quad (9)$$

10) $$E[Y_{1i}|X, A_i = 0] = X_{2i}\beta_1 + \delta_{1\varepsilon}\lambda_{2i} \quad (Non-Adopters\ had\ they\ decided\ to\ adopt\ IWV \quad (10)$$

Situations 7 and 8 are observed in the sample. However, Equation 9 and 10 are the hypothetically expected situations (counterfactual outcome) where the treated happened to be untreated, and the untreated happened to be treated. Accordingly, the expected change in the welfare for households adopted improved varieties, that is, the average treatment effect on the treated plots (ATT) is given as:

$$ATT = (a) - (c) = E[Y_{1i}|X, A_i = 1] - E[Y_{2i}|X, A_i = 1] = X_{1i}(\beta_1 - \beta_2) + \lambda_{1i}\ (\delta_{1\varepsilon} - \delta_{2\varepsilon}) \quad (11)$$

Similarly, the expected per capita consumption of a household not growing improved varieties had they grew an improved variety, that is, the average treatment effect on the untreated households (ATU) is given as:

$$ATU = (d) - (b) = E[Y_{1i}|X, A_i = 0] - E[Y_{2i}|X, A_i = 0] = X_{2i}(\beta_1 - \beta_2) + \lambda_{2i}\ (\delta_{1\varepsilon} - \delta_{2\varepsilon}) \quad (12)$$

Where X1 and X2 are set of explanatory variables affecting consumption expenditure in regime 1 and regime 2, respectively β_1 and β_2 are parameters to be estimated. The transitional and base heterogeneity will also be estimated.

Full information maximum likelihood estimation (FMLE) technique is the appropriate method for endogenous switching regression. It can simultaneously estimate the selection equation (probit model and the outcome equation (the per capita consumption expenditure).

Variable definitions, measurements and expected signs in adoption impact model

Based on the bounds of existing literature on impact analysis on welfare set of explanatory variables are adopted in this study as presented in Table 2.

RESULTS AND DISCUSSION

Descriptive analysis

Distribution of plot size, technology adoption and intensity

Since it is important to describe the data which results in insight on the adoption of agricultural technologies and intensity of use, we demonstrate the distribution of plot size, and technology adoption and intensity in Table 3. It is revealed that about 66% of the samples adopt chemical fertilizer, and 35% of them are non-adopters. This is consistent with Terefe et al. (2013) on the central rift valley of Ethiopia. About 30% of sample respondents appeared to be organic fertilizer (manure) adopters and about only 10% of sample households adopt compost, while about 38% of the samples adopt improved wheat variety. This implies that compared to chemical fertilizer, the adoption of improved seed was found to be small.

Table 3 shows a variation in the application of organic and inorganic fertilizers, and improved high –yield increasing varieties. The low level of organic fertilizer application is a manifestation of the level of technological practices and existing knowledge within the farm households. Table 4 also infer the existence of variation in the intensity of adoption of chemical fertilizer among adopters. On the average adopters use 125 kg of DAP and 95 kg of UREA per hectare of their fertilized land under wheat production. Though variations exist between the two types of chemical fertilizers, the level of chemical fertilizer use per hectare of the wheat plot area is not underestimated.

Technology adoption and productivity

It is worth mentioning to investigate the relationship between productivity (yield) and the application of chemical fertilizer in comparison to pre-existing technological practices. An insightful result on average yield under different technology regimes is presented in

Table 2. Summary of definitions, measurements and expected signs of variables.

Definition of variables	Measurement of variables	Expected variables
Outcome variable		
Consumption expenditure per adult equivalent unit	Per capita consumption expenditure ('000 birr)	
Household characteristics		
Age of household head (age)	Years	±
Gender of household head (sex)	1 for male, 0 otherwise	±
Level of Education of the household head (Edu)	Number of years of formal education	+
Dependency ratio	The ratio of the number of individuals below 14 and above 64 to total household size	_
Active family labour force (AEU)	Adult equivalent	+
Household wealth variables and institutional characteristics		
Farm size (Land)	Total area cultivated in a hectare	+
Plot size under wheat (plot1)	Cultivated plot area in a hectare	+
Livestock ownership(TLU)	Total number of livestock (TLU)	+
Farm support(Farmsup)	1 if the HH get farm support, 0 otherwise	+
Off-farm income (Offarm)	1 if the household has access, 0 otherwise	+
Access to extension service(extention)	Number of extension visit by extension experts	+
Access to credit (credit)	1 if access to credit, 0 otherwise	+
organic fertilizer (Manure)	Manure, compost in Kg/ha	+
Distance from the nearest market	Walking minutes (one way)	-
Farm experience (Farmexp)	Number of years of farming	±
Soil fertility (soil)	1 if fertile, 0 otherwise otherwise	-

Table 3. Distribution of plot size and technology adoption packages (%).

Crop type	Improved seed (% of total plot area)	Fertilizer application (% of plots)		
		Inorganic	Organic	
		Chemical fertilizer	Manure	Compost
Wheat	38	66	30	10

Table 4. Distribution of Chemical Fertilizer Use (Kg/ha).

Fertilizer type (Kg/ha)	
DAP	125
UREA	95
Total	220

Table 5. The average yield is about 1970 kg/ha with significant variation across fertilizer types. Another important feature of the table is the impact of fertilizer use on productivity. There is a positive differential in productivity between adopters and non-adopters of organic fertilizer and improved seed varieties, which implies that fertilizer and improved seed use helps to improve the productivity of smallholder farmers.

A simple mean comparison test between adopters and non-adopters of improved seed variety shows that household characteristics, including education and livestock ownership (TLU), are considerably larger for adopters. The mean distance from input market and distance from the plot area is smaller for the adopters compared to its counterparts, signifying that non-adopters have less access to market and information which in turn results in a slow diffusion of farm technology as well as high transportation cost. Households are also different in terms of their plot characteristics such as plot size and a number of plots of wheat. Adopters of improved wheat varieties have more hectares of wheat land and the number of plots of land under wheat cultivation. However, the data revealed that adopters are highly associated with lower farming experience. It leads us to conclude that farmers with few years of farming tend to adopt more than those with many years of farming. On the other

Table 5. Average yield (kg/ha) under different fertilizer regimes.

Fertilizer regimes											
Chemical fertilizer use			Manure use			Compost use			Improved seed variety		
Yes	No	Diff	Yes	No	Diff	Yes	No	Diff	Yes	No	Diff
1100	1350	-250	1558.5	1252.5	306	1424.6	1401	23.4	1590.6	1427	163.6

Table 6. Descriptive test statistics of difference between adopters and non- adopters of improved wheat varieties (IWV).

Continuous variable	Improved wheat varieties (IWV)			
	Adopters	Non-adopters	Pooled sample	T-value
Age	44	52	48.96	4.85***
Household size (Adult Equivalent)	4.89	4.53	4.67	-0.65
Education	0.81	0.45	0.59	-2.23***
Dependency ratio	0.23	0.51	0.33	0.78
Livestock ownership (TLU)	3.56	3.50	3.53	-2.85***
Distance from nearest input market(min)	7.85	13.67	11.46	9.03***
Distance from the plot area (min)	3.50	7.00	5.67	3.57***
Number of plot	2.35	1.78	2.00	-6.58***
Land (ha)	1.51	0.75	1.03	1.78
Plot size(ha)	.96	.56	.71	2.03***
Farm Experience (years)	8	12	10.48	-2.75***
Extension (no. of visits per cropping season)	10	6	7.52	-3.60***
Wheat yield (kg/ha)	1756.23	1150.25	1380.22	1.98**

And * implies significance at 5 and 1% level of significance.

Table 7. For categorical variables (Chi-square test)

Variable	Adoption decision		Total	$\chi 2$ (P-value)
	Adopters	Non-Adopters	Pooled sample	
Sex (1 if male)	89.73	86.02	87.58	1.16(0.295)
Farm support(1 if yes)	94.56	75.42	84.23	182.10(0.000)
Offfarm (1 if yes)	5.34	7.32	5.26	6.23(0.008)
Credit (1 if yes)	50.00	23	33.26	218.15(0.000)
Soil fertility (1 if fertile)	48.5	42.8	44.96	12.01 (0.001)

hand, adopters are found to be with more access to extension visit and larger farm productivity/ yield per hectare. Farmers who adopt the modern high yield varieties of wheat seed have secured high yield (about 606 kg) than what the non-adopters produce. With regard to institutional factors (credit access and farm support), adopters have more access than non-adopters. This implies that those farmers with access credit or having access to farm support are more likely to participate in adopting new technologies. This is because they can have less financial constraint and more know how to use these technologies. About 50% of adopters were found to have credit access and 94.56% of them have access to farm support. However, only 23% of non- adopters have access to credit service. Tesfaye et al. (2016) have also found a positive implication of credit service on wheat adoption decision. Mohamed and Temu (2008) also argued that credit can facilitate farm households to purchase the needed agricultural inputs and enhance their capacity to affect long-term investment in their farms (Tables 6 and 7).

The probability and intensity of agricultural technology adoption

The probability of agricultural technology adoption

Table 8 deals with the estimated relationship between socio-economic and institutional factors, and smallholder farmer's technology adoption decision. The result has revealed that off-farm employment of the household head, access to credit service, the fertility of soil; total land size and the number of extension visits reinforce farmer's probability of adopting new agriculture technology. The land area the major component of the wealth of rural households has a significant positive impact on the likelihood of adopting an improved variety of wheat. The result demonstrated that the probability of adoption increase by 2.1% when the size of land under cultivation rise by 1 ha. This result is similar to the findings of Asfaw et al. (2011) and Hailu et al. (2014). It is also evident that farmers with financial constraint decide to adopt new technologies provided that they are offered to fill their financial gap, which implies that adoption is greater when farmers are with the opportunity of accessing credit from financial institutions than otherwise could be. The result is consistent with Hailu et al. (2014) and Yu et al. (2011).

The number of extension visits made by extension experts has an imperative role in enhancing farmer's adoption decision. The result has shown that improved wheat variety adoption likely increases by 2.1% for a unit increase in extension visit. It is statistically significant at 1% level of significance. Therefore, we can deduce that the higher the number of extension visit to farmers, the higher the likelihood of preference to adopt a new variety of wheat. It is the major instrument for the dissemination of outputs of agricultural research. It affects agricultural technology adoption in various situations. First, extension training and advisory service to farmers increase human capital and information access. Second, it is mostly complemented with input distribution and farm credit access. Third, it is the major channel through which agricultural research and development outputs are transferred to smallholder farmers. The fertility status of the soil is another factor affecting farmer's adoption decision. The result confirmed that farmers have a high chance of adoption, provided that their plot is fertile land. Their probability of adoption tends to decrease when the soil fertility status is getting poor. The result of this study is also similar to the findings of Asfaw et al. (2012); and Shiferaw et al. (2014).

On the contrary, some institutional, demographic structure and plot specific variables have a detrimental impact on farmer's probability of technology adoption; Farm experience, the number of the plot, distance from input markets and age of the household head are found to influence farmer's adoption decision negatively. The result revealed that the age of the household head is negatively and significantly affecting the probability of adoption at a 1% level of significance. It infers that an increase in the age of the household head by one year will result in the likelihood of adoption of improved wheat variety by 0.01%. The higher the age of the household head is the lesser the probability of introducing the new technology. Likewise, farm experience also influences adoption decision negatively. When number of years of experience increase by a year, the likelihood to adopt the new technology falls by 0.61%. This might be the case that farmers with long years of farming experience are reluctant and stick to their traditional farming, instead of adjusting them to the new *technologies*. This is result is consistent to the descriptive result and the finding of Hailu et al. (2014), Yu et al. (2011) and Kassie et al. (2009).

Land fragmentation measured by the number of plot and average walking distance from the input market in minutes reduces smallholder's interest to adopt a new variety of wheat seeds. It is significant at 5 and 1% level of significance, respectively. Accordingly, as the distance to the nearest market increases by one minute, the probability of adopting improved wheat variety would decrease by 0.03%. The same would also be true that the farther the plot from the homestead, it would be less likely to utilize inputs. The result is in line with our prior expectation and consistent with the theory. The same result was found by Kassie et al. (2012), Shiferaw et al. (2014) and Hailu et al. (2014).

The intensity of technology adoption

It is imperative to try to look at the intensity of technology adoption when we speak of the impact of adoption on households welfare. With this regard, the intensity equation is estimated for improved varieties of wheat, where the result is presented in Table 9. The result demonstrated that household characteristics such as education and household size have a positive and significant effect on the amount of improved wheat variety used. However, age has a detrimental effect on the intensity of technology adoption. As the age of the household head increases the level of adoption tends to decrease. This implies that farmers might become reluctant to take advantage of new technologies and stick to their traditional farming experience as their age goes up, which is in line with prior theoretical expectation. Whereas, the level of adoption was found higher with higher educational level and large family size. The study also revealed that the level of technology adoption (improved wheat variety) by smallholder farm households tends to raise with a better level of livestock asset ownership. Livestock ownership has a positive and significant effect while education has a positive and significant effect. Livestock is a proxy for household wealth and wealthier farmers have more chance of purchasing improved wheat technology. The result also concludes that off-farm employment improves the intensity of adoption. Hence, adopters support themselves with off-farm activities.

Distance from the input market and distance from wheat plot area result in a detrimental impact on the level of technology adoption. As the plot area and the nearest

Table 8. Estimation results of farmers' adoption of improved wheat varieties and average marginal effects after probit (the first hurdle).

Variable	Delta method				
	Dy/Dx	Standard error	Z	P>|z|	(95% Confidence interval)
Sex	0.0854	1.2302	0.06942	0.536	-0.0632082 0.0328484
Age	-0.0091	0.0029	-3.13793	0.002***	0.002807 6584
Education	0.0235	0.0245	0.959184	0.138	-0.0022425 0.0161735
Land	2.1031	0.2133	9.859822	0.003***	0.0535298 0.2539621
Household size	0.0521	0.3242	0.160703	0.078	-0.0551861 0.1892611
Livestock Asset (TLU)	-0.0232	0.0207	-1.12077	0.052	-0.0025281 0.0001699
Off farm employment	0.3523	0.0251	14.03586	0.000***	-0.00227 -0.00103
Distance from the plot area	-1.00E-05	0.00072	-0.01389	0.068	-7.1E-05 0.000151
Distance from input market	-0.0321	0.0021	-15.2857	0.000****	-0.22083 -0.14677
Extension	2.1201	0.1652	12.83354	0.000***	0.346246 0.391223
Access to Credit	0.4721	0.1572	3.003181	0.000***	0.029307 0.097083
Soil fertility	0.768	0.1301	5.903151	0.013**	-0.10289 -0.02038
Plot size (ha)	0.0865	0.0103	8.398058	0.000***	7.08E-05 0.000151
Number of plot	-0.0325	0.0012	-27.0833	0.035**	-0.0071 -0.00373
Farm experience	-0.6132	0.0255	-24.0471	0.003***	0.05353 0.253962
Constant	-0.07	0.008	0.7785	0.000***	0.521562 0.61352

Log likelihood = -166.5232
Number of observation = 150
LR chi2(15)= 67.52
Prob >chi2 =0.0000
Pseudo R2 = 0.2013
*** refers significance at 1%, ** refers to significance at 5% significance level.

Table 9. Estimation results of smallholders' intensity of technology adoption.

GLM	Dependent Variable: Intensity of Adoption (amount of improved variety of wheat seed in kilogram per hectare of land). Second Hurdle Model
Sex	-0.524(0.526)
Age	-0.112*** (0.0042)
Education	0.286*** (0.0025)
Household size	0.323**(0.125)
Off farm employment	1.325** (0.425)
Livestock ownership (TLU)	0.651**(0.158)
Distance from input market	-0.156***(0.0245)
Distance from the plot area	-0.254*** (0.0021)
Extension	1.201**(0.402)
Credit	2.002**(0.850)
Land size	-2.812*** (0.596)
Plot size	0.00985 (0.0245)
Farm experience	-0.052***(0.004)
Number of plot	-0.021(0.025)
Soil fertility	0.891***(0.452)
N=150 Standard error in parenthesis,	**p<0.05, *** p<0.001

input market is far from the homestead, farmers will face higher transportation cost given poor infrastructure and thereby accessibility of new wheat technology becomes difficult. Similar results were found by other studies (Hailu et al, 2014; Kassie et al., 2009).

The intensity of adoption is also found to increase with the higher number of extension visits. Access to the extension has a positive and significant effect on the intensity of adoption which may be due to the fact that access to and the frequency of extension visit is a vital way through which farmers get technical information and other services. Total land size has a negative and significant effect on the intensity of improved wheat variety use. Similarly, farm experience and the number of plot area have a negative effect on the level of improved wheat technology adoption. However, adopters are found to have more access to credit service than non-adopters. Credit gives farmers with the capacity to purchase the demanded technology; hence greater credit accessibility gives them to increase their level of adoption. In the same vein, soil fertility of wheat plot area results in a positive impact on the intensity of adoption.

Welfare impact of technology adoption

Determinants of household per-capita-expenditure

Comparing the household per capita expenditure differential between adopters and non-adopters of improved technology is the major objective of this study. For that matter, we have estimated expenditure functions for the two groups, in order to deduce whether farm households are benefited from adopting improved wheat varieties. For identification, we took government extension, and input market distance as selection instruments of our study. These variables are expected to fulfil to main conditions to be considered as a valid instrument. First, they should not be directly related to the farm household's farm consumption expenditure. They should directly affect the adoption of the Improved wheat variety. For instance, if we take input market distance it directly affects the demand of adopting an improved wheat seed, however; it doesn't have a direct effect on the farm household's expenditure. Because farm inputs are critical ingredients to increase productivity, farmers with difficulty of accessing farm inputs will fail to adopt new technology and vice versa. However, it is difficult to prove the validity of the instruments without undertaking appropriate statistical tests. Hence, we use two main tests for robustness checks. By using robust probit regression the effect of instruments on improved wheat adoption (the dependent variable in the selection equation) is jointly significant at 5% level of significance. The second test is conducted by using OLS regression on the outcome equation of non-adopters with selection instruments and other covariates. The result of this test indicates that the instruments joint effect on the nonadopters consumption expenditure is insignificant.

As we can see in the 1st column of Tables 10 and 11 we have estimated the consumption expenditure function for the pooled sample using OLS estimation technique by considering an improved wheat variety as an explanatory variable. The result shows that the adoption of 9 wheat variety has a positive significant effect on per capita consumption expenditure function. Column (1) of OLS regression in the Table 10 indicates that other factors remain constant; farm households who adopted IWV can

Table 10. Determinants of household welfare.

Model	OLS		Endogenous Switching Regression			
Dependant variable	F (35, 98) =14.53 prob >f = 0.0000 R- squared = 0.193 Root MSE= 3.224		Wald chi2 (35) = 324.74 Log pseudo likelihood = -12188.532 Prob >chi2 = 0.0000			
	Household consumption expenditure for pooled data		Household consumption expenditure for Adopters of IWV		Household consumption expenditure for Non-Adopters of IWV	
Explanatory variables	**Coefficient**	**Robust Std.Err**	**Coefficient**	**Robust Std.Err**	**Coefficient**	**Robust Std.Err**
Adoption of IWV	0.35***	0.08				
Household characteristics						
Age	0.05**	0.02	0.06**	0.02	0.06	0.04
Education	0.006	0.007	0.02	0.03	0.02	0.01
Sex	-0.004	0.07	-0.20	0.19	-0.16	0.29
Household size (AE)	-0.40***	0.14	-0.32**	0.16	-0.78***	0.30
Off -farm employment	-0.16	0.1	0.15	0.12	-0.25	0.21
Livestock ownership	0.03	0.02	0.06***	0.02	0.08	0.05
Farm experience	-0.05**	0.02	0.03***	0.01	-0.02	0.01
Plot Characteristics						
Plot size	-0.14	0.12	-0.12**	0.04	-0.17	0.14
Land size	0.21**	0.11	0.04	0.03	-0.01	0.03
Soil fertility	0.08	0.25	-0.02	0.12	0.08*	0.041
Number of the plot	0.06	0.04	0.03**	0.01	0.02	0.05
Institutional factors						
Credit access	0.24**	0.12	0.28***	0.13	0.12*	0.03
Extension	0.23***	0.08	0.35***	0.16	0.09**	0.03
Distance from input market	-0.04	0.03	-0.08***	0.01	-0.05	0.07
Distance from plot area	-0.12	0.29	-0.25	0.21	0.31	0.25
Sample Size	150		93		57	

Note: Estimation by OLS (first column) and full information maximum likelihood for the remaining columns at the plot-level with robust standard errors in parenthesis. Sample size: 150 plots. ***Significant at 1% level; **Significant at 5% level; *Significant at 10% level.

get a 35% consumption increment than their non-adopter counterparts. However, accepting this result as a correct measure for the effect of IWV on household per capita consumption is not appropriate. Because in this regression it is assumed that there is strict exogeniety in the adoption of IWV. But it is the personal decision of farmers and potentially endogenous. Thus, the estimated results of this model are biased and inconsistent since it fails to account the problem of selection bias and unobservable heterogeneity. Indeed, it fails to identify the structural difference in consumption expenditure between adopters and non-adopters.

To this end, the Endogenous Switching Regression (ESR) model for household per capita consumption functions for adopters and non-adopters was estimated. The last two columns of Tables 10 and 11 indicate the determinants of per capita consumption expenditure for adopters and non -adopters. As per the result, household characteristics, credit access, and extension are found the key determinants of the consumption functions of both adopters and non- adopters. The Wald test of independence is significantly different from zero, which indicates the existence of selection bias and slope heterogeneity between adopters and non- adopters. There are also some factors which affect adopters and non -adopters differently. Thus estimating two separate income functions is mandatory.

Household characteristics

Household characteristics are found significantly influencing household welfare outcomes. Compared to the non-adopters, adopters are found to have higher age level. An increase in the year of the household head results in a 6% increase in household consumption level and a 5% increase in consumption level for total sample households. This implies that households who adopt an

Table 11. Selection instruments and joint significance test.

Model	OLS		Endogenous Switching Regression					
Dependent variable	Household consumption expenditure for pooled data		Adoption of Improved Wheat Variety (IWV)		Household consumption expenditure for Adopters of IWV		Household consumption expenditure for Non-Adopters of IWV	
Explanatory variables	Coefficient	Robust Std.Err	Coefficient	Robust Std.Err	Coefficient	Robust Std.Err	Coefficient	Robust Std.Err
Selection Instruments								
Extension			2.12***	0.16				
Distance from input market			-0.032**	0.002				
Constant	28.80***	8.54	19.25***	2.93				
σ_i					0.05***	0.03	-0.16***	0.12
ρ_i					2.62	0.01	2.69	0.03
WTIE(χ2)			3.25**					
JTI(χ2, F-test)			14.98***(χ2)		0.85			
Sample Size	150		150		93		57	

Note: Estimation by OLS (first column) and full information maximum likelihood for the remaining columns at the plot-level with robust standard errors in parenthesis. Sample size: 4778 plots. ***Significant at 1% level; **Significant at 5% level; *Significant at 10% level: JTI= joint test on selection instruments, WTIE= Wald test of independent equations.

improved variety of wheat have better consumption level than that of their counterparts, despite their age goes up. On the other hand, household size has a strong negative influence on consumption expenditure of non-adopters than adopters. A unit increase in household size results in about 78% reduction in consumption of non- adopters and 32% reduction of consumption for adopters. Probably this will be due to the case when the household members are dependent and not contribute to the income portfolio of the household.

Farm experience is the other significant covariate that affects consumption of households. A one year increase in farm experience of adopters results in a 3% increase in their per capita consumption, however, farm experience doesn't affect the welfare of non- adopters. This is because experienced farmers are more exposed to technology and are better aware of the significance of adoption. Similarly, as prior expectation, an increase in ownership of livestock assets increases the per capita consumption of adopters by 6% than their counterparts. This might be associated with the increase in cash from their livestock assets which can support the access to finance for input for production. The rest of household characteristics, sex, and education and off-farm employment have no significant impact on the consumption level of sample households.

Institutional factors

The result indicated that credit access has a strong significant effect on household per capita consumption/ welfare on both adopters and non- adopters. This might be through the associated productivity growth from credit access and the resulting growth in farm income that adopters and non- adopters increase their welfare.

However, the resulting welfare increment of adopters (24) is 12% higher than that of non- adopters (12%). The effect of extension service on rural household welfare is also positive and significant. Ceteris paribus, adopter's welfare will increase by 35% provided they are privileged to the access to extension service by one more trip, which is significantly higher than non-adopters (9%). According to Birkhaeuser et al. (1991), the extension has the potential of bridging discoveries and mitigation methods from research laboratories and the in-field practices of individual farmers, In addition, it provides, information about cropping techniques, optimal inputs use, high-yield varieties and prices. Access to extension service enhances the adoption of improved agricultural technologies by reducing supply-side constraints that arise due to information market inefficiencies (Wossen et al., 2015).

Plot characteristics

A number of the plot is found has a strong negative and significant impact on household welfare of adopters, while its effect on non-adopters is found neutral. This is because more fragmentation of land might put a challenge on managing croplands during pre and post-harvest period. It will incur much time, money and labour force to manage the weeding and harvesting of crops when the plot is many and fragmented. Especially, improved varieties need strong follow up than the traditional varieties which can adjust to the environment easily. Adopter's welfare will decrease by 3% more than

Table 12. The adoption effects of iwv on household's per capita consumption expenditure.

Decision stage			
	Adopters	**Non- adopters**	**Adoption effect**
adopters	4778.5(62.53)	4268.73(85.45)	TT=509.77***(105.63)
Non- adopters	2985.75(78.9)	1452.2(25.73)	TU=1533.55***(63.36)
Heterogeneity effect	**TH= -1023.78**		

Note:TT=Adoption effect for adopters, TU= Adoption effect for non-adopters, TH (TT-TU) = transitional heterogeneity; ***Significant at 1% level.

their counterparts if their plot land is increased by one more unit. However, plot size has a strong positive impact on the welfare of adopters. Increased in the size of plot land, increase the per capita consumption of adopters by 12% more than the consumption of non-adopters. This is the fact that the large land size allows applying improved technologies and used as security to access credit compared to those with small land size. Similar results have been found in (Hailu et al., 2014).

Average expected per capita consumption expenditure

From our previous result, we have found that adopting IWV has a positive significant effect on household's per capita consumption expenditure. However, this simple measurement is inappropriate as both observed and unobserved factors which may have an effect on the outcome variable may not be considered. Therefore, it is important to compare the value of the outcome variable with the actual and counterfactual cases. In Table 9 the result on the expected consumption expenditure in the actual and counterfactual cases is presented. The result indicates that adoption of IWV do not have the same effect on adopters had they been a non-adopter and non-adopters had they been an adopter.

The number in the first row first cell of Table 12 is the average per capita consumption value (4778.5) for adopters of IWV. The number in the second cell (4268.73) indicates the average per capita consumption for adopters in the counterfactual case. Then the adoption effect on adopters can be found by subtracting the second cell from the first cell (509.77). The result is positive and significantly different from zero. This suggests that the farm household's consumption per adult equivalent for those who adopted IWV is significantly higher than if they did not adopt. By using a similar procedure the adoption effect of IWV on non-adopters can be calculated from the same table. In the second row first cell of the following table, we get the value of average per capita consumption for non-adopters in the counterfactual case, while the second cell in the same row represents the same value in the actual case. Then by taking the difference between the first and the second cell we can get per capita consumption of non-adopters (1533.55). The result indicates that per capita consumption will increase significantly if they adopt IWV than the actual case of non-adoption. Similar studies by Di Falco et al. (2011); also reported the same result with our study.

Conclusions

From the results of the study, we found it possible to draw the following conclusions. First, it was found that household characteristics, plot characteristics and institutional factors are the main determinants of adopting improved wheat verities. Age of household head, off-farm employment, and farm experience were the key household characteristics that determine the likelihood of adoption significantly. Extension service, credit access, soil fertility, plot size and land size affect the probability of adopting IWV positively and significantly. Whereas, farm experience, age of the household head, distance from the input market and a number of the plot (fragmentation) negatively affect the decision of farmers improved wheat technology adoption. Second, the study revealed that adoption of improved wheat variety is found to be less in plots which are located in the farther distance to nearest input market and have more farming experience with many numbers of plots. Plots that are far from the input market fails to get timely access to inputs and accessibility will become costly to get. Indeed farmers cannot visit continuously due to their distance problem. Likewise, households with lots of farm experience are associated with less likelihood of adoption. Similar to the effect on the decision model, variables like; age, off-farm employment, distance from the input market, extension, credit, size and soil fertility, and farm experience have a strong and significant effect on the amount of improved wheat per hectare adopted by households. The intensity of adoption is lower for households with higher age, far from the input market, plots far from the homestead, more land size and high level of farm experience. However, the level of technology adoption is high for households with more education, high household size, off-farm employment, more livestock ownership, more extension service, access to credit service and fertile soil. The study applied the Double Hurdle (DH) model to simultaneously estimate the decision/ Probit and intensity/ truncated model.

Third, factors such as household size, soil fertility, access to credit, and a number of extension visits are the major determinants of households consumption expenditure per adult equivalent for both adopters and non-adopters. Household size measured in adult equivalent; reduce the per capita consumption of both adopters and non-adopters. However, its effect is severe on non- adopters. Soil fertility, access to credit, and the number of extension visits are found to spur household welfare for both adopters and non-adopters, though adapters are better than their counterparts. Other variables like; age, farm experience, asset ownership (TLU), and a number of plot increase the welfare of adopters, leaving non- adopters welfare neutral. On the contrary, the distance of the plot from the nearest input market and plot size reduces adopter's welfare. Fourth, both adopters and non-adopters adopting improved what varieties can improve the farm household's welfare, given they decided to adopt than they would if they had not adopted it. In addition, non-adopters can get the largest payoff relative to adopters if both of the two groups decided to adopt. To recap, the regression result revealed that agricultural technology adoption has a positive and significant effect on the farm by which adopters are better off than non- adopters of the technology.

RECOMMENDATIONS

The findings of this study are indisputably essential to develop policies and strategies that aimed at improving the wellbeing of farm households through improved technology adoption and application of these technologies at a large scale. The result conveyed that the adoption of improved wheat varieties has a positive and significant effect on adopter's welfare. Hence, participation in technology adoption should be further advanced and barriers to access technologies should be settled. Therefore, this study draws the following main policy implications.

(i) Institutional factors like, extension, and credit are found the most important factors which increase the likelihood of adoption. Thus, at most attention should be given by policymakers for the provision of credit service and the number of extension visits for rural farm households. This will enable to increase their willingness and ability to purchase/ participate in new agricultural technologies through relaxing their cash constraint and providing them with better information on the access and application of the technology. Furthermore, the distance to the input market negatively affects the probability of adoption. Hence, alternative ways of accessing complementary inputs which are necessary for effective agriculture should be in place. Mainly improving access to infrastructure might be an alternative.

(ii) Though Farm size affects both the household's welfare and the decision to adopt, it affects adoption decision positively and welfare negatively. As farm size increases the likelihood of adoption increase while farmer's welfare will decline. This implies that farm households are better productive and highly motivated to practice IWV at lower farm size. Therefore, agricultural policies should invest more on mechanisms that enable farmers to be more productive in small land size. So as to augment agricultural productivity and to reduce rural poverty it is better to focus on intensive farming compared to extensive farming

(iii) Credit constraint is a headache of a rural farm household's welfare. Keeping other things stable, by adopting improved/high yield varieties farmers can improve their welfare substantially in terms of per capita consumption expenditure increment. Therefore, it is strategic to promote the adoption of IWV in credit-constrained farm households.

(iv) Since the application of improved technology adoption increase farm household's welfare, increasing the participation of farmers on adoption and their level of adoption vital to spurring agricultural productivity and hence welfare.

(v) Since aged farmers and those with higher years of farm experience have a low rate of technology adoption, their productivity will be lower which would end up with poverty. Therefore, those farmers are needed to be supplemented by strong institutional support and access to credit.

(vi) Household size reduces farmer's per capita consumption expenditure, which might be a higher dependency level. Therefore, it should be better if appropriate family planning mechanism and information on the relevance is addressed timely.

(vii) Despite the positive effect of the adoption of IWV on both adopters and non-adopters, the extent of benefit from the treatment effect is not equal and comparable. This implies the existence of divergence between the two groups. So policymakers should take in to account this heterogeneity when they are attempting to advance the relevance of IWV so as to secure the full potential benefit of the practice.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Asfaw S, Shiferaw B, Simtowe F, Lipper L (2012). Impact of modern agricultural technologies on smallholder welfare: Evidence from Tanzania and Ethiopia. Food Policy 37:283-295.

Asfaw S, Shiferaw B, Simtowe F, Haile M (2011). Agricultural technology adoption, seed access constraints and commercialization in Ethiopia. Journal of Development and Agricultural Economics 3(9):436-477.

Awotide B, Karimov A, Diagne A (2016). Agricultural technology adoption, commercialization and smallholder rice farmers' welfare in

rural Nigeria. Agricultural and Food Economics 4(3):1-24
Bickford R (2019). Ethiopia Grain and Feed Annual Report.Global Agricultural Information Network, USDA foreign agricultural service staff report, ET1903.
Bingxin Y, Alejandro NP (2014). Fertilizer Adoption in Ethiopia Cereal Production. Journal of Development and Agricultural Economics 6(7):319-337.
Birkhaeuser D, Evenson RE, Feder G (1991). The economic impact of agricultural extension: A review. Economic Development and Cultural Change 39(3):607-650.
Cragg JG (1971). Some statistical models for limited dependent variables with application to the demand for durable goods. Econometrica (pre-1986) 39(5):829.
Central Statistical Agency (CSA) (2011). Report on Area and Production of Major Cereals (Private Peasant Holdings, Meher Season). Agricultural Sample Survey 2110/11, Addis Ababa, Ethiopia.
De Janvry A, Sadoulet E (2002). World poverty and the role of agricultural technology: direct and indirect effects. Journal of Development Studies 38(4):1-26.
Di Falco S, Marcella V, Mahmud Y (2011). “Does Adaptation to Climate Change Provide Food Security? Micro Evidence from Ethiopia” American Journal of Agricultural Economics 93(3):829-846.
Gebregziabher G, Holden S (2011). Does Irrigation Enhance and Food Deficits Discourage fertilizer Adoption in a Risky Environment? Evidence from Tigray. Ethiopian Journal of Development and Agricultural Economics pp. 514-528
Gebremedhin B, Swinton SM (2003). Investment in soil conservation in northern Ethiopia: the role of land tenure security and public programs. Agricultural Economics 29(1):69-84.
Greene WH (2000). Econometric Analysis. 4th edition. Englewood Cliffs: NJ: Prentice-Hall.
Hailu BK, Abrha BK, Weldegiorgis KA (2014). Adoption and Impact of Agricultural Technologies on Farm Income: Evidence from Southern Tigray. International Journal of Food and Agricultural Economics 2:91-106.
Hausman JA (1978). Specification tests in econometrics. Econometrica 46:1251-1272
Heckman J (1979). Sample selection as a specification error. Econometrica 47:153-161.
Jaleta M, Kassie M, Marenya P (2015). Impact of Improved Maize Variety Adoption on Household Food Security in Ethiopia: An Endogenous Switching Regression Approach. International conference of agricultural economists, agriculture in the interconnected world, Milan Italy.
Jose A (1989). A double-hurdle model of cigarette consumption. Journal of Applied Econometrics 4(1):23-39.
Kassie M, Shiferaw B, Muricho G (2011). Agricultural technology, crop income, and poverty alleviation in Uganda. World Development 39(10):1784-1795.
Kassie M, Jaleta M, Shiferaw B, Mmbando F, Muricho G (2012). Plot and household-level determinants of sustainable agricultural practices in rural Tanzania. Environment for Development Discussion Paper-Resources for the Future (RFF), (12-02).
Kassie M, Jaleta M, Mattei A (2014). Evaluating the impact of improved maize varieties on food security in Rural Tanzania: Evidence from a continuous treatment approach. Food Security 6(2):217-230.
Kassie M, Yesuf M, Köhlin G (2009). The role of production risk in sustainable land-management technology adoption in the Ethiopian Highlands. rapport nr. Working Papers in Economics 407.
Maertens A, Barrett CB (2013). Measuring Social Networks' Effect on Agricultural Technology Adoption. American Journal of Agricultural Economics 95(2):353-359.
Mekonen T, Karelplein K (2014). Productivity and Household Welfare Impact of Technology Adoption: A Micro-econometric Analysis. United Nation University and Maastricht University.
Mohamed KS, Temu AE (2008). Access to credit and its effect on the adoption of agricultural technologies: the case of Zanzibar. African Review of Money Finance and Banking pp. 45-89.
Nyangena W, Maurice O (2014). Impact of Improved Farm Technologies on Yields, the Case of Improved Maize Varieties and Inorganic Fertilizer in Kenya. The environment for Development, Discussion Paper Series (EfD DP 14-02).
Rogers EM, Shoemaker FF (1971). Communication of innovations: A cross-culture approach. The Free Press, Collier Macmillan Publishing Inc, NY pp. 11-28.
Shiferaw B, Kassie M, Jaleta M, Yirga C (2014). Adoption of improved wheat varieties and impacts on household food security in Ethiopia. Food Policy 44:272-284. Elsevier Ltd.
Shiferaw K, Tewodros AK, You L (2008). Technology Adoption under Seed Access Constraints and the Economic Impacts of Improved Pigeon pea varieties in Tanzania. Agricultural Economics 39(3):309-323.
Tafesse T, Sodo W (2016). Adoption and Intensity of Row-Seeding (Case of Wolaita Zone). Open Access Library Journal 3(03):1.
Teklewold H, Dadi L, Yami A, Dana N (2006). Determinants of adoption of poultry technology: a double-hurdle approach. Livestock Research for Rural Development 18(3):1-14.
Terefe T, Ahmed H, Gebremariam G (2013). Adoption and extent of use of organic fertilizer in Arsi Negelle District, Oromia Regional State of Ethiopia: What are the sources? Advanced Journal of Agricultural Research 1(004):061-071.
Tesfaye S, Bedada B, Mesay Y (2016). Impact of improved wheat technology adoption on productivity and income in Ethiopia. African Crop Science Journal 24(s1):127-135.
Tsehaye A (2016). The Analysis of Fertilizer use and Agricultural Productivity: (Case of La'ilay Maychew Woreda, Tigray, Ethiopia). A paper presented on 14th International Conference on the Ethiopian Economy, organized by Ethiopian Economics Association (EEA).
World Bank (2006). World Development Report: Agriculture for Development, Washington D.C.
Wossen T, Berger T, Di Falco S (2015). Social capital, risk preference and adoption of improved farmland management practices in Ethiopia. Agricultural Economics 46:81-97.
Yu B, Nin-Pratt A, Funes J, Gemessa SA (2011). Cereal production and technology adoption in Ethiopia. Ethiopia Strategy Support Program II (ESSP II), ESSP II Working Paper 31.

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Improvement of growth performance and meat sensory attributes through use of dried goat rumen contents in broiler diets

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The study investigated the use of dried goat rumen contents (DGRC) on growth performance of broiler chickens. Rumen contents were obtained from goats immediately after slaughter during the wet season, sundried, milled and incorporated in experimental diets at levels of 0, 5 and 10%. The 0% DGRC diet was the control. The experimental diets were formulated on iso-caloric and iso-nitrogenous principles in line with the nutritional requirements for growing broiler birds. Experimental birds were first fed on a common starter broiler diet comprising of 21% CP and 3100 Kcal/kg feed from 0 to 21 days of age; thereafter the birds (21-42 days) were allotted to the experimental treatments in a completely randomized design (CRD) with three replications. A cage with 10 birds was the experimental unit. Experimental diets were offered in the morning and evening, water was provided *ad lib*. Feed offered and leftovers were weighed daily, and body weight changes were recorded on a weekly basis. The results showed that birds on the 5% diet had significantly (Linear, Quadratic *P*<0.05) higher final body weights (FBWs), average daily gain (ADG) and better feed conversion ratio (FCR) compared to those o on diets with 0 and 10% DGRC. Apparent and ileal digestibility of nutrients was improved with incorporation of dried goat rumen contents in the diets. Sensory analysis showed that meat from birds on 5% DGRC diet had (*P*<0.05) more oil content and softer meat across diets. It is concluded that, use of dried goat rumen contents (DGRC) in broiler diets improves growth performance and organoleptic qualities of broiler chicken meat.

Key words: Digestibility, growth performance, rumen contents, sensory attributes.

INTRODUCTION

In Uganda, many commercial poultry farmers are grappling with feed related costs that have pushed

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several of them out of the poultry business. In a bid to remain competitive in the poultry industry, poultry farmers near slaughter houses mostly in the urban settings have ventured into the use of rumen contents as alternative protein source to fish meal (FM) which is a more expensive feed ingredient (Van Huis et al., 2013). Fish meal is a predominant principal source of animal protein in animal feed industry due to its higher biological value and essential amino acids profile (Shahid and Talat, 2005) has become a target of adulteration by many feed dealers. This is in a bid by feed dealers to make more profits at the expense of the poultry farmers. Eventually, the production of birds has been poor and poultry farmers' returns to investment have also decreased with many failing to break even as a consequence of rampant feed ingredient adulteration. The inclusion of rumen contents in poultry diets comes as not only a sustainability strategy by poultry farmers (Vahid et al., 2017; Sugiharto, 2019), but also pivotal to the safe disposal of abattoir wastes through recycling (Dairo et al., 2005; Esonu et al., 2006). However, rumen content use in poultry diets is done with limited information in regard to processing, and level of use in poultry diets which further compromises production performance of the birds. Despite having relatively good crude protein (CP) and minerals levels, energy and vitamins especially vitamin B complex (Agbabiaka et al., 2012), rumen contents contain high dietary fiber which affects dietary energy levels. More so, rumen contents tend to have a repulsive smell and an inherent color which affects its acceptability by the animals. Previous studies have reported variations in rumen content composition. For instance, Ravindra et al. (2017) reported that use of buffalo rumen content in poultry diet contain a crude protein (CP) of 8.5% and crude fiber (CF) of 34.1%. On the other hand, a study by Sakaba et al. (2017) found that cattle rumen content contained CF of 48.1% and CP of 14.73%, whereas Sheep rumen contents were found to contain CF and CP of 48.7 and 15.5% respectively. All these reports indicate nutritional variability of rumen content with respect to animal type. Moreover, during the dry season, animals tend to consume forages that are coarse and high in fiber but with little nutritional composition which results in rumen content of high crude fiber (CF), low crude protein (CP) and consequently, of low nutritional value to poultry. However, small ruminants and particularly goats have a higher degree of quality forage selection than grazing large ruminants (Taylor and Kotman, 1990). Goats tend to go for tender and more nutritious plant portions of plants (low lignification, high CP and low tannin) which translates to finer rumen contents with less fiber and of potentially high nutritional value to chickens. The objective of the present study was to improve the utilization of rumen contents in poultry through evaluation of dried goat rumen content (DGRC) as a protein substitute to fish meal on broiler performance, carcass characteristics and sensory attributes.

MATERIALS AND METHODS

Experimental site

The study was conducted at Tatton Agriculture Park (TAP), Egerton University, Njoro, Kenya. The farm lies on a latitude of 0° 23' south, longitude 35° 35' East and an altitude of 2238 m above sea level and receives a bimodal mean annual rainfall of 1000-1200 mm. Long rains are received between April and August and short rains between October and December. According to Egerton University weather station, mean annual temperatures range between 10 and 22°C (Egerton University, Civil and Environmental Engineering Department).

Rumen content collection and processing

Goat rumen contents were collected from Kampala city abattoirs immediately following slaughter during the wet season of September- January 2018 and March to April 2019. The rumen content was then sun dried to a moisture content of about 12%, bagged stored. Thereafter it was milled in a hammer mill through a 1.5 mm screen.

Proximate analysis

Ground rumen content samples were analyzed for dry matter (DM), nitrogen (N), gross energy (GE), ether extract (EE), calcium (Ca), phosphorus (P), crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) (AOAC, 2005). Dry matter was determined according to AOAC International (2005) standard procedures. Nitrogen was determined by Kjedhal's method (AOAC International, 2005, method 968.06) using a CNS-2000 carbon, nitrogen, and sulfur analyzer (Leco Corporation, St. Joseph, MI). The CP values were determined by multiplying the assayed N values by 6.25. GE was determined using an adiabatic bomb calorimeter (Gallenkamp, London, UK), standardized with benzoic acid. Ether extract content was determined following Soxhlet extraction procedure. Calcium (Ca) and phosphorus (P) were determined using atomic absorption spectrophotometer. Nitrogen free extract (NFE) was calculated by subtracting the sum of % ash, % crude ether extract (EE), % crude fibre (CF) and % crude protein (CP) from 100.

$$\%NFE = 100 - (\%Ash + \%CF + \%EE + \%CP)$$

Amino acid (AA) analysis of the feed ingredients

Amino acid analysis was performed at Laboratory of Gastrointestinal Microbiology, National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University. The protein samples were hydrolyzed in gas phase using 6 M HCl at 115°C for 24 h. The liberated amino acids were converted into phenylthiocarbamyl derivatives and analyzed by high-pressure liquid chromatography (HPLC) on a PicoTag 3.9 × 150 mm column (Waters, Milford, MA, USA).

Dietary formulations

The ingredients used in formulating experimental diets are shown in Table 1. In the diets, dried goat rumen content (DGRD) substituted fish meal at levels of 0, 5 and 10%. The diet with 0% dried goat rumen contents was the control. The experimental diets were

Table 1. Dietary composition of broiler finisher diets (22-42 days).

Ingredients	**Dietary treatment**		
	T1	**T2**	**T3**
DGRC	0.0	5.0	10.0
BM	59.0	67.0	68.2
WP	17.1	7.1	2.0
FM	10.0	8.0	6.0
SBM	12	11	11.9
DCP	0.9	0.9	0.9
Lime stone	0.2	0.2	0.2
Salt	0.3	0.3	0.3
*Vitamin premix	0.5	0.5	0.5
Total/kg	**100**	**100**	**100**
Calculated (%)			
DM	89.6	89.8	90.9
CP	20.6	20.8	20.2
Ca	0.5	0.5	0.5
P	0.6	0.6	0.6
CF	4.2	4.9	5.3
ME Kcal/Kg	3141	3112	3100

*To supply Vitamins A 12000000 iu; D3 2500000 iu; E 20000 mg; K3 2000 mg; B1 2000 mg; B2 5000 mg; B6 4000 mg; B12 15 mg; Niacin 30000 mg; Pantothenic acid 11000 mg; Folic acid 1500 mg; Biotin 60 mg; Choline chloride 220000 mg; Antioxidant 1250 mg; Mn 50000 mg; Zn 40000 mg; Fe 20000 mg; Cu 3000 mg; I 1000 mg; Se 200 mg; Co 200 mg. [1] DGRC: dried goat rumen contents; BM: broken maize; WP: wheat pollard; SB: soybean meal; FM: Fish meal; DM: dry matter; CP: crude protein; CF: crude fibre; Ca: calcium; P: phosphorous; ME: metabolisable energy.

iso-caloric (3100 Kcal/Kg) and iso-nitrogeneous (20% CP) and contained equal levels of calcium (Ca), phosphorus (P), sulphur amino acids, lysine and sodium in line with the dietary nutritional requirement for growing broiler birds (NRC, 2001).

Management of birds, feeding and performance measurements

Day old broiler chicks were purchased from KENCHICK, a Kenyan company that specializes in poultry related businesses. The chicks were brooded for 21 days under a common starter diet; after which they were weighed and allotted to 9 cages (10 chicks per cage) such that the mean bird weight per cage was similar (0.52±0.01 g). The three dietary treatments were then randomly allotted to 9 cages in a completely randomized design (CRD) with three replicates. Birds were allocated a space of 530 and 640 cm^2 in the brooder and grower cages respectively. Feed and clean water were provided *ad libitum* basis during the entire experimental period. Vaccination against major diseases like Gumboro, fowl pox and Newcastle were carried out in line with the recommended veterinary vaccination schedule. Experimental diets were offered to the birds in the finisher stage starting on the 22^{nd} day, as to allow time for the bird's caecum to develop fully to size capable of handling fiber in the experimental diets. Body weight (BW) was taken on a weekly basis while feed intake (FI) on a daily basis throughout the experimental period. Mortalities were recorded whenever they occurred. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated by dividing the change in weight and total feed intake by weight gain of live birds in a week respectively.

Digestibility of experimental diets

From the 35^{th} to 39^{th}, feed intake and total faecal output were measured per cage over a period of 4 consecutive days for determination of nutrient retention and apparent metabolizable energy (AME). On the 42^{nd} day, 12 birds per treatment were euthanized by intracardial injection of sodium pentobarbitone and contents of the lower half of the ileum were expressed by gentle flushing with distilled water. Digesta from birds within a cage was pooled, resulting in 3 samples per dietary treatment; the samples were frozen immediately after collection and stored for analysis.

Sample chemical analysis

The samples of excreta and ileal contents were freeze dried. Samples of diets, ileal contents and excreta were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at −4°C until chemical analyses.

Determination of apparent metabolisable energy and ileal digestibility

Apparent metabolizable energy (AME) values of the diets were calculated from the equation below

$$AME\ feed\frac{MJ}{Kg} = \frac{(Feed\ intake\ x\ GE\ diet) - (Excreta\ out\ put\ x\ GE\ excreta)}{Feed\ intake}$$

Where GE is given in kilocalories per kilogram, and feed intake and faecal output in kilograms DM per day. Nitrogen-corrected AME was determined by correction for zero nitrogen retention by simple multiplication with 8.22 kcal per gram nitrogen retained in the body as described by Hill and Anderson (1958).

The apparent ileal digestibility of DM, nutrients (CP and fat), and GE was calculated following the formula below, Chromium III Oxide external marker ratio in the diet and ileal digesta:

$$\text{Apparent ileal digestibility}(\%) = \frac{\left(\frac{NT}{indicator}\right) diet - \left(\frac{NT}{Indicator}\right) ileal\ digesta}{\left(\frac{NT}{Indicator}\right) diet} x\ 100$$

Where, (NT/Indicator) diet = ratio of component and indicator in the diet, and (NT/Indicator) ileal content = ratio of component and indicator in ileal contents. Component can be DM, CP, fat, or GE.

Apparent total tract retention of DM, EE, CP, calcium, phosphorus, and CF were calculated as follows:

$$\text{Retention \%} = \frac{(Feed\ Intake\ x\ Component\ in\ diet) - (\text{Excreta out put x Component in excreta})}{(Feed\ Intake\ x\ Component\ in\ diet)} x\ 100$$

Components are DM, EE, CP, calcium, phosphorus, CF. Feed intake and excreta output are given in kilograms per day and components as a percentage (Adeola et al., 2008).

Slaughtering procedure

At the 42^{nd} day, 4 birds per treatment per replicate were numbered randomly, selected, weighed, and slaughtered in accordance with the animal welfare law (Anderson, 2004). Prior to slaughter, feed was withdrawn for 12 h but water was provided *adlibitum* basis in order to clear the digestive tract. The birds were slaughtered following the cervical dislocation method, then plucked and eviscerated. Breast and thigh meat were used for sensory evaluation.

Sensory evaluation of broiler meat samples

Cooked meat samples (breast and thigh) were evaluated for sensory attributes at the Department of Dairy, Food Science and Technology (DAFTEC), Egerton University. A commercial broiler from a local supermarket was purchased and used as a reference sample during orientation and evaluation. A trained 20-member panel (instructors and undergraduate and post graduates students) was used to evaluate the meat samples using a 15-cm line scale. The panelists were trained in sensory evaluation according to Stone and Sidel (2004). During the orientation sessions, the panel agreed on the attributes to use for evaluation, evaluated several samples, and rated the intensities of the reference sample (agreed upon by consensus by the panel). The reference was used as a warm-up sample and was provided to the panelists with its intensities during the testing sessions. Boiled chicken meat was prepared following deboning and cutting into small pieces of approximately 2 x 2 cm. Meat from each carcass was cooked separately according to the treatments. The meat pieces were put into the cooking pot and water was added to cover it. The cooking lasted for 50 min. The cooked meat pieces were then presented for descriptive sensory analysis. Samples were randomized according to the diet of the chicken and then by meat type (breast meat or thigh). Each panelist was provided with 6 pieces on a white sensory evaluation plates labeled with 3-digit blinding codes. Cooked samples were evaluated for color, glossiness, juiciness, texture, chewiness, fattiness, chicken flavor, and overall quality. Water was provided for cleansing and rinsing the palate between samples. The panelists recorded attributes intensities on the scale by placing a slash perpendicular to the line at the point that best described the attribute. The numerical intensity was measured in centimeters with a ruler from the left-hand side of the scale.

Data analysis

Data for growth, digestibility and carcass data were first subjected to normality and homogeneity of variance tests and thereafter were analyzed using the GLM procedures of SAS (2010) as a completely randomized design (CRD). Treatment effects were determined with orthogonal contrasts arrangement. Separation of means where significant differences occurred was done using Tukey's test at $P \leq 0.05$. The sensory data did not meet the conditions for parametric statistical tests and therefore non-parametric statistical tests were applied. The differences between groups for sensory data were tested with Kruskal-Wallis test.

RESULTS

Proximate analysis of feed ingredients and experimental diets

Table 2 shows the results of the laboratory analysis of the feed ingredients used in the formulation of experimental diets. Dried goat rumen contents (DGRC) had lower crude protein (CP) and Metabolizable energy (ME) compared to rest of the ingredients used; however, DGRC was higher in crude fiber (CF) and Phosphorus (P). Amino acid (AA) profile and dried goat rumen content (DGRC) were comparable to wheat pollard (WP). Overall, total amino acids (TAA) were higher for fish meal (FM) followed by broken maize (BM).

Proximate composition of experimental diets

Chemical composition of the experimental diets is shown in Table 3. Dry matter (DM), Crude protein (CP) and ether extract (EE) was similar across diets. Crude fiber (CF) in the dietary treatments differed slightly and was higher in diet with 10% dried goat rumen content inclusion levels (DGRC). Despite the slight disparities in the CF content of the diets, CF of the diets was within the range (2-5%) for optimum broiler performance as reported by NRC. Metabolizable Energy (ME) of the dietary treatments ranged between 31500-3200 Kcal/kg feed.

Effects of DGRC inclusion levels on apparent and ileal nutrient digestibility of diets in broilers

Apparent and ileal nutrient digestibility for experimental diets in broiler chickens is shown in Table 4. The results in the present study indicated that inclusion of DGRC levels in broiler diets had a significant ($P<0.05$) effect on apparent and ileal digestibility in broiler chickens. The

Table 2. Proximate composition of feed ingredients used in experimental dietary formulations**.**

Composition (%)	BM	WP	SBM	FM	DGRC
DM	89.51	86.00	89.23	93.10	97.3
CP	10.64	10.61	44.41	58.21	16.2
CF	1.95	7.43	3.51	12.01	20.7
Ca	0.01	0.001	0.20	2.97	1.70
P	0.23	0.06	0.65	2.62	3.80
ME	3400.01	2900.32	2800.41	3290.03	1190.16
Amino acids					
Ala	0.13	0.10	0.12	0.18	0.09
Arg	0.11	0.07	0.07	0.10	0.09
Asn	0.00	0.00	0.00	0.00	0.00
Asp	0.16	0.14	0.18	0.18	0.18
Gln	0.01	0.00	0.00	0.01	0.00
Glu	0.27	0.08	0.15	0.27	0.06
Gly	0.12	0.07	0.04	0.12	0.05
His	0.00	0.02	0.02	0.00	0.02
Ile	1.98	1.81	1.86	2.01	1.76
Leu	0.09	0.03	0.07	0.12	0.00
Lys	0.11	0.06	0.09	0.15	0.07
Met	0.09	0.07	0.04	0.15	0.06
Phe	0.03	0.03	0.03	0.05	0.03
Ser	0.00	0.00	0.00	0.00	0.00
Thr	0.11	0.07	0.00	0.14	0.03
Trp	0.05	0.05	0.03	0.05	0.05
Tyr	0.09	0.09	0.09	0.09	0.09
Val	0.12	0.09	0.11	0.15	0.09
Total AA	3.47	2.77	2.90	3.79	2.66

[1] BP: broken maize; WP: wheat pollard; SB: soybean meal; FM: Fish meal; DGRC: dried goat rumen contents; DM: dry matter; CP: crude protein; CF: crude fiber; NDF: neutral detergent fiber; EE: ether extract; Ca: calcium; P: phosphorous; ME: metabolisable energy; AA: Amino acids Ala: Alanine; Arg: Arginine; Asn: Asparagine; Asp: Aspartic acid; Gln: Glutamine; Glu:Glutamic acid; Gly:Glycine; His:Histidine ; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Phe: Phenylalanine; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine.

Table 3. Chemical composition of the experimental diets (laboratory analysis).

Parameter	Dietary treatment		
	T1	T2	T3
DM%	89.73 ±0.26	87.11± 0.26	87.99 ±0.26
CP%	21.01 ±3.14	21.09± 3.14	21.11 ±3.14
EE%	7.00 ±0.01	8.00 ±0.01	8.00 ± 0.01
CF%	2.9 ±0.02	3.50 ±0.02	4.50 ±0.02
Ash%	5.25 ±0.22	5.05 ±0.22	4.77± 0.22
Ca%	1.98 ±0.07	1.90±0.07	1.80±0.07
P%	0.36 ±0.54	1.18 ±0.54	0.29± 0.54
NFE%	64.74 ±1.74	61.20 ± 1.74	66.56 ± 1.74
ME Kcal/Kg	3275.14 ±7.17	3242.06 ± 7.17	3236.75 ± 7.17

DM: dry matter; CP: crude protein; CF: crude fiber; NDF: EE: ether extract; Ca: calcium; P: phosphorous; NFE Neutral free extract; ME: metabolisable energy. Values presented in means and standard error of means (Means±SE); T1=0% DGRC; T2=5% DGRC; T1=10% DGRC.

Table 4. Apparent nutrient and ileal digestibility of diets containing DGRC in broilers.

Apparent nutrient digestibility	Dietary treatment		
	T1	T2	T3
DM	71.27[b]	93.92[a]	71.27[b]
CP	60.31[c]	89.67[a]	85.29[b]
CF	59.14[c]	71.80[a]	67.82[b]
EE	64.34[c]	87.42[a]	77.60[b]
Ca	74.04[c]	86.72[a]	80.12[b]
P	69.44[b]	75.53[a]	74.92[a]
Ash	60.35[c]	82.35[a]	78.61[b]
NFE	82.2[c]	89.97[a]	86.06[b]
ME	78.89[c]	92.22[b]	81.31[b]
SEM	0.63	0.88	2.48
Linear	0.9655	0.0003	0.0898
Quadratic	0.1628	<0.0001	0.0012
Apparent ileal digestibility			
DM	79.94[b]	91.22[a]	68.22[c]
CP	79.17[b]	91.85[a]	68.26[c]
CF	65.23[c]	90.37[a]	76.48[b]
EE	70.02[b]	79.42[a]	67.60[c]
Ca	76.69[b]	92.56[a]	61.25b[c]
P	77.68[a]	77.83[a]	62.06[b]
Ash	83.55[a]	83.57[a]	72.47[b]
NFE	76.41[ab]	78.22[a]	69.86[b]
ME	81.13[a]	83.35[a]	72.00[b]
SEM	2.11	2.75	2.123
Linear	0.0234	0.0617	0.1949
Quadratic	0.0040	0.4498	0.0433

[abc]Means with different superscript within row differ significantly (P<0.05). BP: broken maize; WP: wheat pollard; SB: soybean meal; FM: Fish meal; DGRC: dried goat rumen contents; DM: dry matter; CP: crude protein; CF: crude fiber; NDF: neutral detergent fiber; EE: ether extract; Ca: calcium; P: phosphorous; ME: metabolisable energy; SEM: Standard error of the mean.

experimental diets with 5% DGRC levels had the highest apparent digestibility coefficients for DM,CP,CF, EE, Ca, Ash and NFE followed by diets with 10% DGRC levels with exception P and ME which were comparable. The diets without DGRC had the lowest apparent digestibility in those parameters compared with other studied experimental diets with DGRC. Generally, the results showed an increasing trend of apparent digestibility coefficients for all nutrients studied nutrients with inclusion of 5% DGRC level in the experimental diets but showed a decreasing trend with incorporation with 10% DGRC level in the diet with exception of P and ME. In addition, the results indicated that diets with 5% DGRC inclusion level in the experimental diets had the highest ileal digestibility whereas those diets with 10% DGRC inclusion level had lowest ileal digestibility for DM, CP, CF, EE, Ca and NFE. Therefore, the diets with 5% DGRC level in the experimental diets showed an increasing trend of ileal digestibility for DM, CP, CF, EE, Ca and NFE but inclusion of 10% DGRC level in diets showed a decreasing trend of ileal digestibility compared to those diets with 5% DGRC level though had higher digestibility coefficients than control diets.

Performance of broiler chickens fed dried goat rumen content

The performance of birds fed diets with dried goat rumen content is shown in Table 5. Incorporation of dried goat rumen contents in broiler diets lead to improved growth performance of birds. Birds on 5% dried goat rumen content (DGRC) had a significantly higher average final body weight (Linear, Quadratic $p<0.05$), average daily gain (ADG) (Quadratic, $p<0.05$) and a lower average daily feed intake (ADFI) compared to the control diet (Table 5). Similarly, the feed conversion ratio (FCR) was significantly lower (Linear, Quadratic, $p<0.05$) for the

Table 5. Performance of broiler chickens fed experimental diets.

Parameter (n=90)	Level of DGRC			SEM	*p*-value	
	0%	5%	10%		Linear	Quadratic
AIBW (kg/bird/day)	0.52[a]	0.52[a]	0.52[a]	0.01	0.9601	0.9080
AFBW (kg/bird/day)	1.59[c]	1.84[a]	1.64[b]	0.02	0.0031	<0.0001
ADFI (g/bird/day)	115.81[a]	88.94[b]	87.40[c]	0.45	<.0001	<0.0001
ADG (g/bird/day)	52.76[b]	60.08[a]	52.29[b]	0.23	0.1636	<0.0001
FCR	2.19[a]	1.49[c]	1.67[b]	0.05	<.0001	<0.0001

[abc]Means with different upper case letters within a row differ significantly at P<0.05; DGRC dried goat rumen content; AIBW average initial body weight; AFBW average final body weight; ADFI average daily feed intake; ADG average daily gain; FCR feed conversion ratio SEM standard error of the mean.

birds fed on diets containing 5% DGRC across diets than birds on the control diet (0% DGRC). Average daily feed intake (ADFI) was significantly higher (Linear, Quadratic $p<0.05$) for birds fed on 0% dried goat rumen content (DGRC) compared to other dietary treatments. However, as the level of DGRC increased in the diet, average daily feed intake (ADFI) of birds decreased. Despite the higher average daily feed intake (ADFI) exhibited by the birds fed on 0% dried goat rumen content (DGRC) inclusion level, the birds showed lower average daily weight gain (ADG). Although there were differences in average daily feed intake (ADFI) exhibited by the birds on different dietary treatments, DGRC in the experimental diets was readily accepted by the birds across the two treatments (5 and 10%) inclusion levels. No death was registered among birds across the three dietary treatments.

Effect of dried goat rumen contents (DGRC) on sensory characteristics of broiler meat

Table 6 shows the effects of incorporating dried goat rumen contents (DGRC) in broiler diets on broiler meat sensory characteristics. The results showed that inclusion of DGRC in broiler diets affected oiliness, wetness, hardness, juiciness and ease of swallow of broiler meat. Birds fed diets with 5% dried goat rumen content had meat with the highest ($P<0.05$) oiliness followed by those fed diets with 10% dried goat rumen whereas those birds fed diets with 0% (control) dried goat rumen had the significantly ($P<0.05$) lowest. However, the present study (Table 6) revealed that inclusion of DGRC in broiler diets had no significant ($P>0.05$) effect on color, flavor, bitterness, sweetness, fishy flavor, springiness, fatty mouth feel, ease of swallow, tooth pack and fibrousness of broiler chicken meat.

DISCUSSION

Chemical composition of feed ingredients and experimental diets

The higher crude fiber (CF) and phosphorus (P) exhibited by diets with dried goat rumen contents is in line with the findings of Djordjevic et al. (2006). Dry matter (DM), crude protein (CP), calcium (Ca) and phosphorus (P) values of DGRC were higher than those reported by Efrem et al. (2016). This may be due to nutritional differences with respect to season and the type of animal from which the rumen contents were gotten from. In this study, goat rumen contents were collected from the abattoir during the wet season, and thus, the forages eaten by the goats prior to slaughter may have been young and tender with high concentration of minerals (Agbabiaka et al., 2012). Goats are browsers and concentrate selectors; they tend to go for tender leaves and grasses that are more nutritious resulting in their rumen content being finer, less fibrous and more nutritious than that of large ruminants (bulky feeders). This may further explain why goat rumen content was lower crude fiber (CF) in relation to results of Efrem et al. (2016). The composition of rumen contents is also influenced by pre-slaughtering conditions exposed to the animals and the length of holding period between feeding and slaughter (Abouheif et al., 1999).

Despite the experimental diets being formulated to meet the nutritional requirements for growing birds at isocaloric and iso-nitrogenous principles (Table 3), there were differences in fiber contents of the diets as a result of dried goat rumen content (DGRC) incorporation. Even though the fiber content of the diet with 10% dried goat rumen content (DGRC) inclusion level (Table 3) was higher, it was within the limit (2-5%) to elicit normal growth responses of the birds. Several authors have reported that rumen contents contain high fiber content which tend to increase the total fiber of the diets (Esonu et al., 2004; Khan et al., 2014).

Effect of DGRC inclusion levels in broiler diets on nutrient digestibility

Improvements in nutrient digestibility leads to increased nutrient availability which eventually improves the performance of birds. In this study, incorporation of dried goat rumen contents (DGRC) at 5% improved the digestibility (Table 4) of crude fibre (CF), calcium (Ca) and phosphorous (P). This implied that, there is a limit to

Table 6. Effect of dried goat rumen contents (DGRC) diets on sensory characteristics of broiler meat.

Attribute	Mean Rank			P-value
	T1	T2	T3	
Color	126.98	134.99	135.52	0.993
Color uniformity	133.45	124.03	138.43	0.446
Flavor	118.27	141.32	137.91	0.097
Fishy smell	143.65	131.54	122.31	0.177
Umami	125.07	131.19	141.23	0.366
Bitterness	137.64	132.41	127.45	0.675
Sweatiness	123.13	142.58	131.79	0.238
Oiliness	109.19	144.34	143.98	0.002*
Wetness	107.31	146.81	142.00	0.001*
Springiness	134.99	132.17	130.34	0.94
Hardness	153.60	122.32	121.57	0.006*
Juiciness	115.45	146.41	135.64	0.025*
Fibrousness	147.54	123.60	125.04	0.066
Chew count	143.35	119.53	131.62	0.144
Sustained Juiciness	123.99	137.79	135.72	0.433
Easy of swallow	116.69	144.97	134.37	0.045*
Fatty feel	117.96	140.09	138.04	0.103
Tooth pack	143.38	123.70	128.83	0.206

*Mean rank significant at (P<0.05); T1= 0% dried goat rumen contents (DGRC); T2=5% dried goat rumen contents (DGRC); T3= dried goat rumen contents (DGRC)

which dried goat rumen contents can be incorporated in diets for growing birds beyond which digestibility of nutrients becomes compromised. The apparent ileal digestibility coefficients (Table 4) revealed the same trend; birds on 5% dried goat rumen content (DGRC) diet had better digestibility coefficients in relation to dry matter (DM), crude protein (CP) and phosphorus than those on 0% DGRC and 10% DGRC diets. However, despite the differences existing in apparent ileal digestibility (AID), the coefficients seemed to have been over estimated by the model. This result concurs with the findings of Garcia et al. (2007).

Effects of inclusion of DGRC in broiler diets on performance of broiler chickens

The improved growth performance of broilers on diets with DGRC compared to those on control diets (Table 5) observed in the present study is in agreement with the the results of Esonu et al. (2006) who reported a general increase in growth rates of birds as rumen contents were increased in the diets. However, the significant decrease in average daily feed intake (ADFI) of birds with increase in DGRC in the diets may be attributed to increase in dietary fiber content (Ubua et al., 2019) and more so, to the unpleasant smell of rumen contents. Dietary fiber limits feed intake (FI) more especially in young birds because their gastrointestinal tract (GIT) cannot digest fiber more easily (Ubua et al., 2019). As the level of DGRC increased in the diets, this could have led to a commensurate increase of unpleasant smell in the diets which could have eventually translated into reduced feed intake by the birds (Odonsi, 2003; Said et al., 2015). Despite the reduced average feed daily intake (AFDI) exhibited by the birds fed diets with DGRC, their growth performance was not compromised. Birds on 5% DGRC diet had better body weights (BWs) with across diets. This may be partly attributed to better feed utilization by the birds (Table 4) and more so, rumen contents are largely comprised of partially digested forages with appreciable quantities of microbial protein, tnnins, volatile fatty acids (VFAs) and a vast array of minerals which could have promoted good chick growth and more so, improved health gut development (Rodriguez et al., 2012; Sugiarto et al., 2014; Alagbe, 2017; Hidanah et al., 2018; Sebola et al., 2019). Even though the diets with DGRC were high in fiber, this could have been within the limits (2-5%) as not to compromise nutrient availability and retention by the birds.

Several studies have reported a decline in performance of birds as the levels of rumen content (RC) was increased in the diets (Colette et al., 2013; Elfaki et al., 2015; Tesfaye et al., 2013). In this study, even though the growth performance of birds declined significantly as the level of dried goat rumen content (DGRC) in the diets was increased up to 10%, this did not differ from the control diet with 0% DGRC. This indicates that, even the

10% DGRC diet could be effective in replacing the control diet (0% inclusion of DGRC) without compromising the bird's performance and at even a better feed conversion ratio (FCR). The lower FCR exhibited by the birds fed on diets with DGRC is in line with the findings of Makinde et al. (2008). These results are handy and comes at a time when poultry farmers are grappling with adulteration of feed ingredients especially those of animal protein origin (fish meal) which are more expensive (Shahid and Talat, 2005; Mwesigwa et al., 2013; Mohanta et al., 2013; Vahid et al., 2017). Rumen contents are less expensive and readily available at most slaughter houses. Therefore, efficient utilization as livestock feed ingredients would not only save farmers a great deal of costs but also safeguard the environment from pollution (Katongole et al., 2009). The mechanisms through which DGRC based diets improved the performance of the birds cannot be fully elucidated by the collected data. However, digestive and absorptive capacity of the birds could have been increased, hence encouraging a greater flow and absorption of nutrients in the small intestines.

Effect of dried goat rumen contents (DGRC) diets on sensory characteristics of broiler meat

The appearance of meat in terms of texture, juiciness, wateriness, firmness, tenderness, odor and flavor are the most important meat factors that determine judgment by consumers before and after purchasing a meat product (Nasir et al., 2017). In the present study, Juiciness, oiliness, flavor and hardness of broiler meat were improved by the addition of dried goat rumen contents (DGRC) in the diets (Table 6). These results implies that inclusion of DGRC in broiler diets imparted fat which may have influenced taste, juiciness and flavor in meat and that could increase acceptability of meat to most consumers (Nasir et al., 2017 Cofrades et al., 2000). Further, the results suggested that inclusion of DGRC in broiler diets increased oiliness to meat and that led to tender meat that could increase acceptability of meat to most consumers. The degree of oiliness of meat is positively correlated with meat softness (de lavergne et al., 2015; Damian et al., 2016). This phenomenon partly explains why broiler meat from birds fed on DGRC content in this study had more tender meat (Table 6) compared with meat from the control group. These results suggest that inclusion of DGRC in the broiler diets could improve sensory characteristics and tenderness of meat, and eventually improve its market demand.

In the present study, inclusion of DGRC in the diet had no significant effect on general color of the meat. These results suggested that DGRC had not influenced myoglobin activities for storing and delivering oxygen in the muscle (Joo et al., 2013). However, in the present study, it was revealed that inclusion of DGRC in the broiler diets had an influence on skin color at the time of slaughter. The birds fed diets incorporated with DGRC had yellowish skin color compared with their counterparts fed the control diets. These results could be associated with carotenoid deposition in the skin, resulting primarily from xanthophylls in the DGRC and that could be of significance to consumer's meat acceptability. Yellow color or appearance of the meat is the most important factor of meat consumer's acceptability. Most of consumers often link yellow color with freshness and nutritional value (Joo et al., 2013). The results suggested that inclusion of DGRC in broiler diets could produce meat with attractive color to consumers and eventually increase its market demand.

CONCLUSION

From the results, it can be concluded that inclusion of DGRC in broiler diets improved nutrient digestibility coefficients for proximate components, minerals and energy contents of broiler diets, suggesting its use could improve utilization and feeding value of broiler diets. It also improved growth performance, feed intake and feed conversion ratio, suggesting that its use could improve broiler performance. Improved sensory characteristics, tenderness and color of broiler meat suggested that DGRC use could improve consumers' acceptability and eventually market demand of broiler chicken meat.

REASEARCH APPROVAL

Permission to carry out this research was granted by the National Commission for Science and Technology of Kenya, under permit No: NACOSTI/P/19/96187/28085. Ethical of approval was granted by Institute of Primate Research of Kenya under Reference No. ISERC/02/19

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abouheif MA, Kradiees MS, Alselbood BA (1999). The utilization of rumen content-brely meal in diets of growing lambs. Asian-Australasian Journal of Animal Science 12(8):1234-1240.

Adeola O, Shafer DJ, Nyachoti CM (2008). Nutrient and energy utilization in enzyme- supplemented starter and grower diets for white pekin ducks. Poultry Science 87(2):255-263.

Agbabiaka LA, Madubuike FN, Uzoagba CU (2012). Performance of catfish (Clariasgariepinus, Burchell, Burchell, 1822) fed enzyme supplemented dried rumen digesta. Journal of Agricultural Biotechnology and Sustainable Development 4(2):22-26.

Alagbe JO (2017). Effect of dietary inclusion of Polyalthia longifolia leaf meal as phytobiotic compared with antibiotics on performance, carcass characteristics and haematology of broiler chicken. Scholarly Journal of Agricultural Science 7(3):68-74.

Anderson E (2004). Animal rights and the values of Non-human life. In: Animal Rights Current Debates and New Directions, CR, Sunstein, Nussbaum M, (Eds.). Oxford: Oxford University Press. pp. 277-98.

Association of Official Analytical Chemists (AOAC) (2005). Official Methods of Analysis of the Association of Analytical Chemists International, 17th Edn. AOAC International, Gaitherburg, MD, USA.

Cofrades S, Guerra MA, carballo J, Fern´andez-Mart´in F, Jim´enez-colmenero F (2000). Plasma protein and soy fiber content effect on Bologna sausage properties as influenced by fat level. Journal of Food Science 65(2):281-287.

Colette NTN, Fotsa JC, Etchu KA Ndamukong, KJN (2013). Effects of dried rumen content and castor oil seed cake diets on haematological indices, serum biochemistry and organoleptic properties of broiler birds. Sky Journal of Agricultural Research 2(9):120-125.

Dairo FA, Aina SOO, Asafa AR (2005). Performance evaluation of growing rabbits fed varying levels of rumen content and blood rumen content mixture. Nigerian Journal of Animal Production 32:67-72.

Damian F, Seon-Tea J, Robyn W (2016). Consumer acceptability of intramuscular fat. Korean Journal Food Science and Animal Resources 36(6):699-708.

de Lavergne MD, van de Velde F, van Boekel M, Stieger M (2015). Dynamic texture perception and oral processing of semi-solid food gels: Part II: Impact of breakdown behaviour on bolus properties and dynamic texture perception. Food Hydrocology 49:61-72.

Djordjevic N, Popovic Z, Grubic G (2006). Chemical composition of the rumen contents in Roe deer (Capreolus capreolus) as potential quality indicator of their feeding. Journal of Agricultural Science 51:133-140.

Efrem G, Getachew A, Mengistu U, Yoseph M (2016). Sun dried bovine rumen content (SDRC) as an ingredient of a ration for White Leghorn layers. East African Journal of Sciences, 10(1):29-40.

Elfaki MOA, Abdelatti KA (2015). Nutritive evaluation of rumen content from cattle, camel, sheep and goat. Global Journal of Animal Scientific Research 3(3):617-621.

Esonu BO, Ogbonna UD, Anyanw GA, Emelanom OO, Uchegbu, MC, Etuk EB, Udedibe ABI (2006). Evaluation of performance, organ characteristics and economic analysis of broiler finisher fed dried rumen digesta. International Journal of Poultry Science 5:1116-1118.

Esonu BO, Azubuike, JC, Emenalom OO, Etuk EB, Okoli IC, Ukwu HO, Nneji, CS (2004). Effect of enzyme supplementation on the performance of broiler finisher fed microdesmispeberula leaf meal. International Journal of Poultry Science 3(2):112-116.

Garcia AR, Batal AB, Dale NM (2007). A comparison of methods to determine amino acid digestibility of feed ingredients for chickens. Poultry Science 86(1):94-101.

Hidanah S, Sabdoningrum EK, Wahjuni RS, Chusniati S (2018). Effects of meniran (*Phyllanthus niruri* L.) administration on leukocyte profile of broiler chickens infected with Mycoplasma gallisepticum. Veterinary World 11(6):834-839.

Hill FW, Anderson DL (1958). Comparison of metabolisable energy and productive energy determinations with growing chicks. Journal of Nutrition 64(4):587-603.

Joo ST, Kim GD, Hwang YH, Ryu YC (2013). Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Science 95(4):828-836.

Katongole CB, Sabiti EN, Bareeba FB, Ledin I (2009). Performance of growing indigenous goats fed diets based on urban market crop wastes. Tropical Animal Health and Production 41(3):329-336.

Khan MW, Pasha TN, Koga A, Anwar S, Abdullah M, Iqbal Z (2014). Evaluation and utilization of rumen content for fattening of Nili-ravi male calves. Journal of Animal and Plant Science 24(1):40-43.

Makinde O, Sonaiya B, Adeyeye S (2008). Conversion of abattoir wastes into livestock feed: Chemical composition of sun-dried rumen content blood meal and its effect on performance of broiler chick. Conference on International Research on Food Security, Natural Resource Management and Rural Development, University of Hohenheim, Tropentag October 7-9, 2008, pp. 2-7.

Mohanta KN, Subhramanian S, Korikanthimath VS (2013). Evaluation of different animal protein sources in formulating the diets for Blue Gourami, Trichogastertrichopterus finger links. Journal of Aquaculture Research and Development 4:2-7.

Mwesigwa R, Mutetikka D, Kugonza DR (2013). Performance of growing pigs fed diets based on by-products of maize and wheat processing. Journal of Animal Health and Production 45(2):441-446.

Nasir AM, Aasima R, Faneshwar K, Vijay S, Vivek S (2017). Determinants of broiler chicken meat quality and factors affecting them: A review. Journal of Food Science and Technology 54(10):2997-3009.

National Research Council (NRC) (2001). Nutrient requirements of poultry (7th Ed.). National Research Council, National Academy of Science Press, Washington.

Odunsi AA (2003). Blend of bovine blood and rumen digesta as a replacement for fishmeal and groundnut cake in Layer diets. International Journal of Poultry Science 2(1):58-61.

Ravindra S, Rajkumar SP, Yadav VJ, Yadav DK (2017). Effect of supplemental microbial protein feed on broiler growth traits. International Journal of Current Microbiology Applied Science 6(9):1140-1144.

Rodriguez, ML, Rebole A, Velasco S, Ortiz LT, Trevino J, Alzueta C (2012). Wheat- and barley-based diets with or without additives influence nutrient digestibility and intestinal microflora. Journal of Science Food and Agriculture 92(1):184-190.

Said IF, Reham MA, Sherif MS, Hassan AA, Mona AE (2015). Impact of feeding dried rumen content and olive pulp with or without enzymes on growth performance, carcass characteristics and some blood parameters of molar ducks. International Journal of Agriculture Innovations and Research 4:2319-1473.

Sakaba AM, Hassan AU, Harande IS, Isgogo MS, Maiyama FA, Danbare BM (2017). Proximate composition of rumen digesta from sheep slaughtered in Zuru Abattoir, Kebbi State, Nigeria. Journal of Agricultural Science and Practice 2(5):86-89.

SAS (2010). SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC. USA.

Sebola NA, Mlambo V, Mokoboki HK (2019). Chemical characterisation of Moringa olifera (MO) leaf and the apparent digestibility of MO leaf meal-based diets offered to three chicken strains. Agroforestry System 93:149-160.

Shahid R, Talat NP (2005). Effect of different levels and source of fish meal on the performance of broiler chicks. International Journal of Scientific and Engineering Research 6:78-89.

Stone H, Sidel JL (2004). Sensory Evaluation Practices, 3rd (edn). Elsevier Academic Press, San Diego, California.

Sugiarto A, Rosyidi D, Hasanuddin A (2014). Protein digestibility, performance and carcass quality of broiler chickens fed diets supplemented with centrifuged rumen contents. Livestock Research for Rural Development. Volume 26, Article #31. Retrieved October 26, 2019, from http://www.lrrd.org/lrrd26/2/sugi26031.htm

Sugiharto S, Yudiarti T, Isroli I, Widiastuti E, Wahyuni HI, Sartono TA (2019). Recent advances in the incorporation of leaf meals in broiler diets. Livestock Research for Rural Development. Volume 31, Article #109. Retrieved October 24, 2019, from http://www.lrrd.org/lrrd31/7/sgu_u31109.html

Taylor CA, Kothmann MM (1990). Vegetation response to increasing stocking rate under rational stocking. Journal of Range Management 43:123-126.

Tesfaye E, Animut G, Urge M, Dessie T, (2013). Moringa olifera Leaf meal as an alternative protein feed ingredient in broiler ration. International Journal of Poultry Science 12(5):289-297.

Ubua JA, Ozung PO, Inagu PG (2019). Dietary inclusion of Neem (Azadirachta indica) leaf meal can influence growth performance and carcass characteristics of broiler chickens. Asian Journal of Biological Science 12(2):180-186.

Vahid J, Boldaji F, Dastar B, Hashemi SR, Ashayerizadeh A (2017). Effects of fermented cottonseed meal on the growth performance, gastrointestinal microflora population and small intestinal morphology in broiler chickens. British Poultry Science 58:402-408.

Van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, Vantomme P (2013). Edible insects: future prospects for food and feed security (No. 171). Food and agriculture organization of the United Nations (FAO). 187 p.

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Full Length Research Paper

Multivariate analysis in the evaluation of sustrate quality and containers in the production of Arabica coffee seedlings

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Coffee growing is recognized as an activity of great economic and social importance for Brazil. Obtaining good quality coffee seedlings is a major factor in the implantation of a productive and lasting crop. In view of the aforementioned, the objective of this study was to evaluate the quality of Arabica coffee seedlings produced with different substrates in different containers with multivariate analysis techniques. The experimental design used was in randomized blocks, in a 3 x 4 subdivided plot scheme, with 3 replications. The plot levels were: Tube of 120 cm³, Tube of 280 cm³ and Polyethylene bag with volume of 615 cm³. In the subplots the different levels of substrate were randomized: Conventional (S1), leguminous compound (S2), composed of grass + cured bovine manure (S3) and Vermicompost (S4). Treatments 10; 1; 4; 7 evaluated presented potential for diffusion of technology in the process of seedling formation.

Key words: *Coffea arabica*, composting, agroecology.

INTRODUCTION

Coffee growing is recognized as an activity of great economic and social importance for Brazil. Obtaining good quality coffee seedlings is a major factor in the implantation of a productive and lasting crop. Sustrate quality influences the soil bulk density/porosity and decomposion thus enhances the nutrient cycling in the soil and finally the production/productivity (Upadhyay et al., 1989; Bargali et al., 1993, 2015; Bargali, 1996;

Pandey et al., 2006). Due to this importance, several studies have been carried out in order to seek the production of seedlings with superior quality and low costs (Vallone et al., 2010). According to Morgado et al. (2000), containers and their different volumetric capacities may influence the quality of the seedlings and also on the cost of production, because using containers larger than the recommended volume results in additional expenditure for the viveirista.

The viveirista should be attentive in the production of the substrate so that the final product reaches physical and chemical characteristics required by the plant. According to Silva et al. (2010), the substrate should have good aeration, good porosity, water retention capacity, ability to exchange cations and be free of pests and inoculates as a source of disease propagation. According to Vallone et al. (2010), the substrate is responsible for 38% of the total cost of the project. Currently, the replacement of industrialized materials with organics enriched with macro and micronutrients has become frequent for substrate production. And the production of substrate within the property is common in the sector, with expenses reaching up to 38% of the total cost of the seedling (Vallone et al., 2010).

Considering these factors, coffee is a perennial crop, and for its implementation it is necessary to plan all phases, from seedling formation to crop management. Any limitation in this period can severely compromise exploration, resulting in a decline in productivity and lower crop durability. Thus, the planting of vigorous coffee seedlings ensures a good "catch", decreases spending on the replanting operation and contributes with rapid initial growth of plants in the field, constituting a fundamental factor for a successful cultivation (Alves and Guimarães, 2010).

According to Henrique et al. (2011), vigorous seedlings have bright green leaves, thick stem and root system with abundant absorbent roots. However, the diameter of the stem is one of the most relevant parameters to estimate the seedling quality and the setting rate after transplantation in the field.

The objective of this study was to evaluate the quality of Arabica coffee seedlings produced with different substrates in different containers.

MATERIALS AND METHODS

The experiment was carried out at the Federal Institute of Espírito Santo (IFES) - Campus de Alegre, located in the municipality of Alegre-ES, in a seedling nursery with sombrite coverage 50%. The geographical coordinates of the nursery are 20º45'44" south latitude and 41º27'43"longitude West, with altitude of 134 m. According to Köppen classification, the climate of the region is of the type "Aw", dry winter and rainy summer with average annual temperature of 23ºC and precipitation annual period around 1,200 mm. The rainy season in the region is concentrated from November to March.

The experimental design used was in randomized blocks, in a 3 x 4 subdivided plot scheme, with 3 replications. The plot levels were: Tube of 120 cm³, Tube of 280 cm³ and Polyethylene bag with volume of 615 cm³. In the subplots, the different levels of substrate were randomized: Conventional (S1), leguminous compound (S2), composed of grass + cured bovine manure (S3) and Vermicompost (S4). The containers used present as a primordial characteristic the non-release of toxins in the cultivation substrate.

The substrates used were: S1 - conventional - made from ravine land with bovine manure, in the proportion of 3:1 (volume/volume) plus the complementation of fertilization with NPK recommended for culture (Prezotti et al., 2013); S2 - organic leguminous compound, consisting of the basis of legumes (guandu beans) with bovine manure in the proportion of 1:1 (volume/volume), after the maturation process of the material reaching 90 days; S3 - organic grass compound, derived from the process of composting bovine manure and snapping of grasses of gardens in the proportion of 1:1, as described by Souza et al. (2013) and; S4 - vermicompost, resulting from the organic compound grasses.

After the composting process, the compound was taken to a vermicomposteira (3 m long by 0.80 m wide and 0.50 m) for the formation of the vermicoposto. The compound was covered by a layer of 10 cm of dry straw of mowed grass to maintain moisture and darkness, essential to the creation of earthworms, which cannot receive sunlight. The internal temperature of the construction site was maintained between 16 and 22ºC and humidity of 60%, through regas on alternate days. The species used was red worm of California (*Eisenia foetida*) and the process of vermicomposting lasted approximately 40 days. The chemical characterization of the substrates was performed at the Soil Fertility Laboratory of the Soil Department of the Federal Rural University of Rio de Janeiro (Table 1).

The cultivar "Catuai IAC 44 - Arabica" was evaluated. Two seeds per container were used, sowing at 1.0 cm deep. Thinning was carried out shortly after the appearance of the first pair of true leaves, eliminating the less vigorous plants (Matiello et al., 2005). The irrigations were carried out twice a day (morning and afternoon), by microaspersion, until the end of the experimental phase.

At 165 days after sowing, the characteristics evaluated were: shoot and root dry mass (g plant^{-1}), number of leaves, shoot height (cm plant^{-1}), leaf area (cm² plant^{-1}), Dickson quality index, shoot/root ratio, root length (cm plant^{-1}), total nitrogen (plant^{-1}%) and total crude protein (% plant^{-1}). The dry mass of shoot and root were obtained in a digital scale after drying in a greenhouse forced circulation at 75°C until constant weight. Height was measured with millimeter rule, considering the region between the collection and the apical yolk.

Leaf area (PA) was measured by the mathematical model AF=0.667 from Barros et al. (1973), where CNC is the length of the central rib of the sheet. Dickson's quality index was obtained by the formula: IQD = [total dry mass / (height/diameter ratio + shoot/root ratio)) recommended by Dickson et al. (1960).

Total nitrogen was obtained by the Kjeldahl method, which is based on the decomposition of organic matter through the digestion of the sample at 400ºC with concentrated sulfuric acid, in the presence of copper sulfate as a catalyst that accelerates the oxidation of the matter organic. Nitrogen present in the resulting acid solution was determined by steam drag distillation, followed by

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Table 1. Chemical characterization of substrates used in the production of coffee seedlings.

Substrates	N	P_2O_5	K_2O	Mg	Ca	C	pH
	g kg^{-1}	mg dm^{-3}		$Cmol_c.dm^{-3}$		g kg^{-1}	H_2O
S1	18.0	38.0	18.07	5.0	26.0	40.7	6.2
S2	33.0	28.1	15.36	5.7	2.9	158.0	8.8
S3	15.0	16.0	30.6	5.3	27.9	62.0	7.4
S4	15.0	36.3	36.72	7.9	5.3	113.0	6.7

S1 - conventional; S2 - composed of legumes + cured bovinemanure; S3 - grass compound + cured bovine manure; S4 - vermicomposto.

titration with diluted acid (Nogueira and Souza, 2005).

The expression (PBT = NT x FN) was used to determine the total crude protein (PBT), where NT is the total nitrogen and FN is the factor of 6.25 (Nogueira and Souza, 2005). The protein content of a food is measured from the nitrogen content present in the sample analyzed. The analysis is performed by the Kjeldahl Method, where the percentage of nitrogen obtained is multiplied 6.25 and then expressed as Crude Protein (CP). This analysis is based on the fact that all proteins have 16% nitrogen, and that all nitrogen of the food is in the protein form (Nogueira and Souza, 2005).

In the next stage, the computational application R Core Team (2017) was used to determine the generalized Euclidean distance in order to obtain the matrix of dissimilarities between the treatments where the combinations between qualitative and quantitative factors were made, depending on the distance between individuals and the grouping was grouped by the hierarchical method of medium group connection (UPGMA).

The previous characterization of the treatments was performed with the combinations between the containers and substrates used soon after using the principal component analysis (PCA), which is an exploratory multivariate technique. It was processed with the covariance matrix of the original variables, obtaining from it the self-values that built the auto-vectors. These are linear combinations of the original variables and are called main components. The discriminatory power of each variable in a component was measured by the formula:

$$r_{xj}(cp_h) = \frac{a_{jh}\sqrt{\lambda_h}}{S_j}$$

Where, sj =standard deviation of variable j, ajh = coefficient of variable j in the h-thésimo main component, and λh = h-ésima root characteristic (autovalue) of the covariance matrix (Hair et al., 2009). All analyses were processed in the computational program R Core Team (2017) after standardization of variables (null mean and unit variance).

RESULTS AND DISCUSSION

From the dendrogram obtained by the hierarchical method of distance means (Figure 1), considering the cut by the method Mojena (1977) to 45% of the maximum fusion level, it was possible to verify that the 12 treatments were separated into two dissimilar groups: Group I: T10; T1; T4 and T7. Group II: T11; T2; T12; T6; T3; T9; T5; T8.

In the evaluation of the treatments studied, treatment 10 (bag 615 ml + vermicompound) and 8 (tube 280 ml + Grass compound + cured bovine manure) were the most discrepant among them, while the lowest divergence was presented by T10 treatments and T1 (bag 615 mL + conventional substrate). These divergences can be noted using the mean distance between groups method in order to detect more divergent groups, as well as Dardengo et al. (2013). The highest quality indices were observed in T10; T1; T2, T7, and T4, and these treatments can be used for seedling production, such as T10, or even submitted for local selection, with an increase in the quality index.

Main component 1 (CP1) and main component 2 (CP2) contributed 64.13 and 17.03%, respectively, of the remaining variance. Thus, these agronomic variables of coffee seedlings highlighted in the first two main components CP1 and CP2 are considered important for the selection of treatment between container and substrates for the Alegre-ES region.

Figure 2 shows that negative correlations are responsible for the discrimination of treatments located on the left of CP1 (T11, T10, T1, T4 and T7) and positive correlations by treatment discrimination on the right of CP1 (T2, T3, T5, T6, T8, T9) while positive correlations by treatment discrimination was on the right of CP1 (T2, T3, T5, T6, T8, T9 and T12). The variables with positive correlation are responsible for the discrimination of treatments located at the top and bottom of CP2 while the variables with negative correlation are responsible for the discrimination of treatments located in the upper and lower part of CP2 (T10, T11, T7, T2 and T6).

You can note that the variables associated with seedling production are left-facing in CP1. When observing in Figure 1 the association between the groups of variables and the treatments formed between substrates and containers, it was seen that treatments 10, 11, 7, 4 have potentials to have higher total fresh mass values (FTM), nitrogen of the aerial part (NTPA), shoot length (CPA), number of leaves (NF), total length (CT), height and diameter ratio (ALT/DC), ratio of dry mass shoot sand root (MSPA/MSR) and Dickson quality index (IQD).

Regarding CP2, treatments 10, 11, 7, 4 present potentials, although weak, to have higher values of shoot dry mass (FTM), leaf area (PA), root length (CR), root nitrogen (NTR), crude aerial protein (PBPA) and crude root protein (PBR). In T1 occurs the reverse in which they

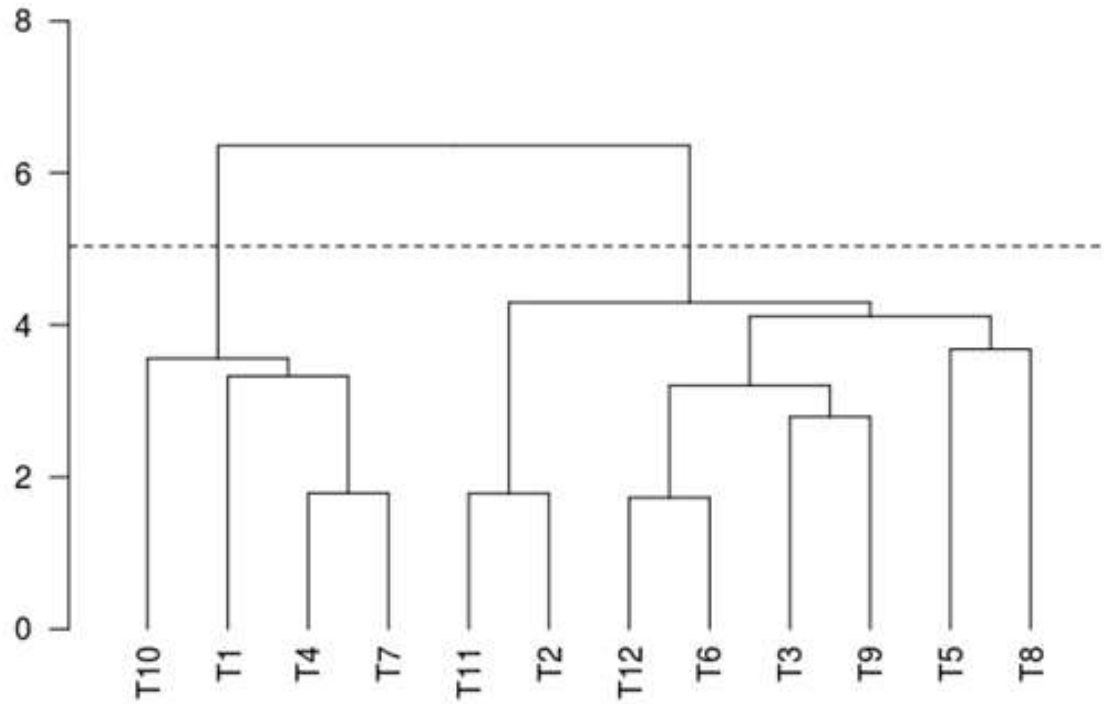

Figure 1. Representationof the dissimilarities between 12 treatments formed between substrates and coffee containers through theEuclidiana generalized distance.

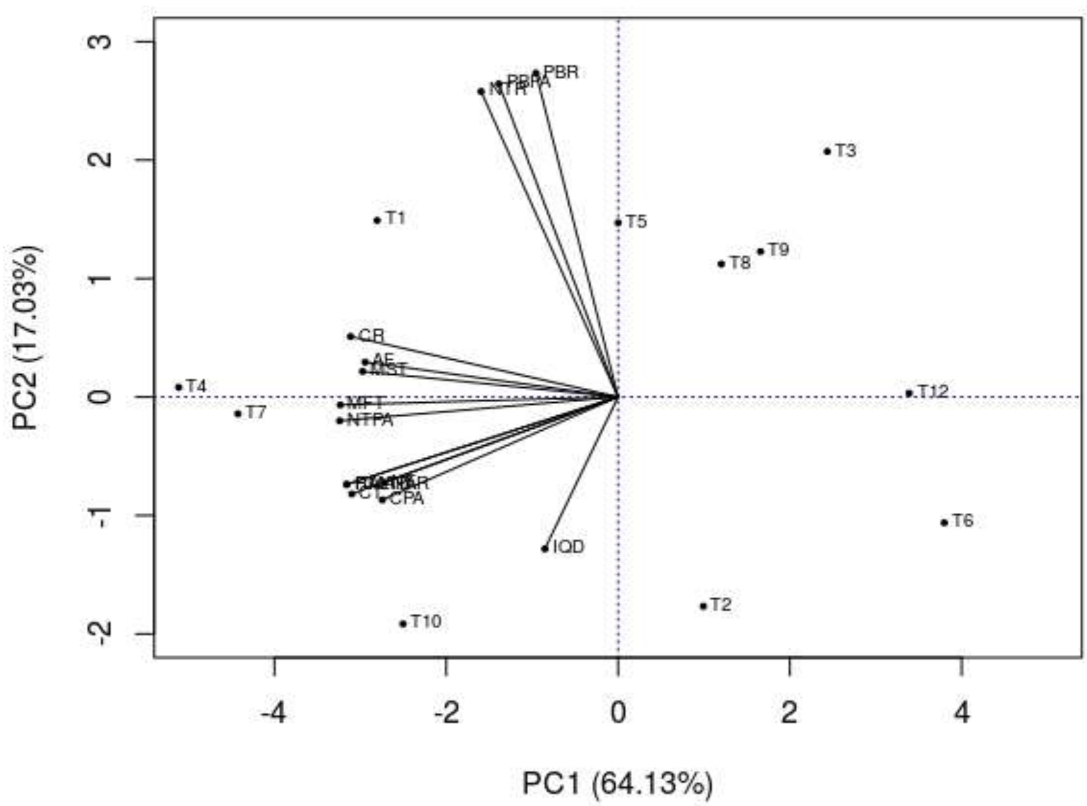

Figure 2. Analysis of main components in the evaluation of the quality of Arabica coffee seedlings produced with different substrates and containers.

Table 2. Analysis of correlations related to the characteristics evaluated in response to the types of substrates and containers.

Variable	CT	MFT	CR	CPA	RALT/D	MSA/R	IQD	AF	MST	NTPA	NTR	PBPA	PBR
NF	0.712	0.799	0.653	0.553	0.717	0.717	0.521	0.593	0.544	0.728	0.199	0.149	0.084
CT		0.818	0.873	0.873	0.941	0.941	0.318	0.630	0.716	0.847	0.278	0.195	0.038
MFT			0.903	0.573	0.868	0.868	0.241	0.839	0.837	0.961	0.311	0.343	0.089
CR				0.455	0.733	0.733	0.090	0.766	0.807	0.837	0.385	0.423	0.216
CPA					0.658	0.658	0.295	0.462	0.501	0.603	0.301	0.110	0.174
ALT/D						1.000	0.287	0.654	0.760	0.892	0.221	0.225	-0.061
MSA/R							0.287	0.654	0.760	0.892	0.221	0.225	-0.061
IQD								-0.186	-0.224	0.105	0.033	-0.101	-0.011
AF									0.947	0.905	0.251	0.311	0.151
MST										0.926	0.270	0.338	0.109
NTPA											0.275	0.324	0.060
NTR												0.472	0.876
PBPA													0.404
PBR													

present potentials to have higher values of dry mass shoot (MST), leaf area (PA), root length (CR), root nitrogen (NTR), crude shoot protein (PBPA), crude root protein (PBR), weak for the variables total fresh mass (FTM), nitrogen of the aerial part (NTPA), shoot length (CPA), number of leaves (NF), total length (CT), height and diameter ratio (ALT/DC), ratio dry mass shoot sand root (MSPA/MSR) and Dickson quality index (IQD).

The other treatments, although located on the right side, tend to have samples with less expressive characters, differentiating from treatments 10, 11, 7, 4 and 1. The main component technique has been used for the characterization of vegetable germplasm benches, such as coriander, onion and beans (Rodrigues et al., 2002; Leite et al., 2005; Magalhães et al., 2010) and has led to identification of important characteristics to be evaluated through previous studies of its contribution to variability (Pereira, 1989). This has enabled discarding of low-contribution characters for genotype discrimination or even evaluated treatments and, thus, it is possible to reduce labor, time and costs (Cruz et al., 2004).

Table 2 presents the correlation analysis according to the model developed by Wright (1934) to better understand the associations between different variables. According to Silva et al. (2010), characters with high positive correlations indicate the presence of an influence on another causing dependence. Thus, the total length obtained dependence on the number of leaves with 0.712 and total fresh matter obtained dependence on number of leaves and total length with 0.799 and 0.818, respectively.

In root length (CR), dependence was observed between CT and MFT with 0.873 and 0.903. For shoot length, the only dependence generated was with the variable total length (CT) with 0.873. For the correlations between, the ALT/D ratio were significant for NF, CT, MFT and CR with values of 0.717, 0,941, 0.868 and 0.733, respectively. Importance should be given to the root system of seedlings, in addition to morphological parameter studies to ensure better field performance. The roots are closely linked to seedling survival since every physiological process started in this soil water and plant environment system (Carneiro, 1995).

The height of the shoot is easy to measure and, therefore, has always been used efficiently to estimate the quality pattern of seedlings in nurseries (Gomes, 2013), and is also considered as one of the most important parameters to estimate growth in the field (Eloy et al., 2013), in addition to the fact that their measurement does not lead to destruction, being technically accepted as a good measure of the performance potential of the seedlings (Mexal and Lands, 1990).

In other research papers, the highest heights corresponded, in the field, to the highest survival rate and the highest initial growth for Pinus Radiata Pawsey and Pseudotsuga Menziesii (Richter, 1971; Pawsey, 1972). For the ratio dry mass shoot and root (MSA/R), there was cause and effect for variables: NF, CT, MFT, CR and ALT/D in which the effects were highly positive with 0.717, 0,941, 0,868, 0.733 and 1,000 showing high dependence among these evaluated character sets. The ratio of dry matter weight of the shoot/dry matter weight of the roots, despite being considered as an efficient and safe index to assess the quality of seedlings. Parviainen (1981) may be contradictory to express growth in the field (Burnett, 1979).

In the evaluation of Dickson's quality index (IQD), no cause and effect were observed between the correlations in which the values were low for variables; NF, CT, MFT, CR, CPA, ALT/D and MAS/R. The values obtained in the correlations in the present study are in accordance with those cited by Dardengo et al. (2013). In the evaluation in the leaf area correlation (PA) between the other variables, it was observed that only two characteristics presented dependence that were MFT (0.839) and CR (0.766).

Positive effect of CT characters were observed to evaluate the total dry mass (FTM), MFT, CR, ALT/D, MAS/R and AF with 0.716, 0,837, 0,807, 0,760, 0.760 and 0.947, respectively, but the same did not occur with Dickson's quality index (IQD) that obtained negative effect with -0.224 showing that when higher, the lower FTM values will be those of IQD. According to Ribeiro Júnior and Melo (2009), when the coefficient is negative, high values of one variable will be associated with low values of the other.

In the NTPA feature it was observed that it causes highly positive effect of NF characters; CT, MFT, CR, ALT/D, MAS/R, AF and MST with values of 0.72, 0,84, 0,96, 0,83, 0,89, 0,89, 0.90 and 0.92, respectively. In the bromatological character it is evident that the effect generated between NTPA in IQD and very low with 0.10 showed null effect. For the NTR and PTBA characters, no high-effect correlation scans were expressed and null effect may be considered. The same does not occur with the PBR character that correlates with values of 0.87 NTR. For the characteristics of IQD importance, MAS/R and ALT/D correlation are negative with -0.011, -0.061 and -0.061 showing that this characteristic has its null expression.

Conclusions

The analysis of main components, as an exploratory tool, allowed to identify the important variables in the characterization of treatments for seedling formation. Thus, it was possible to identify treatments 10, 1, 4, 7 as promising and with potential for the diffusion of technology in the process of seedling formation.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interest.

REFERENCES

Alves JD, Guimarães RJ (2010). Sintomas de desordens fisiológicas em cafeeiro. Semiologia do cafeeiro: sintomas de desordens nutricionais, fitossanitárias e fisiológicas. Lavras: UFLA, pp. 169-215.

Bargali SS, Singh SP, Singh RP (1993). Pattern of weight loss and nutrient release in decomposing leaf litter in an age series of eucalypt plantations. Soil Biology and Biochemistry 25:1731-1738.

Bargali SS (1996). Weight loss and nitrogen release in decomposing wood litter in an age series of eucalypt plantation. Soil Biology and Biochemistry 28:699-702.

Bargali SS, Kiran S, Lalji S, Ghosh L, Lakhera ML (2015). Leaf litter decomposition and nutrient dynamics in four tree species of Dry Deciduous Forest. Tropical Ecology 56(2):57-66.

Barros RS, Maestri M, Vieira M, Braga Filho LJ (1973). Determinação da área de folhas do café (Coffea arabica L. cv. Bourbon Amarelo). Revista Ceres (Brasil) 20(107):44-52.

Burnett AN (1979). New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. Canadian Journal of Forest Research 9(1):63-67.

Carneiro JDA (1995). Produção e controle de qualidade de mudas florestais (No. 634.956 C280p). Universidade Federal do Paraná, Curitiba, PR (Brasil) Universidade Estadual do Norte Fluminense, Campos, RJ (Brasil) Fundacao de Pesquisas Florestais do Paraná, Curitiba, PR (Brasil).

Cruz CD, Regazzi AJ, Carneiro PCS (2004). Modelos biométricos aplicados ao melhoramento genético (volume 1). Viçosa, Editora UFV. 1:480p.

Dardengo MCJ, Sousa EFD, Reis EFD, Gravina GDA (2013). Crescimento e qualidade de mudas de café conilon produzidas em diferentes recipientes e níveis de sombreamento. Coffee Science, Lavras 8(4):500-509.

Dickson A, Leaf AL, Hosner JF (1960). Quality appraisal of white spruce and white pine seedling stock in nurseries. The Forestry Chronicle 36(1):10-13.

Eloy E, Caron BO, Schmidt D, Behling A, Schwers L, Elli EF (2013). Avaliação da qualidade de mudas de Eucalyptus grandis utilizando parâmetros morfológicos. Floresta 43(3):373-384.

Gomes DR, Caldeira MVW, Delarmelina WM, de Oliveira Gonçalves E, Trazzi PA (2013). Lodo de esgoto substrato para produção de mudas de Tectona grandis L. Cerne 19(1):123-131.

Hair JF, Black WC, Babin BJ, Anderson RE, Tatham RL (2009). Análise multivariada de dados. Bookman Editora.

Henrique PC, Alves JD, Deuner S, Goulart PDFP, do Livramento DE (2011). Aspectos fisiológicos do desenvolvimento de mudas de café cultivadas sob telas de diferentes colorações. Pesquisa Agropecuária Brasileira 46(5):458-465.

Leite RLBDL, Sinigaglia ECC (2005). Divergência genética entre populações de cebola com base em marcadores morfológicos. Ciência Rural 35:2.

Magalhães BCHC, Pinheiro EAR, Nóbrega GN, de Lima Duarte JM (2010). Desempenho agronômico e divergência genética de genótipos de coentro. Revista Ciência Agronômica 41(3):409-416.

Matiello JB, Santinato R, Garcia AWR, Almeida SR, Fernandes DR (2005). Cultura de café no Brasil: novo manual de recomendações (No. 633.730981 C968). Ministério da Agricultura, da Pecuária e do Abastecimento, Brasília, DF (Brasil).

Mexal JG, Landis TD (1990). Target seedling concepts: height and diameter. In Proceedings, western Forest nursery association, pp. 13-17.

Mojena R (1977). Hierarchical grouping methods and stopping rules: An evaluation. The Computer Journal 20(4):359-363.

Morgado IF, Carneiro JGA, Leles PSS, Barroso DG (2000). Nova metodologia de produção de mudas de E. grandis Hill ex Maiden utilizando resíduos prensados como substratos. Revista Árvore 24(1):27-33.

Nogueira ARA, Souza GB (2005). Manual de laboratórios: solo, água, nutrição vegetal, nutrição animal e alimentos. São Carlos: Embrapa Pecuária Sudeste 313 p.

Pandey CB, Sharma DK, Bargali SS (2006). Decomposition and nitrogen release from *Leucaena leucociphala* in Central India. Tropical Ecology 47(1):149-151.

Parviainen JV (1981). Qualidade e avaliação de qualidade de mudas florestais. Seminário de Sementes e Viveiros Florestais 1:59-90.

Pawsey CK (1972). Survival and early development of Pinus radiata as influenced by size of planting stock. Australian Forest Research 5(4):13-24.

Pereira AV (1989). Utilização de análise multivariada na caracterização de germoplasma de mandioca (Manihot esculenta Crantz). Piracicaba^ eSP SP: ESALQ.

Prezotti LC, Oliveira J, Gomes J, Dadalto G (2013). Manual de recomendação de calagem e adubação para o Estado do Espírito Santo: 5ª aproximação. 305p.

R Core Team (2017). R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL http://www.R-project.org/., page R Foundation for Statistical Computing.

Richter J (1971). Das umsetzen von dougiasien in kulturstadium. Allgemeine Forst- und Jagdzeitung 142:63-69.
Rodrigues LS, Teixeira MG, da Silva JB (2002). Divergência genética entre cultivares locais e cultivares melhoradas de feijão. Pesquisa Agropecuária Brasileira 37(9):1275-1284.
Silva JI, Vieira HD, Viana AP, Barroso DG (2010). Desenvolvimento de mudas de Coffea canephora Pierre ex A. froehner em diferentes combinações de substrato e recipiente. Coffee Science, Lavras 5(1):38-48.
Souza MPS, Costa AC, Carreço RLB, de Lima WL (2013). 14102-A valorização do lixo orgânico no setor de Agroecologia no Instituto Federal do Espírito Santo–Campus de Alegre. Cadernos de Agroecologia 8:2.
Upadhyay VP, Singh JS, Meentemeyer V (1989). Dynamics and weight loss of leaf litter in Central Himalayan forests: abiotic versus litter quality influences. The Journal of Ecology, pp. 147-161.
Vallone HS, Guimarães RJ, Mendes ANG, Cunha RLD, Carvalho GR, Dias FP (2010). Efeito de recipientes e substratos utilizados na produção de mudas de cafeeiro no desenvolvimento inicial em casa de vegetação, sob estresse hídrico. Ciência e Agrotecnologia, Lavras 34(2):320-328.
Wright S (1934). “The Method of Path Coefficients.” The Annals of Mathematical Statistics 5:161-215.

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Effect of time of *Azolla* incorporation and inorganic fertilizer application on growth and yield of Basmati rice

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***Azolla* tissue contains 5% N, which is slowly released into the soil upon decomposition. Timing of incorporation is therefore important for maximum benefit to a crop. The effect of time to incorporate *Azolla* biomass on growth and yield of rice was investigated in Mwea-Kenya. Treatments consisted of 7.5 t ha^{-1} *Azolla* biomass applied at transplanting, 7.5 t ha^{-1} *Azolla* applied at 21 days after transplanting (DAT) and 30 kg N ha^{-1} inorganic N applied in splits at O, 21 and at 55 DAT. There were control treatments without *Azolla* and without inorganic N application. The treatments were laid out in a Randomized Complete Block Design (RCBD) with three replications. Phosphorus and potassium were applied at 50 Kg ha^{-1} each as P_2O_5 and K_2O. Plant height and tiller numbers were recorded at 21 (rooting/tillering), 32 (tillering), 42 (maximum tillering), 60 (flowering) and 75 DAT (heading) while yield parameters were determined at physiological maturity (120 DAT). Data were analysed using SAS software and means separated using the least significant difference test ($p \leq 0.05$). *Azolla* incorporation at transplanting significantly enhanced panicle m^{-2}, grain weight and grain yield while incorporating it at 21 DAT only significantly enhanced panicle m^{-2}. Higher environmental temperatures enhanced *Azolla* effect. The effect of Inorganic N significantly increased plant height, tiller number, grain weight and spikelets $panicle^{-1}$. However, percentage grain filling was reduced. The effect of interaction between *Azolla* application and inorganic N was significant on spikelets $panicle^{-1}$ and grain weight. Observations therefore indicate that the effect of *Azolla* on yield and yield components was more when incorporated at transplanting.**

Key words: *Azolla,* incorporation time, inorganic fertilizer, rice yields.

INTRODUCTION

Azolla is a fern found in still or slow-moving water bodies (Campbell, 2011). It has a symbiotic association with nitrogen fixing blue-green algae, *Anabaena azollae* (Bocchi and Maglioglio, 2010). The association enables it to fix nitrogen, which it releases upon decomposition thus making *Azolla* an important source of bio-fertilizer (Wager, 1997). Nitrogen, Phosphorus and potassium constitute 4-5, 1.5 and 3% of *Azolla* respectively on a dry

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weight basis. In addition, *Azolla* can provide 1.8–3 tons ha^{-1} dry matter per crop, (IRRI, 1990). According to Kannaiyan (1993) about 20 t/ha *Azolla* is capable of providing 40 kg N ha^{-1} inorganic nitrogen requirement upon incorporation in the soil. According to IRRI (1990) about 50% of the nitrogen is released within the first 6-8 weeks of incorporation into the soil.

Due to its nitrogen fixing capability, *Azolla* has been used as a bio-fertilizer in rice paddies for increased productivity (Kamalasanana et al., 2002). For centuries, the potential of *Azolla* and its nitrogen-fixing partner *Anabaena azollae* has been exploited to increase rice yields in China and Asian countries (Armstrong, 1979; Carrapiço et al., 2000). However, the advent of the industrial revolution resulted in increased use of inorganic fertilizers leading to reduction in the traditional use of *Azolla* as a green manure (Carrapiço et al., 2000). In China, at least 3.2 million acres of rice paddies were planted with *Azolla* by 1980. In Northern Italy at Po Valley, *Azolla* incorpored in paddies produced equivalent of 30-40 Kg N/ha (Bocchi and Maglioglio, 2010). In India, Singh and Singh (1987) reported significant increase in rice yields from *Azolla* application at transplanting and *Azolla* dual incorporation. Research in Guinea Bissau on the comparative effects of *Azolla* on rice yields showed that incorporating 7 tons ha^{-1} *Azolla* biomass gave an equivalent effect of 43.5 kg N ha^{-1} (Carrapiço et al., 2000). Findings by Malyan et al. (2019) showed that the use of *Azolla* reduces the need for application of urea fertilizer by 25% in rice production with no effects on yields. According to Razavipour et al. (2018), *Azolla* use increases tillers, grain weight, yield of rice and is a desirable management practice in rice production Field studies at Ahero Irrigation Research Station in Kenya (1980) confirmed the positive benefit of 4.8 tons ha^{-1} *Azolla* when used with inorganic nitrogen (AIR Report, 57). However, the need for extra fields for mass multiplication of *Azolla* proved uneconomical. In West Kano Irrigation Scheme, incorporation of *Azolla* + urea gave significantly higher grain yields and plant height (Serrem et al., 2013).

Azolla is a major source of nitrogen when grown in paddies and incorporated into the soil as green manure. The process of incorporating *Azolla* in the soil can be done either at transplanting or during active tillering, thus making it a dual crop with the paddy rice (Bocchi and Maglioglio, 2010). According to IIRR (Low input Rice Production-LIRP Technology Kit), three methods are commonly used; (i) *Azolla* is grown with the rice crop in paddies and incorporated as green manure; (ii) *Azolla* is incorporated once at 20 days after transplanting; (iii) *Azolla* is incorporated during subsequent cropping. *Azolla* in the soil provides organic matter, which improves soil quality and provides nutrients for the current and subsequent crops (Ferentinos et al., 2002). However, the timing of *Azolla* application and the benefit to the crop is affected by the environmental conditions (Wagner, 1997).

Although *Azolla* is beneficial to rice production, its use has not been widely accepted due to several constraints including labour for its incorporation (Carrapiço et al., 2000). Consequently, farmers continue to use inorganic fertilizers. Inorganic fertilizers have been shown to improve yields initially but their impacts are however not sustainable over a long period of time (Patro et al., 2011). This is because of creation of nutrients imbalance which consequently leads to a reduction in soil fertility and crop yields (Singh et al., 2001). Application of *Azolla* combined with inorganic nitrogen gives optimum grain yields (Kannaiyan, 1993). Ito and Watanabe (1985) showed that early incorporation of *Azolla* in the soil increases nitrogen availability. Farmers in Mwea incorporate *Azolla* in the soil during weeding as a management strategy (Oyange et al, 2019). Considering the abundance of *Azolla* in Mwea paddies, its integration with inorganic fertilizers and timely incorporation in the soil can help reduce the cost of inorganic fertilizers and consequently the cost of paddy rice production. The objective of the study was to determine the effect of timing of *Azolla* incorporation on paddy rice growth and yield.

MATERIALS AND METHODS

Site description

The study was done at Mwea Irrigation Scheme during the year 2015 and 2016. Mwea lies within agro-ecological zones LM3 and LM 4 (Marginal cotton zones). Rainfall pattern is bimodal; long rainy season begins from March to May and the short rainy season from October to November. Annual mean rainfall is about 930 mm, out of which 510 mm is received during long rainy season, with 66% reliability. The mean temperature is 22°C with a minimum of 17°C and a maximum of 28°C. During the experimental period, the average temperature was 23°C with relative humidity of 78% (Appendix Table 1). The average and maximum temperatures were higher during growth stage but lower during heading and maturity stages for second than first season. Relative humidity was lower for the first season than for the second season. The experimental plots had black cotton soils, imperfectly drained, with ideal pH (Table 1). The N and P levels were near threshold while the K levels were low. *Azolla* tissue N, P and K levels were 4.0, 0.45 and 1.1%, respectively for Mwea (Table 2).

Experimental design

Treatments consisted of 7.5 t ha^{-1} *Azolla* incorporated at transplanting, 7.5 t ha^{-1} *Azolla* applied at 21 DAT and no *Azolla* application combined with inorganic N application of 0 and 30 kg N ha^{-1} as Sulphate of Ammonia. The treatments were laid out in a RCBD with a split plot arrangement. Inorganic N was applied in three equal splits each of 10 kg N ha^{-1} at transplanting, 21 DAT and 50 DAT respectively. Nutrient P and K were applied at Mwea Irrigation Agricultural Development Centre (MIAD) standard rates of 50 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} K_2O as triple super phosphate and muriate of potash, respectively. Irrigation was carried out to maintain a water depth of 2-5 cm above the ground. Basmati 370 rice variety sourced from MIAD was grown at a spacing of 30 cm x 15 cm. One seedling per hill was transplanted 21 days after sowing and the field was kept weed free by manual weeding at 21, 32 and 45 DAT.

Table 1. Soil nutrient status at Mwea paddy fields.

	S1	S2	S3	S4	S5	S7	Average	Classification
pH	6.26	6.84	6.06	5.96	5.97	5.74	6.14	Ideal
N%	0.105	0.119	0.144	0.130	0.133	0.151	0.130	*
P(ppm)	15.0	13.0	13.0	12.0	14.0	14.0	13.5	*
K (me %)	0.085	0.17	0.17	0.127	0.127	0.085	0.127	Low
E.C µS/cm	663	475	451	323	172	235	387	Ideal

S= Sample, * = the level is near the threshold of that particular element.

Table 2. Tissue nutrient content of *Azolla* accessions (dry weight basis) in Kenya.

Accession	N (%)	P (%)	K (%)
Mwea	4.0	0.45	1.1
Ahero	5.1	0.21	2.2
West Kano	4.8	0.18	1.6
Bunyala	3.4	0.23	1.5
Taveta 1	3.2	0.20	1.3
Taveta 2	3.4	0.22	2.0
TARDA 2	3.4	0.40	1.9
Mean	3.9	0.27	1.6
P-value	<0.001	<0.001	<0.001
LSD (0.05)	0.2	0.14	0.2
CV (%)	1.9	2.1	3.8

Data collection

Data collected included plant height, tiller numbers, grain yield and grain yield components (panicle number, spikelets per panicle, 1000 grain weight, % filled grains and % ripened grains). Ten hills per plot were sampled to determine plant height and tiller numbers at 21, 35, 42, 60 and 75 DAT, corresponding to rooting, tillering, maximum tillering, flowering and heading stages respectively. Soil samples from the experimental site were analyzed for N, P, K and pH, prior to crop establishment. *Azolla* biomass (100 g) each was collected from the canal drains within the six major irrigation schemes in Kenya namely: Mwea, Ahero West Kano Bunyala, Taveta and TARDA for N, P and K analysis.

Data analysis

Data collected were subjected to analysis of variance using SAS statistical package and means separated using the least significant difference (LSD) test at p≤0.05. Linear regression analysis was done to determine the linear regression relationship between yield and yield components.

RESULTS

Soil nutrient status in Mwea

The soil N, P and K averaged 0.13%, 13.5 ppm and 0.13%, respectively. The pH was on average 6.1. The N and P levels were within threshold limits while the pH was ideal.

Azolla plant tissue nutrient levels

The total *Azolla* plant tissue N% on a dry weight basis ranged between 3.14 and 5.06% (Table 2). Mwea Azolla accession had tissue N levels of 4.0%

Effect of time of incorporation on tilers and plant height

Time of *Azolla* incorporation in paddy rice plots had no significant effect on tiller numbers and plant height during both seasons. However, application of 30 kg N ha^{-1} significantly increased panicles/m^2, grain weight, and % grain filling during the first season, while spikelets/panicle significantly increased during the second season. The effect of interaction between time of *Azolla* application and inorganic N on tiller numbers and plant height was not significant.

Time of *Azolla* incorporation significantly affected the number of spikelets/panicle, neck node, panicle/m^2, grain weight and grain yield. Application of 7.5 t ha^{-1} *Azolla* at transplanting gave significantly more spikelets/panicle higher grain weight and grain yield than when 7.5 t ha $^{-1}$ *Azolla* was incorporated at 21 DAT (Table 3). However,

Table 3. Effect of time of *Azolla* biomass application on number of tillers and plant height in Mwea Irrigation Scheme during 2015/2016.

Variable	Season 1					
	Tiller numbers m^{-2}			Plant height (cm)		
Treatment	30 DAT	60 DAT	75 DAT	30 DAT	60 DAT	75 DAT
No *Azolla* (control)	302.9	367.4	351.5	53.6	93.9	117.1
7.5 t ha^{-1} *Azolla* at transplanting	278.1	365.9	345.2	49.3	87.4	115.3
7.5 t ha^{-1} *Azolla* at 21 DAT	258.9	382.9	357.7	50	91.2	119.2
Mean	280	372.1	351.4	51	90.9	117.2
P-value	0.25	0.86	0.94	0.07	0.37	0.46
LSD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	15.4	15.6	18	6	8.3	4.5
0 kg N ha^{-1} (control)	296.8	345.4	329.4	51.4	88.9	117
30 kg N ha^{-1}	263.2	398.7	373.5	50.6	92.8	117.4
Mean	280	372.1	351.4	51	90.9	117.2
P-value	0.13	0.08	0.17	0.59	0.304	0.89
LSD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	15.4	15.6	18	6	8.3	4.5
N x *Azolla* - P-value	0.16	0.71	0.93	0.6	0.4	0.73
			Season 2			
No Azolla (control)	124.4	395.2	435.1	34.8	48.4	118.7
7.5 t ha-1 Azolla at transplanting	125.2	378.5	442.6	35.6	51	122
7.5 t ha-1 Azolla at 21 DAT	127.4	382.9	402.2	35.9	52.6	124.4
Mean	125.7	385.5	426.6	35.4	50.7	121.7
P-value	0.98	0.82	0.83	0.87	0.21	0.11
LSD (0.05)	39.1	60	154.3	4.4	4.9	5.4
CV (%)	NS	NS	NS	NS	NS	NS
0 kg N ha^{-1} (control)	109.9	373.1	420	34	48.5	119
30 kg N ha^{-1}	141.5	398	433.3	36.8	52.8	123.9
Mean	125.7	385.5	426.6	35.4	50.7	121.7
P-value	0.05	0.28	0.82	0.12	0.04	0.05
LSD (0.05)	31.5	49	126	3.6	4	4.4
CV (%)	24.2	NS	NS	NS	7.6	3.5
N x Azolla - P-value	0.93	0.82	0.8	0.98	0.62	0.6

incorporation of 7.5 t ha^{-1} *Azolla* at 21 DAT during second season, resulted in significantly longer neck node, more panicle m^{-2} than basal application of the *Azolla*. The treatment significantly increased the number of spikelets $panicle^{-1}$ during the first season. The effect of interaction between time of *Azolla* incorporation and inorganic nitrogen application was significant on spikelets $panicle^{-1}$ and grain weight during the second season (Table 4). Linear regression relationship between spikelets $panicle^{-1}$ and yield (Figures 1 and 2) showed a positive significant relationship (r^2 = 0.149). There was also a strong positive linear relationship (r^2=0.42) between panicle m^{-2} and yield (Figure 1).

DISCUSSION

The soils in Mwea had N, P and K levels within threshold limits (Table 1). The pH was ideal (6.1), and within acceptable levels for maximum P availability; Fe and Al ions had no detrimental effects on other nutrients (Miller, 2016). The total *Azolla* plant tissue N% on a dry weight basis ranged from 3.14 to 5.06% (Table 2). This is consistent with the findings of Watanabe and Berja (1983), which showed 4-5% tissue N and 0.7-1.85 ppm P. The tissue P level was in conformity with the reported amounts of 0.1- 0.5% (Better Crops, 1999). The soil N levels were near the threshold limits thus necessitating external application

Azolla incorporation in the soil at transplanting significantly increased spikelets $panicle^{-1}$, grain weight and yield while incorporation in the soil at 21 DAT significantly enhanced number of panicles, neck node length, and grain weight (Table 5). Time of *Azolla* incorporation had no significant effect on plant height and tiller numbers both seasons (Table 4). The significant

Table 4. Effect of time of incorporation of *Azolla* biomass on neck node, spikelets, panicles and panicle length in Mwea Irrigation Scheme during 2015/2017.

Treatment	Season 1			
	Neck node (cm)	Number of Spikelets/ panicle	Number of Panicles m^{-2}	Panicle length (cm)
No *Azolla* (control)	3.6	75.2	465.1	25.7
7.5 t ha^{-1} *Azolla* at transplanting	3.7	84.9	428.8	26.4
7.5 t ha^{-1} *Azolla* at 21 DAT	3.5	80.1	470.3	25.7
Mean	3.6	80	454.7	26.1
P-value	0.205	0.016	0.83	0.11
LSD (0.05)	NS	6.1	NS	NS
CV (%)	3.7	5.9	28.4	2
0 kg N ha^{-1} (control)	3.6	76.1	425.6	26.1
30 kg N ha^{-1}	3.6	83.9	483.9	26
Mean	3.6	80	454.7	26.1
P-value	0.68	0.01	0.36	0.58
LSD (0.05)	NS	5	NS	NS
CV (%)	3.7	5.9	28.4	2
N x *Azolla* - P-value	0.37	0.55	0.81	0.46
	Season 2			
No Azolla (control)	3.72	75.7	344.5	24.7
7.5 t ha-1 Azolla at transplanting	3.81	74.4	362.1	25.2
7.5 t ha-1 Azolla at 21 DAT	3.94	75.5	409.3	25.8
Mean	3.8	75.2	372	25.2
P-value	0.05	0.91	0.03	0.15
LSD (0.05)	0.17	NS	47.1	NS
CV (%)	3.5	7.1	9.8	3.5
0 kg N ha-1 (control)	3.8	74.5	345.7	25
30 kg N ha-1	3.8	75.9	398.2	25.4
Mean	3.8	75.2	372	25.2
P-value	0.44	0.58	0.01	0.4
LSD (0.05)	NS	NS	38.4	NS
CV (%)	3.5	7.1	9.8	3.5
N x Azolla - P-value	0.65	0.03	0.73	0.57

DAT= days after transplanting.

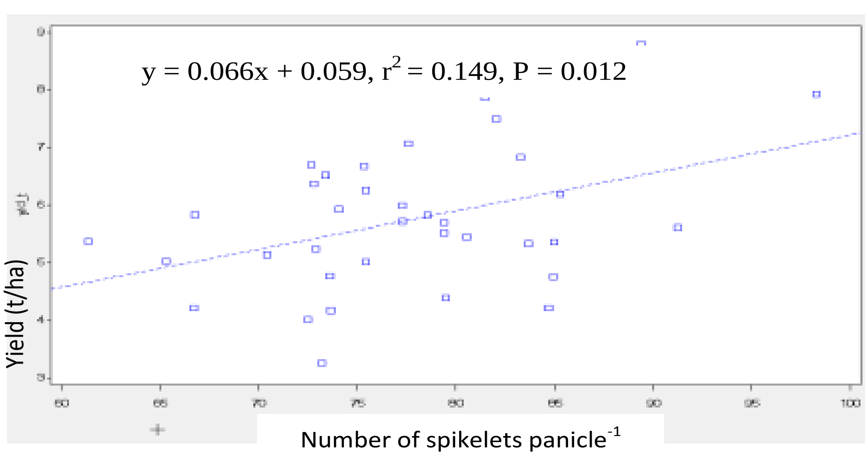

Figure 1. Linear regression relationship between spikelets panicle^{-1} and grain yield at Mwea Irrigation Scheme.

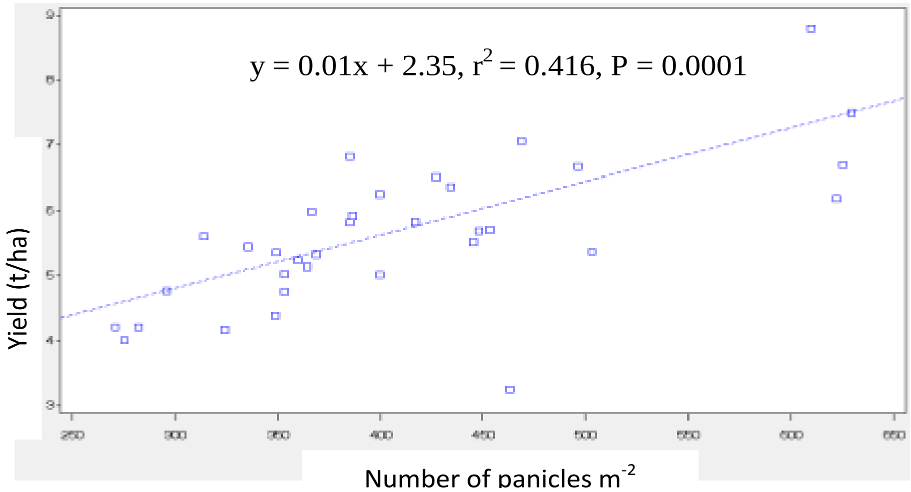

Figure 2. Linear regression relationship between number of panicle (m^{-2}) and grain yield at Mwea Irrigation Scheme.

Table 5. Effect of time of *Azolla* incorporation on grain weight, % grain filling and yield of paddy rice in Mwea Irrgation Scheme during 2015/2016.

Treatment	Season 1		
	Grain weight (g)	**% filled grains**	**Yield (t/ha)**
No *Azolla* (control)	0.0213	0.68	5.18
7.5 t ha^{-1} *Azolla* at transplanting	0.0216	0.77	6.21
7.5 t ha^{-1} *Azolla* at 21 DAT	0.0217	0.71	5.7
Mean	0.022	0.72	5.7
P-value	0.712	0.430	0.440
LSD (0.05)	NS	NS	NS
CV (%)	4.08	14.6	23.6
0 kg N ha^{-1} (control)	0.0216	0.73	0.51
30 kg N ha^{-1}	0.0215	0.71	6.3
Mean	0.022	0.72	5.7
P-value	0.770	0.610	0.080
LSD (0.05)	NS	NS	NS
CV (%)	4.08	14.6	23.6
N x *Azolla* - P-value	0.77	0.4	0.27
	Season 2		
No *Azolla* (control)	0.0226	0.86	5.2
7.5 t ha^{-1} *Azolla* at transplanting	0.0237	0.86	6.4
7.5 t ha^{-1} *Azolla* at 21 DAT	0.0229	0.88	5.69
Mean	23	0.87	5.8
P-value	0.002	0.660	0.030
LSD (0.05)	0.01	NS	0.83
CV (%)	1.6	3.7	11.2
0 kg N ha^{-1} (control)	0.0228	0.89	5.5
30 kg N ha^{-1}	0.0233	0.85	6.1
Mean	0.023	0.87	5.80
P-value	0.010	0.050	0.080
LSD (0.05)	0.004	0.034	NS
CV (%)	1.6	3.7	11.2
N x *Azolla* - P-value	0.01	0.63	0.25

effect of *Azolla* incorporation on yield and yield components but not on growth stages of rice crop can be attributed to a comparatively slow rate of nutrients release by *Azolla*. Watanabe et al. (1991) reported that the rate of mineralization in *Azolla* is gradual. The slow rate of mineralization is due to existence of lignified tissues, which make decomposition to be slow, leading to gradual availability of tissue nutrients (Watanabe et al., 1991). According to Ito and Watanabe (1985), 60% of the tissue N is released within the first four weeks.

Inorganic nitrogen application significantly affected both vegetative and reproductive components of rice plant. Plant height, numbers of tillers, grain weight and spikelets/panicle were significantly increased while percentage grain filling was reduced. Inorganic nitrogen application increased spikelets/panicle in the first season and number of panicles, grain weight and % filled grains in the second season. Inorganic N application has been reported to enhance growth, tillers and yield of paddy rice (Yesuf and Balcha, 2014; Chaturvedi, 2005). The enhancement of growth and yield components can be attributed to supply of readily available nitrogen source throughout the growing period. In this study, N was applied in equal splits at 0, 21 and 53 DAT respectively. This consequently benefitted both vegetative and reproductive phases of rice crop and led to the significant increase realized. Enhanced vegetative growth increases solar radiation reception by the plant canopy (Marshall and Roberts, 2000) and this had a positive effect on plant height, tiller numbers and yield components. Yoshida (1972) reported that increased reproductive tillers concurrently increased rice yields.

A positive correlation between the number of spikelets/panicle, number of panicles m^{-2} and yield suggests the beneficial effect of effective timing of *Azolla* incorporation. It also suggests that *Azolla* should be incorporated at transplanting for maximum benefit to farmers, especially where temperatures are relatively low. The effect of interaction between time of incorporation and inorganic N application was not significant for all parameters except for spikelets panicle^{-1}.

The significant effects of *Azolla* incorporation were more pronounced during the second season. This can be attributed to relatively higher temperature and relative humidity, which may have enhanced mineralization of *Azolla* during vegetative stage of the second season. During the first season, average temperatures were lower (22°C) at growth stage and higher at reproductive (23.5°C) stage while in the second season, temperatures were higher at growth stage (22.9°C) and lower at reproductive stage (22.1°C). Relative humidity was also higher in second season (79%) than in the first (69%). Consequently, *Azolla* mineralization could have been faster in the second season leading to the response observed. These results are in concurrence with the findings of Subedi and Shrestha (2015) who reported that the rate of nutrient release upon decomposing *Azolla* increases with increasing environmental temperatures and relative humidity.

Conclusion

Time of *Azolla* application affects growth and yield of paddy rice. Application of *Azolla* at planting is more beneficial to paddy rice as it increases both yield and yield components of paddy rice

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahero Research Station, Ministry of water and Irrigation (1980). Technical Report no 57

Armstrong WP (1979). A marriage between a fern and *cyanobacteria*. Environmental South West 50:20-24.

Better Crops (1999). Better crops with plant food. Potash and Phosphate Institute (PPI) 83:1. http:/www.ipni.net/publication/bettercrops.nsf

Bocchi S, Malgioglio A (2010). *Azolla-Anabaena* as a bio-fertilizer for rice paddy fields in the Po valley, a temperate rice area in Northern Italy. International Journal of Agronomy, pp. 152-158

Campbell R (2011). *Azolla* growth in farm dams, Agriculture Victoria. Online-http/agriculture.vic.gov.au/ agriculture/ farm). Date accessed, 14/3/2016.

Carrapiço F, Teixeira G, Diniz M (2000). *Azolla* as a bio-fertiliser in Africa. A challenge for the future. Revista de Ciências Agrárias, 23(3-4):120-138.

Chaturvedi I (2005). Effects of nitrogen fertilizers on yield and quality of hybrid rice (Oryza sativa). Journal of Central European Agriculture 6(4):611-618.

Ferentinos L, Smith J, Valenzuela H (2002). Sustainable agriculture, green manure crops. Available online at: https://scholarspace.manoa.hawaii.edu/bitstream

International Rice Research Institute (IRRI) (1990). Low - external input rice production (LIRP) Technology Information Kit, P. 292.

Ito O, Watanabe I (1985). Availability to rice plants of nitrogen fixed by *Azolla*. Soil Science Plant Nutrients 31(I):91-104.

Kamalasanana P, Premalatha S, Rajamony S (2002). *Azolla* – A sustainable feed substitute for livestock. Leisa India magazine 4:1. Available online at: http. *www.leisa.info.*

Kannaiyan S (1993). Nitrogen contribution by *Azolla* to rice crop. Proceedings of the Indian National Science Academy 59(4):309-314.

Malyan SK, Bhatia A, Kumar SS, Fagodiva KR, Pugazhendh A, Duc AP (2019). Mitigation of greenhouse gas intensity by supplementing with *Azolla* and moderating the dose of nitrogen fertilizer. Biocatalysis and Agricultural Biotechnology P. 20. doi.org/10.1016/ j.bcab.2019.101266

Marshall B, Roberts JA (2000). Leaf development and canopy growth. Sheffield Academic Press; Boca Raton, FL.

Miller JO (2016). Soil pH affects nutrient availability, FS-1054. University of Maryland Extension. Available on line at: https://extension.umd.edu/anmp.

Oyange WA, Chemining'wa GN, Kanya JI, Njiruha P (2019). *Azolla* Fern in Mwea Irrigation Scheme and Its Potential Nitrogen Contribution in Paddy Rice Production. Journal of Agricultural Science 11(18):30-44

Patro H, Dash D, Ramesh C T, Shahid M (2011). Effect of organic and inorganic sources of N on growth attributes, grain and straw yield of rice (*Oryza sativa*). International Journal of Pharmacy and Life Sciences 2(4):655-660.

Razavipour T, Moghaddam SS, Doaei S, Noorhosseini SA, Damalas

CP (2018). *Azolla* (*Azolla filiculoides*) compost improves grain yield of rice (*Oryza sativa* L.) under different irrigation regimes. Agricultural Water Management 209:1-10.

Serrem CK, Ng'etich WK, Kemei MK (2013). Soil fertility improvement using crop residues and Azolla for sustainable production of rice and fish in irrigated rice-fish farming system in the Lake Victoria basin of Kenya. Joint proceedings of the 27th Soil Science Society of East Africa and the 6th African Soil Science Society, 20-25th October, 2013

Singh AL, Singh PK (1987). Influence of *Azolla* management on the growth, yield of rice and soil fertility. Plant and Soil 102:41-47.

Singh SK, Varma SC, Singh RP (2001). Effect of integrated nutrient management on yield, nutrient uptake and changes in soil fertility under rice (*Oryza sativa*) – lentil (*Lens culinaris*) cropping system. Indian Journal of Agronomy 46(2):191-197.

Subedi P, Shrestha J (2015). Improving soil fertility through *Azolla* application in low land rice: A review. Azarian Journal of Agriculture 2:35-39.

Wagner MG (1997). *Azolla*, a review of its biology and utilization. The Botanical Review 63:1-26.

Watanabe I, Berja NS (1983). The growth of four species of *Azolla* as affected by temperature. Aquatic Botany 15:175-185

Watanabe I, Padre B, Ramirez C (1991). Mineralization of *Azolla* N and its availability to wetland rice. Soil Science and Plant Nutrition 37(4):679-688.

Yesuf E, Balcha A (2014). Effects of nitrogen application on grain yield and nitrogen efficiency of rice (*Oryza sativa* L.). Asian Journal of Crop Science 6:273-280.

Yoshida S (1972). Physiological aspects of grain yield. Annual Review of Plant Physiology 23:437-464.

APPENDIX

Appendix Table 1. Average temperatures, relative humidity and rainfall during 2015/2016.

Season 1																
Month	**Nov**		**Dec**				**Jan**				**Feb**				**March**	
Week	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2
Max Temp. (°C)	28.5	29.8	28.1	28.4	29.7	26.6	31.0	31.4	31.1	31.1	31.7	32.1	32.3	33.1	33.2	32.7
Min Temp.(°C)	17.2	16.0	15.3	14.9	14.9	15.3	13.7	13.6	14.6	14.1	14.3	15.0	16.5	15.1	13.8	14.6
Av Temp.(°C)	22.9	22.9	21.7	21.6	22.3	20.9	22.4	22.5	22.8	22.6	23.0	23.5	24.4	24.1	23.5	23.6
RH (%)	77.0	79.0	72.0	63.0	64	60.0	59.0	34.0	55.0	52.0	53.0	65.0	64.0	34.0	53.0	64.0
Rainfall (mm)	0.0	0.0	0.0	0.1	0.0	0.8	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Season 2																
Month	**Sep**				**Oct**				**Nov**				**Dec**			
Week	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4
Max Temp. (°C)	27.2	28.8	29.0	29.4	29.9	30.6	30.7	31.0	30.3	29.7	26.9	27.4	28.0	28.1	29.1	28.9
Min Temp.(°C)	16.2	16.1	14.4	17.7	18.0	17.3	16.3	16.9	18.0	17.5	16.8	17.2	14.9	15.9	14.1	15.0
Av Temp.(°C)	21.7	22.4	21.7	23.6	23.9	23.9	23.5	23.9	24.2	23.6	21.8	22.3	21.5	22.0	21.6	22.0
RH (%)	82.0	78.4	80.0	78.0	80.9	75.3	71.9	74.1	79.3	85.3	88.7	85.4	80.3	79.8	76.4	77.8
Rainfall (mm)	0.0	0.0	0.0	6.3	0.6	0.1	0.0	0.8	2.1	0.8	13.5	4.2	0.2	2.8	1.0	0.5

W= week, temp=temperature, Max= maximum, Min=Minimum, Av= average, RH relative humidity.

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Ultraviolet B radiation affects growth, physiology and fiber quality of cotton

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The effects of artificial biologically effective UV-B radiation on a range of growth and physiological parameters in two cotton (*Gossypium hirsutum* L.) cultivars (Romanos and Allegria) were recorded. Three levels of biologically effective UV-B were used: (1) zero (2) ambient and (3) elevated (determined as that associated with a notional 15% depletion of stratospheric ozone). Plants were grown under artificial light in growth chambers and subjected to the biologically effective UV-B radiation treatments. Compared to the zero level, the ambient and elevated biologically effective UV-B radiation significantly reduced plant height, leaf chlorophyll content, net photosynthetic rate, stomatal conductance, bract length, petal length, anthers number, pollen germination, seed cotton weight, fibre strength, fibre elongation, fibre micronaire, fibre maturity index, fibre spinning consistency index, mean fibre length, fibre yellowness and fibre uniformity index. Both the ambient and the elevated UV-B radiation also significantly increased stomatal density, short fibre index and fibre reflectance.

Key words: UV-B radiation, cotton, photosynthesis, stomatal density, flower characteristics, pollen germination, seed cotton weight, fibre quality.

INTRODUCTION

Chlorofluorocarbons (CFCs) released into the atmosphere in earlier years have depleted the stratospheric ozone layer resulting in increased levels of UV-B radiation reaching the Earth's surface (Rowland, 1990). As CFC levels gradually decline, the amount of UV-B radiation in the northern mid-latitudes is projected to return to its pre-1980 level by about 2065 (McKenzie et al., 2011).

Depending on location and season, crops grown between latitudes 40° N and 40° S currently receive a solar UV-B radiation dose of some 2-10 kJm^{-2} per day (Reddy et al., 2003). In several crops including cotton, a variety of plant processes are affected by UV-B radiation (Reddy et al., 2003; Kakani et al., 2003a). These processes include those associated with crop production, such as pollination, boll formation, boll development and lint yield.

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Plants perceive UV-B by the specific ultraviolet-B photoreceptor UV Resistance Locus 8 (UVR8) that at the molecular level has been identified in *Arabidopsis thaliana* to enhance photomorphogenesis (Lee, 2016). It has also been reported that UV-B radiation affects crop yield and quality parameters (Wargent and Jordan, 2013).

In Greece, the cotton crop is an important component of trade and economy. However, the understanding of cotton's response to elevated UV-B is limited, especially in relation to the plant's growth and development, as well as in relation to fibre yield and quality (Gao et al., 2003).

The objectives of the present study were: i) to estimate the effects of UV-B radiation on cotton plant height, leaf chlorophyll content, net photosynthetic rate and stomatal conductance, stomatal density, stomatal length, stomatal width, bract length, petal length, staminal column length, anthers number, pollen germination and seed cotton weight in two commercial cultivars; and (ii) to quantify the effects of UV-B radiation on various key fibre properties.

MATERIALS AND METHODS

Two commercial cotton (*Gossypium hirsutum* L.) cultivars (cv., that is, Romanos and Allegria) were used in the present study.

Growth chambers

The experiment was conducted in UV-B plant growth chambers at a controlled environment facility in University of Thessaly, Volos City, Greece. The chambers were lined internally with polyethylene sheets (TUV 3999, Crete plastics, Heraklion, Crete, Greece), which absorbed all UV-A and UV-B radiation. Sheets were replaced every two weeks. The chamber air conditioner comprised a cooling system, a heating system, a ventilation system and a control system. The minimum and maximum temperatures were 21-29°C during the light period and 15-19°C during the dark period. The relative humidity range was 41-65% (measured by a HOBO LCD data logger).

Each chamber was illuminated with multiple lamps emitting photosynthetic active radiation (PAR), UV-B radiation (280-315 nm) and ultraviolet A radiation (UV-A; 315-400 nm). The lamps were mounted at different heights above the plant canopy. Each chamber contained four PAR lights, being two of a metal halide type (MH; Osram HQI-TS 1000 W) and two of a high-pressure sodium type (HPS; Phillips SON-T 1000 W). Both lamp types were emitting some UV-A radiation. The average PAR measured using a 6200 quantum sensor (LI-COR, Lincoln, Nebraska, USA) just above the canopy ranged from 497.00 ± 90 μmol m^{-2} s^{-1} to 1042.00 ± 110 μmol m^{-2} s^{-1} during the experimental period (Table 1). The heights of the PAR lamps above the canopy were adjusted to the median plant height at least once per week to maintain a constant PAR exposure.

UV-B and PAR treatments

Three levels of biologically effective ultraviolet-B radiation ($UV\text{-}B_{BE}$) per photoperiod duration were used: (1) 0 $UV\text{-}B_{BE}$ (control; 0 kj $m^{-2}day^{-1}$ $UV\text{-}B_{BE}$) (2) Am $UV\text{-}B_{BE}$ (mean ambient $UV\text{-}B_{BE}$ radiation) and (3) $UV\text{-}B_{BE}$ 15 (mean enhanced $UV\text{-}B_{BE}$ radiation with a 15% reduction in stratospheric ozone). In the control chambers, the UV-B tubes were inactivated so that they did not emit UV-B radiation. The intensities of visible light and the degree of mutual shading from the UV tubes were similar across all chambers.

Solar UV-B and PAR values at ground level were determined for the city of Volos (Greece) using the MODerate resolution Imaging Spectroradiometer (MODIS) (http://modis.gsfc.nasa.gov/) onboard the satellites Terra and Aqua. The Radiative Transfer Model (LibRadtran) was used to simulate GHI and DNI for different scenarios of atmospheric parameters. Spectra of solar UV-B irradiance reaching the ground were calculated. These spectra were weighted with the Caldwell (1971) generalised plant action spectrum (normalised at 300 nm) to determine the biologically effective UV-B radiation dose. The average values of photoperiod duration and photosynthetically active radiation (PAR) were also calculated.

Throughout the period from seedling emergence to boll harvest, the mean Am $UV\text{-}B_{BE}$ and UV- B_{BE}15 were changed according to the duration of photoperiods. Also, the mean PAR values during the photoperiod and the mean photoperiod length were changed (Table 1). The mean UV-A radiation values were supplemented by four Phillips TLD 36W/08 lamps, which together with the metal halide and high-pressure sodium lamps provided a total UV-A flux of 1.27 ± 0.05 W m^{-2} at plant height. The $UV\text{-}B_{BE}$ radiation was delivered to the plants during the photoperiod; the doses are listed in Table 1.

In each chamber, the heights of the PAR, UV-B and UV-A lamps were adjusted to median plant height once per week. Two types of polyethylene sheets (Crete plastics, Heraklion, Crete, Greece) were used: a) TUV 3942 filter that blocks UV-B and transmits UV-A and longer wavelength radiation in control chambers and b) TUV 3999 filter. The $UV\text{-}B_{BE}$ radiation was provided by five parallel fluorescent tubes (TL 40 W/12 RS-Philips, Holland) perpendicular to the plant rows and 0.6 m above the canopy. The energy of the emitted UV-B and UV-A radiations were checked, adjusted and delivered according to photoperiod durations, using a computer, UV-B and UV-A sensors and microcontrollers. The fluorescent UV-B light was filtered through 75 μm thick cellulose diacetate sheets (Clarifoil, Coventry, UK). Cellulose diacetate was used to eliminate any UV-C radiation but to transmit $UV\text{-}B_{BE}$ and longer wavelengths, including UV-A radiation. Cellulose diacetate filters were replaced every 2 days to ensure uniformity of UV-B and UV-A transmission due to photodegradation. UV-B and UV-A irradiances were measured using SKU 430 and SKU-420 sensors (Skye Instruments, Llandrindod Wells, UK), respectively.

Plant culture and growth conditions

The experiment began one day after emergence (1 DAE) and continued to harvest (172 DAE) (Table 1). Cotton seeds of the two cultivars were sown in a substrate of 80:20 (w/w) soil:peat in rectangular steel bins on wheels, equipped with drain holes 810 (high) x 770 (long) x 230 mm (wide). The main characteristics of the soil were: colour 10YR 3/3, sand 31%, silt 34%, clay 35%, texture Clay Loam, organic matter 1.77 g/100 g soil, $CaCO_3$ 10.1%, pH 7.8 (H_2O 1:1), phosphate 10 ppm (Olsen). The exchangeable cations were: K 0.45, Na 0.17, Ca 30.15 and Mg 7.83 me/100 g soil (Mitsios et al., 2000). The peat used was Floradur R: pH 5.0-6.5, N 50-300, P2O5 80-300 and K2O 80-400 mg/l) produced by Floragard Vertriebs GmbH für Gartenbau. After germination, seedlings were thinned to four plants per pot, with main stems about 170 mm apart. Plants received about 3 L of tap water per day via a dripper. A complete fertiliser (Hakaphos N:P:K, 12:32:14+3% Mg, Compo Hellas) was added on three occasions on 27, 57 and 99 DAE. To minimize positional effects, pots were completely randomized within each chamber every third day.

Table 1. Experimental structure.

DAE	Am UV-B_{BE} (kJ m^{-2} day^{-1})	UV-B_{BE} 15 (kJ m^{-2} day^{-1})	PAR (µmol m^{-2} s^{-1})	Photoperiod (h:min)
1-7	4.46	5.95	947.00	14:24
8-37	5.87	7.63	1042.00	15:00
38-68	5.95	7.58	1023.00	14:43
69-99	4.84	6.16	956.00	13:43
100-129	3.24	4.16	812.00	12:27
130-160	1.87	2.51	663.00	11:04
161-172	0.85	1.23	497.00	9:53

Days after seedling emergence (DAE); biologically effective ultraviolet-B radiation (UV-B_{BE}); photosynthetic active radiation (PAR). Treatments were ambient UV-B_{BE} (Am UV-B_{BE}) and the calculated level of UV-B_{BE} radiation associated with a notional 15% depletion of stratospheric ozone (UV-B_{BE} 15).

Measurements

Plant height was determined on eight plants per UV-B_{BE} treatment and cultivar on 92 and 170 days after emergence. Total chlorophyll contents were estimated in the same leaves after net photosynthetic rates and stomatal conductance determination. Contents of total chlorophyll (chlorophyll a and chlorophyll b) of fully-expanded leaves from the top of the main stem were determined in SPAD units by a chlorophyll meter (SPAD 502, Minolta LTD, Ojaka, Japan). Eight leaves, derived from 8 plants by different pots, were used per UV-B treatment and cultivar. Eight average values were taken from each leaf.

Net photosynthetic rate (Pn) and stomatal conductance (gs) were measured per UV-B treatment and cultivar on the youngest, fully expanded mainstem leaves from the top of the main stem of eight plants, from different pots. These measurements were made using a LI 6200 portable photosynthesis system (LI-COR, Lincoln, NE USA).

Portions of the adaxial and abaxial surfaces of the youngest, fully expanded mainstem leaves (counting from the top of the main stem) were coated with clear nail varnish, in the mid-area between the central vein and the edge of the leaf. The chosen leaves were those for which gas exchange and chlorophyll content were measured. After drying, the peels were removed using fine forceps and placed on a slide. The numbers of stomata per mm^2 (stomatal density), stomatal length and stomatal width were counted using a light microscope (x400 magnification). The numbers of stomata were counted in three fields on each leaf surface of eight replicate leaves per treatment and cultivar. The leaves were selected from eight plants from different pots. Stomatal length and stomatal width of randomly chosen stomata was also measured in three different fields on each leaf surface of eight leaf samples from eight plants, under the same magnification using an ocular micrometer. Therefore, the length or width of 24 stomata for each UV-B treatment, cultivar and leaf surface was measured.

Total chlorophyll content, photosynthetic, stomatal conductance, stomatal density, stomatal length and stomatal width were measured on 66 and 128 days after emergence.

Lengths of bracts, petals, maximum measurable length of staminal columns and anther number for five flowers in each treatment and cultivar were counted on 71 and 91 days after emergence

Early in the morning on 67 and 88 days after emergence, when the anthers were beginning to dehisce and pollen grains were at the same developmental stage, five flowers were randomly selected from five plants per treatment and cultivar by plants grown outdoors. Flower peduncles were immediately placed with their cut ends in a porous material (Oasis floral foam blocks) impregnated with tap water. The flowers were placed under lamps of 0 UV-B_{BE}, Am UV-B_{BE}, and UV-B_{BE} 15 radiation at 0.60 m. Then, 5-6 anthers were taken from each flower and transferred to a drop of liquid germination medium on a glass slide. This medium was made up according to Brewbaker and Kwack (1963) and modified to contain H_3BO_3 0.1 gl^{-1}, Ca $(NO_3)_2.4H_2O$ 0.3 gl^{-1}, Mg SO_4 0.2 gl^{-1}, KNO_3 0.1 gl^{-1}, KH_2PO_4 0.1 gl^{-1} and sucrose 100 gl^{-1}. Pollen grains were distributed uniformly in the drop of liquid medium using a needle. Slides were kept placed in moist Petri dishes, covered and incubated at 22±2°C for 2 h before storage at 4°C pending observation of germination by light microscopy. Pollen grains were considered to have germinated artificially when the pollen tube length was at least equal to or greater than one pollen grain diameter. Six microscope fields were examined per flower, cultivar and UV-B_{BE} level, with each field containing 30-70 pollen grains. Germination percentage was determined as the fraction of the total grains present.

Seed cotton weight per plant was determined at 171 days after emergence. Samples of seed cotton from eight plants were gathered by hand and put in plastic bags. Qualitative characteristics of cotton fibres were determined in the high volume instrument machine in the laboratory of a local textile industry, after harvesting the raw cotton from eight plants.

Statistical analyses

The collected data was computerized and analyzed using ANOVA to determine least significant differences (LSD tests). The software package IBM SPSS Statistics V23.0 was used for all statistical computations.

RESULTS AND DISCUSSION

The UV-B treatments significantly affected all traits of both cultivars (Tables 2 to 6). The pollen germination and fibre quality results reported in the present study are the first describing ambient and supplemental UV-B_{BE} radiation effects on cotton.

Plant height

Throughout the experimental period, plant height was reduced by increased UV-B_{BE} irradiance. In both cultivars, decreases in plant height due to UV-B_{BE} followed the

Table 2. Effect of three levels of UV-B_{BE} on plant height, leaf chlorophyll content, net photosynthetic rate and stomatal conductance of two cotton cultivars (Romanos and Allegria).

UV-B treatment	Cultivar	Plant height (cm)	Leaf chlorophyll content (SPAD units)	Net photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (mmol $m^{-2}s^{-1}$)
0 UV-B_{BE}	Romanos	100.3 ± 5.210^{e}	43.5 ± 0.837f	15.60 ± 0.440^{d}	520.69 ± 24.595f
	Allegria	82.0 ± 4.552^{b}	40.6 ± 0.803^{e}	13.35 ± 0.448^{b}	446.74 ± 24.279^{d}
Am UV-B_{BE}	Romanos	96.2 ± 5.072^{d}	38.9 ± 0.814^{d}	14.70 ± 0.391^{c}	459.59 ± 25.256^{e}
	Allegria	78.7 ± 4.472^{a}	37.3 ± 0.871^{b}	12.47 ± 0.421^{a}	396.11 ± 25.465^{b}
UV-B_{BE} 15	Romanos	94.9 ± 5.048^{c}	37.9 ± 0.778^{c}	14.48 ± 0.378^{c}	437.79 ± 25.244^{c}
	Allegria	77.6 ± 4.394^{a}	36.4 ± 0.859^{a}	12.17 ± 0.421^{a}	382.19 ± 25.966^{a}
UV-B treatment (UV-B)		***	***	***	***
Cultivar (CV)		***	***	***	***
Time (T)		***	***	***	***
UV-B × CV		ns	***	ns	***
UV-B × T		ns	ns	ns	***
CV × T		***	*	*	ns
UV-B × CV × T		ns	*	ns	ns

Values are means ± SE across all samplings, n=8. Different letters within each column denote significant difference at 0.05 probability level. * P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; P > 0.05 ns (not significant).

pattern: plant height under 0 UV-B_{BE} > Am UV-B_{BE} > UV-B_{BE} 15. Throughout the sampling period, compared with the controls, the plant height reductions in cv. Romanos were 4.09% (Am UV-B_{BE}) and 5.38% (UV-B_{BE}15) and in cv. Allegria were 4.02% (Am UV-B_{BE}) and 5.37% (UV-B_{BE}15) (Table 2). These results are consistent with those of Gao et al. (2003), who reported reductions in cotton plant height under field conditions due to enhanced UV-B radiation. The exclusion of solar UV-B radiation increased the specific leaf weight compared to the control and increased plant height with a significant biomass increase (Zhu and Yang, 2015).

In addition, UV-B treatments decreased plant height. The reduction in plant height following exposure to UV-B radiation has been attributed to inhibition of biosynthesis or signaling of various hormones (Vanhaelewyn et al., 2016), to reductions in indole-3-acetic acid (IAA) by increasing peroxidase and IAA oxidase activities that can cause growth suppression (Huang et al., 1997). The reductions are due to a shortening of internode length, rather to fewer internodes (Reddy et al., 2003).

Chlorophyll (a+b) content

In both cultivars, the mean chlorophyll content under exposure to the three UV-B_{BE} levels followed the following reduced range: chlorophyll content of 0 UV-B_{BE} > chlorophyll content of Am UV-B_{BE} > chlorophyll content of UV-B_{BE} 15. Leaf chlorophyll content was higher in cv. Romanos than in cv. Allegria in all UV-B_{BE} equivalent regimes. Compared to controls, the leaf chlorophyll content reductions in cv. Romanos were 10.57% (Am UV-B_{BE}) and 12.87% (UV-B_{BE} 15) and in cv. Allegria were 8.13% (Am UV-B_{BE}) and 10.34% (UV-$_{BBE}$ 15) (Table 2).

Kakani et al. (2003b) reported that chlorophyll content reduction after exposure to UV-B ranged from 10 to 78% among the dicot species. Enhanced UV-B radiation exposure significantly reduced total chlorophyll content, depending on crop cultivars/species, treatment doses, length and intensity of UV-B radiation, and variation in the PAR/UV-B ratio. The decrease in chlorophyll content was due to thylakoids and grana rupture on UV-B radiation exposure. Also, the declines in pigments of chlorophyll and photosynthesis led in reduced biomass and yield for most crop plants. Enhanced UV-B radiation due to depletion of stratospheric O_3 decreases the financial yield and product quality of field crops.

Ambient UV-B radiation resulted in increased UV-B absorbing leaf compounds, while chlorophyll a, b, and (a+b) content reduced. However, chlorophyll bleaching and damage by solar UV-B radiation to the photosynthetic apparatus induced a decrease in photosynthetic rate (Zhu and Yang, 2015). It has been reported that chl-a and chl-b concentration in plants exposed to UV radiation has been significantly reduced. This is due to enhanced chlorophyll photodegradation, to lower chlorophyll synthesis rates resulting from decreased expression of genes encoding chlorophyll-binding proteins, or to break down of chloroplasts structural integrity (Sarghein et al., 2008). The previously mentioned are the possible reasons for the reduction in the present study

Table 3. Influence of three levels of UV-B_{BE} on stomatal density, stomatal length and stomatal width of two cotton cultivars (Romanos and Allegria).

UV-B treatment	Cultivar	Surface	Stomatal density (no. per mm^2)	Stomatal length (μm)	Stomatal width (μm)
0 UV-B_{BE}	Romanos	Adaxial	65.5 ± 4.047[a]	27.2 ±0.099[b]	20.3 ± 0.060[abc]
		Abaxial	178.4 ± 1.386[e]	26.4 ± 0.069[a]	20.2 ± 0.131[abc]
	Allegria	Adaxial	67.4 ± 0.708[a]	28.5 ± 0.137[cd]	21.1 ± 0.149[c]
		Abaxial	166.4 ± 1.112[d]	28.2± 0.095[c]	20.3 ± 0.054[abc]
Am UV-B_{BE}	Romanos	Adaxial	82.3 ± 0.702[bc]	27.3± 0.087[b]	20.2 ± 0.114[abc]
		Abaxial	196.5 ± 1.075[g]	26.5 ± 0.109[a]	20.0 ± 0.111[ab]
	Allegria	Adaxial	79.3 ± 0.958[b]	28.5 ± 0.109[cd]	20.9 ± 0.101[bc]
		Abaxial	182.1 ± 1.358[ef]	28.3 ± 0.061[cd]	20.3 ± 0.054[abc]
UV-B_{BE} 15	Romanos	Adaxial	86.8 ± 0.760[c]	27.3± 0.086[b]	19.5 ± 0.687[a]
		Abaxial	201.6 ± 1.315[g]	26.5± 0.109[a]	20.1± 0.122[ab]
	Allegria	Adaxial	83.4 ± 0.713[bc]	28.6± 0.075[d]	20.9± 0.113[bc]
		Abaxial	186.3 ± 1.381[f]	28.3 ± 0.058[cd]	20.3 ± 0.051[abc]
UV-B treatment (UV-B)			***	ns	ns
Cultivar (CV)			***	***	***
Surface (S)			***	***	*
Time (T)			***	***	ns
UV-B x CV			ns	ns	ns
UV-B x S			ns	ns	ns
UV-B x T			ns	ns	ns
CV x S			***	***	***
CV x T			ns	ns	ns
S x T			ns	*	ns
UV-B x CV x S			ns	ns	ns
UV-B x CV x T			ns	ns	ns
UV-B x S x T			ns	ns	ns
CV x S x T			ns	ns	ns
UV-B x CV x S x T			ns	ns	ns

Values are means ± SE across all samplings, n = 24. Different letters within each column denote significant difference at 0.05 probability level. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; P > 0.05 ns (not significant).

Net photosynthetic rate

Compared to controls plants, the reduction of Pn under Am UV-B_{BE} exposure was during the sampling 5.77% in Romanos and 6.59% in Allegria, while under UV-B_{BE}15 exposure the reductions were 7.18% for cv. Romanos and 8.84% for cv. Allegria. In both cultivars, net photosynthetic rate reduced under Am UV-B_{BE} and even more under UV-B_{BE}15. The variation of Pn following exposure to UV-B radiation in both cultivars usually followed the following pattern throughout the sampling period: Pn under 0 UV-B_{BE} > Pn under Am UV-B_{BE} > Pn under enhanced UV-B_{BE}15 (Table 2).

It is inferred that the photosynthesis decline was closely correlated with a decline in stomatal conductance (Kakani et al., 2004). It was also shown that increased UV-B significantly decreased net photosynthetic rate and stomatal conductance (Yao and Liu, 2006). UV-B radiation exclusion significantly increased the net photosynthetic rate, stomatal conductance and activity of Rubisco. There was also a suppressive action of ambient UV-B on growth and photosynthesis; and dicots (as cotton) were more susceptible than monocots in this suppression (Kataria et al., 2013).

The effect of UV-B radiation on photosynthesis has been shown to depend on species of crops, cultivars, experimental conditions, dosage of UV-B, ratio of PAR to UV-B radiation, stages of plant growth and interactions between UV-B radiation and other stresses of the environment. The decrease in photosynthesis by UV-B

Table 4. Differences in flower characteristics and pollen germination of two cotton cultivars under three UV-B_{BE} radiation treatments.

UV-B treatment	Cultivar	Bract length (cm)	Petal length (cm)	Staminal column length (mm)	Anthers (no. per flower)	Pollen germination (%)
0 UV-B_{BE}	Romanos	45.37 ± 0.604^{bc}	58.90 ± 0.344^{c}	18.50 ± 0.582^{a}	89.70 ± 1.012^{d}	69.13 ± 0.709^{e}
	Allegria	47.07 ± 0.540^{c}	59.86 ± 0.451^{c}	19.10 ± 0.526^{a}	91.50 ± 0.910^{d}	72.95 ± 0.824^{f}
Am UV-B_{BE}	Romanos	43.50 ± 0.518^{ab}	56.18 ± 0.469^{b}	18.10 ± 0.526^{a}	81.30 ± 0.844^{b}	46.59 ± 0.685^{c}
	Allegria	44.87± 0.527^{bc}	57.26 ± 0.523^{b}	18.70 ± 0.448^{a}	84.20 ± 0.940^{c}	48.99 ± 0.750^{d}
UV-B_{BE}15	Romanos	43.00 ± 0.415^{a}	54.74 ± 0.486^{a}	18.00 ± 0.577^{a}	78.70 ± 0.831^{a}	37.55± 0.718^{a}
	Allegria	44.43 ± 0.500^{ab}	56.58 ± 0.471^{b}	18.50 ± 0.500^{a}	81.50 ± 0.910^{b}	42.88± 0.743^{b}
UV-B treatment (UV-B)		***	***	ns	***	***
Cultivar (CV)		***	***	ns	***	***
Time (T)		*	***	ns	***	*
UV-B × CV		ns	ns	ns	ns	ns
CV × T		ns	ns	ns	ns	ns
UV-B × T		ns	ns	ns	ns	***
UV-B × CV × T		ns	ns	ns	ns	ns

Data are the mean ± SE across all samplings (n_1= 15, n_2 = 25, n_3 = 5, n_4 = 5, n_5 = 30). Means within a column marked by the same letter indicate a lack of significant difference at 0.05 probability level. * P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; P > 0.05 ns-not significant differences.

Table 5. Seed cotton weight and fibre quality responses of two cotton cultivars (Romanos and Allegria) to three levels of UV-B_{BE}.

UV-B treatment	Cultivar	Seed cotton weight (g plant^{-1})	Fibre strength (g tex^{-1})	Fibre elongation (%)	Fibre micronaire	Fibre maturity Index (%)	Fibre spinning consistency index
0 UV-B_{BE}	Romanos	69.87 ± 1.151^{d}	32.84 ± 0.171^{e}	5.28 ± 0.118^{d}	4.91 ± 0.058^{c}	0.86 ± 0.008^{c}	133.25 ± 0.250^{d}
	Allegria	58.28 ± 0.847 ^{b}	32.25 ± 0.161^{d}	5.78 ± 0.077^{e}	5.21 ± 0.052^{d}	0.88 ± 0.008^{c}	133.75 ± 0.313^{d}
Am UV-B_{BE}	Romanos	67.84 ± 1.068^{c}	30.18 ± 0.166^{b}	4.34 ± 0.080^{b}	4.54 ± 0.050^{a}	0.83±0.007^{ab}	128.88±0.227^{b}
	Allegria	55.81 ± 0.873^{a}	30.83±0.176^{c}	5.08±0.077^{d}	4.71 ± 0.055^{b}	0.84 ± 0.007^{b}	129.75 ± 0.313^{c}
UV-B_{BE} 15	Romanos	67.46 ± 0.911^{c}	29.40 ± 0.144^{a}	4.04 ± 0.080^{a}	4.44 ± 0.050^{a}	0.82 ± 0.008^{a}	127.88 ± 0.227^{a}
	Allegria	55.21 ± 0.799^{a}	30.45 ± 0.222^{c}	4.79 ± 0.083^{c}	4.55 ± 0.076^{a}	0.83 ± 0.007^{ab}	129.00 ± 0.267^{b}
UV-B treatment (UV-B)		**	***	***	***	***	***
Cultivar (CV)		***	***	***	***	***	***
UV-B × CV		ns	ns	ns	ns	ns	ns

Values are means ± SE across all samplings, *n* = 8. Means within a column marked by the same letter indicate a lack of significant difference at 0.05 probability level. **P* ≤ 0.05; ***P* ≤ 0.01; ****P* ≤ 0.001; *P* > 0.05 ns (not significant).

Table 6. Fibre properties of two cotton cultivars (Romanos and Allegria) in response to three levels of $UV\text{-}B_{BE}$.

UV-B treatment	Cultivar	Mean fibre length (mm)	Short fibre index (%)	Fibre reflectance (Rd)	Fibre yellowness (+b)	Fibre uniformity index (%)	Moisture (%)
0 $UV\text{-}B_{BE}$	Romanos	31.13 ± 0.147[d]	6.55 ± 0.116[ab]	74.08 ± 0.195[a]	7.50 ± 0.098[cd]	84.41 ± 0.116[d]	5.65 ± 0.076[cd]
	Allegria	30.26 ± 0.320[c]	6.36 ± 0.094[a]	75.03 ± 0.234[b]	7.88 ± 0.222[d]	85.70 ± 0.171[e]	5.31 ± 0.097[a]
Am $UV\text{-}B_{BE}$	Romanos	30.04 ± 0.155[bc]	7.53 ± 0.106 [c]	80.21 ± 0.202[c]	6.80 ± 0.141[ab]	81.95 ± 0.164[b]	5.39 ± 0.067[ab]
	Allegria	29.66 ± 0.168[ab]	6.61 ± 0.090[b]	80.25± 0.175[c]	7.34 ± 0.118[c]	82.69 ± 0.190[c]	5.70 ± 0.076[d]
$UV\text{-}B_{BE}15$	Romanos	29.63 ± 0.153[ab]	7.73 ± 0.119[c]	82.69 ± 0.151[d]	6.61 ± 0.242[a]	81.20 ± 0.165[a]	5.53 ± 0.065[bcd]
	Allegria	29.44 ± 0.153[a]	6.76 ± 0.091[b]	82.76 ± 0.185[d]	7.09 ± 0.123[bc]	81.83 ± 0.274[b]	5.51 ± 0.072[bc]
UV-B treatment (UV-B)		***	***	***	***	***	ns
Cultivar (CV)		***	***	*	***	***	ns
UV-B × CV		ns	***	*	ns	ns	***

Numbers in the table represent means ± SE across all samplings (n = 8). Values within a column followed by different letters indicate significant differences at 0.05 probability level. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; P > 0.05 ns (not significant).

doses ranged from 3-90% in crop plants. Decrease in photosynthesis was due to impact of UV-B on Photosystem II along with reduction in pigments and leaf area. However, the declines in pigments of chlorophyll and photosynthesis led in reduced biomass and yield for most crop plants (Kakani et al., 2003b).

Photosynthesis, however, can also be depleted by stomatal density and opening, reduced stomatal conductance or reduced chlorophyll content, following exposure of certain plants to UV radiation (Salama et al., 2011). Most of the rice cultivars plants grown in the greenhouse, exposed to enhanced UV-B radiation showed reduced photosynthetic rate, pollen germination, fertility and yield (Mohammed and Tarpley, 2011). UV-B radiation causes harm to the photosynthetic apparatus of green plants at various sites. UVB radiation has been shown to cause a decrease in photosynthetic activity primarily associated with the PSII protein degradation, chlorophyll and carotenoid destruction, reduced stomatal function activity and impacts on Rubisco activity (Kataria et al., 2014).

Stomatal conductance

During the sampling period, stomatal conductance was higher in cv. Romanos at similar $UV\text{-}B_{BE}$ intensities than in cv. Allegria. Also, gs under exposure to UV-B levels followed in both cultivars the following reduced scale: gs under 0 $UV\text{-}B_{BE}$ > gs under Am $UV\text{-}B_{BE}$ > gs under enhanced $UV\text{-}B_{BE}15$. Compared to control plants, the reduction of stomatal conductance under Am $UV\text{-}B_{BE}$ exposure was during the sampling 11.73% in Romanos and 11.33% in Allegria, while under $UV\text{-}B_{BE}15$ exposure, the reductions were 15.92% for cv. Romanos and 14.45% for cv. Allegria. Plants under 0 UV - B_{BE} showed a significant increase in the net rate of photosynthesis and stomatal conductance (Table 2). The results of Kataria et al. (2013) were similar after exclusion of the UV-B.

It has been reported that UV-induced changes in stomatal conductance reduced CO_2 assimilation, because it affects the opening or closing of stomata through alterations in the stomatal aperture. It has been postulated that high fluences of UV-B either stimulated stomatal opening or stomatal closing in *Vicia faba,* depending on the metabolic state of the guard cell, and neither of these responses is readily reversed. High-UV-B acted on the guard cell aperture control and changed the mesophyll photosynthesis. High-UV-damaged the PSII in the guard cells, affecting photophosphorylation and hence ion transport, controlled osmotic solute flux, notably K^+, from guard cells and the resultant changes in guard cell turgor and stomatal aperture. The plasmalemma based enzyme complexes facilitate the solute fluxes leading to stomatal opening and closure (Jansen and Van

Den Noort, 2000). It is remarkable that UVR8 controls the stomatal closure by means of a mechanism involving both H_2O_2 and NO generation, which increased in UV-B-irradiated stomata, although stomatal closure required only NO (Tossi et al., 2014).

Stomatal density

Stomatal density was always higher on abaxial than on adaxial leaf surface of two cultivars. In both cultivars, both Am UV-B_{BE} and enhanced UV-B_{BE} 15 radiation increased the number of stomata on the adaxial or abaxial surfaces compared with the controls. The exposure of plants to UV-B_{BE} radiation in both cultivars increased the stomatal density, with values to follow the following pattern across radiation levels: stomatal density under UV-B_{BE} 15 > stomatal density under Am UV-B_{BE} > stomatal density 0 UV-B_{BE}. Compared with the zero UV-B controls, high UV-B treatment increased the number of stomata on the adaxial surfaces of cv. Romanos by 32.52% and of cv. Allegria by 23.74%. The corresponding increases on the abaxial surfaces were 13.00% for cv. Romanos and 11.96% for cv. Allegria (Table 3).

It has been reported that the UV-B enhancement included changes in the stomatal density, leaf area, leaf thickness, wax deposition, elongation of the stem and pattern of branching, in plant–pathogen and plant–predator interactions and gene expression as well as in the synthesis of secondary metabolites (Prado et al., 2012). In the present study, the increased stomatal density by UV-B radiation may have provided cotton cultivars with greater CO_2 concentrations, which will increase their photosynthetic rates. Leaf area and net photosynthetic rates in cotton were reduced by enhanced UV-B radiation (Zhao et al., 2004). Thus, the distribution of the number of stomata over a smaller leaf area surface in both cultivars, increased stomatal density. Indeed, it has been reported that the stomatal density and conductance affect the CO_2 uptake and, therefore, photosynthesis (Zheng and Van Labeke, 2017).

Stomatal length and width

There were no significant differences in stomatal length and stomatal width among UV-B levels (Table 3).

Flower characteristics

Bract length, petal length and anther number were significantly reduced by the ambient UV-B_{BE} and UV-B $_{BE}$15 treatments compared with greatest values for plants in the controls (Table 4). Throughout the sampling period, compared with the controls, the anther reductions in cv. Romanos were 9.36% (Am UV-B_{BE}) and 12.26% (UV-B_{BE} 15) and in cv. Allegria were 7.98% (Am UV-B_{BE}) and 10.93% (UV-B_{BE}15), respectively (Table 4). There were also no significant differences between the flowers exposed to ambient UV-B_{BE} and UV-B_{BE}15 radiation treatments in the staminal column length (Table 4).

Pollen germination

Compared to the controls, both Am UV-B_{BE} and enhanced UV-B_{BE} 15 radiation reduced the mean pollen germination over cultivars sampling. With respect to cultivars, pollen germination in cv. Allegria was on average higher than in cv. Romanos. The data showed significant reductions in pollen germination for both genotypes. The pollen germination reductions in cv. Romanos were 32.61% (Am UV-B_{BE}) and 45.68% (UV-B_{BE} 15) and in cv. Allegria were 32.85% (Am UV-B_{BE}) and 41.22% (UV-B_{BE} 15) (Table 4).

According to Llorens et al. (2015), pollen grains are shielded by bracts or petals in entomophilous plants. As a consequence of having constitutively greater levels of UV-B protective compounds, ovaries are better shielded against UV-B radiation than other floral parts. Pollen is the reproductive tissue most susceptible to UV-B, particularly during anther dehiscence and pollen tube penetration of the stigma. There is also a tendency in annual species to reduce fruit and/or seed production as UV-B doses increase.

Increased UV-B radiation reduced *in vitro* the rate of pollen germination and tube length as well as its ability to fertilize in the field. Oxygen species (O_2•− and H_2O_2) production increased with UV-B radiation and their ongoing accumulation resulted in lipid peroxidation and reduced antioxidant activity in maize (Wang et al., 2010).

He et al. (2007) observed that *Paulownia tomentosa* pollen exposed *in vitro* to UV-B radiation reduced pollen germination and tube growth, but also increased NO synthase activity and NO production in pollen grain and tube. UV-B radiation in maize pollen grains induced a significant increase in UV-B absorbing pigments (plants adaptation to complete their reproductive cycle) (Santos et al., 1998).

Seed cotton weight

Seed cotton weight was reduced by the UV-B, but the magnitude of the response was not similar across cultivars. Plant exposure to UV-B radiation resulted in reduced seed cotton weight compared with the controls, an effect that was more evident under the enhanced UV-B_{BE}15 level.

Compared to the 0 UV-B_{BE} level, the reductions in seed cotton weight under Am UV-B_{BE} exposure were 2.91% in cv. Romanos and 4.24% in cv. Allegria, while the decreases were 3.45% in cv. Romanos and 5.27% in cv.

Allegria under the enhanced UV-B_{BE}15 exposure (Table 5). Similarly, the supplemental UV-B irradiance declined significantly the unginned cotton yield (Gao et al., 2003). In addition, the exposure of plants to UV-B_{BE} radiation resulted in lower seed weight and the magnitude of the reduction was dependent on UV-B_{BE} level.

Lint quality traits

In comparison with the controls, fibre qualitative characteristics were reduced or increased by exposure to UV-B. The two cultivars also performed differently. Maximum reductions under UV-B_{BE}15 were observed on fibre strength 10.48% (Romanos), elongation 23.49% (Romanos), micronaire 9.57% (Romanos), maturity index 4.65% (Romanos), spinning consistency index 4.03% (Romanos), mean length 2.71% (Allegria), yellowness 11.87% (Romanos) and uniformity index 3.8% (Romanos). Also, compared with the controls, maximum increases under UV-B_{BE}15 were observed on fibre short index 18.01% (Romanos) and reflectance 10.3% (Allegria) (Tables 5 and 6).

Gao et al. (2003) found similar negative effects on cotton fibre quality under enhanced UV-B. Exposure to ambient UV-B radiation reduces the crops photosynthesis, growth, production of dry matter, yield and quality of grain (Gao et al., 2010). Changes in the yield and quality of wheat induced by increased UV-B throughout the whole growth stage (Yao et al., 2014). In addition, due to elevated UV-B, the seed quality of soybean cultivars was deteriorated (Choudhary and Agrawal, 2015). Under increased UV-B radiation, the protein content of maize grains was increased, but the content of oil and starch were not influenced (Yin and Wang, 2012). Furthermore, the most important flavor compounds of holy basil (*Ocimum sanctum* L) plants cultivated in the field after the biologically effective supplemental ultraviolet-B radiation treatment significantly increased (Kumari and Agrawal, 2011).

Conclusions

Both ambient UV-B_{BE} and enhanced UV-B_{BE} 15 irradiances significantly affected most of the cotton growth, physiological and fibre quality traits measured in the present study, with the higher UV-B dose generally having the strongest effect. Compared to the control, plants exposed to biologically effective UV-B radiation showed lower values in most traits, including pollen germination and fibre elongation, micronaire, spinning consistency index, and uniformity index. On the contrary, the values of stomatal density, short fibre index and fibre reflectance were increased due to the ambient and enhanced UV-B radiation compared to the control. There have been differences in cultivar response to UV-B_{BE} in several cases, suggesting differential genotypic sensitivity of cotton to increased levels of UV-B radiation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Brewbaker JL, Kwack BH (1963). The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany 50(9):859-865.

Caldwell MM (1971). Solar UV irradiation and the growth and development of higher plants. Photophysiology 6:131-177.

Choudhary KK, Agrawal SB (2015). Effect of elevated ultraviolet-B on four tropical soybean cultivars: quantitative and qualitative aspects with special emphasis on gas exchange, chlorophyll fluorescence, biomass and yield. Acta Physiologiae Plantarum 37(2):31.

Gao W, Schmoldt DL, Slusser JR (2010). UV Radiation in Global Climate Change, Measurements, Modelling and Effects on Ecosystems. Tsinghua University Press, Beijing.

Gao W, Zheng Y, Slusser JR, Heisler GM (2003). Impact of enhanced ultraviolet-B irradiance on cotton growth, development, yield, and qualities under field conditions. Agricultural and Forest Meteorology 120(1-4):241-248.

He JM, Bai XL, Wang RB, Cao B, She XP (2007). The involvement of nitric oxide in ultraviolet-b-inhibited pollen germination and tube growth of Paulownia tomentosa *in vitro*. Physiologia Plantarum 131(2):273-282.

Huang S, Dai Q, Peng S, Chavez AQ, Miranda ML, Visperas RM, Vergara BS (1997). Influence of supplemental ultraviolet-B on indoleacetic acid and calmodulin in the leaves of rice (*Oryza sativa* L.). Plant Growth Regulation, 21(1):59-64.

Jansen MA, Van Den Noort RE (2000). Ultraviolet-B radiation induces complex alterations in stomatal behaviour. Physiologia Plantarum 110(2):189-94.

Kakani VG, Reddy KR, Zhao D, Gao W (2004). Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. Physiologia Plantarum 121(2):250-257.

Kakani VG, Reddy KR, Zhao D, Mohammed AR (2003a). Effects of ultraviolet-B radiation on cotton (*Gossypium hirsutum* L.) morphology and anatomy. Annals of Botany 91(7):817-826.

Kakani VG, Reddy KR, Zhao D, Sailaja K (2003b). Field crop responses to ultraviolet-B radiation: a review. Agricultural and Forest Meteorology 120(1-4):191-218.

Kataria S, Guruprasad KN, Ahuja S, Singh B (2013). Enhancement of growth, photosynthetic performance and yield by exclusion of ambient UV components in C3 and C4 plants. Journal of Photochemistry and Photobiology B: Biology 127:140-152.

Kataria S, Jajoo A, Guruprasad KN (2014). Impact of increasing Ultraviolet-B (UV-B) radiation on photosynthetic processes. Journal of Photochemistry and Photobiology B: Biology 137:55-66.

Kumari R, Agrawal SB (2011). Comparative analysis of essential oil composition and oil containing glands in *Ocimum sanctum* L. (Holy basil) under ambient and supplemental level of UV-B through gas chromatography–mass spectrometry and scanning electron microscopy. Acta Physiologiae Plantarum 33(4):1093-1101

Lee JH (2016). UV-B signal transduction pathway in Arabidopsis. Journal of Plant Biology 59(3):223-230.

Llorens L, Badenes-Pérez FR, Julkunen-Tiitto R, Zidorn C, Fereres A, Jansen MA (2015). The role of UV-B radiation in plant sexual reproduction. Perspectives in Plant Ecology, Evolution and Systematics 17(3):243-254.

McKenzie RL, Aucamp PJ, Bais AF, Björn LO, Ilyas M, Madronich S (2011). Ozone depletion and climate change: impacts on UV radiation. Photochemical and Photobiological Sciences 10(2):182-198.

Mitsios IK, Toulios L, Charoulis A, Gatsios FA, Floras SA (2000). Soil survey and soil map of Thessaly University farm, in Velestinon area.

Zymel editions, Athens. (In Greek).
Mohammed AR, Tarpley L (2011). Morphological and physiological responses of nine southern US rice cultivars differing in their tolerance to enhanced ultraviolet-B radiation. Environmental and Experimental Botany 70(2-3):174-84.
Prado FE, Rosa M, Prado C, Podazza G, Interdonato R, González JA, Hilal M (2012). UV-B Radiation, its Effects and Defense Mechanisms in Terrestrial Plants. In. Parvaiz A, Prasad MNV (Eds.), Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change. New York, NY: Springer Science Business Media pp. 57-83.
Reddy KR, Kakani VG, Zhao D, Mohammed AR, Gao W (2003). Cotton responses to ultraviolet-B radiation: experimentation and algorithm development. Agricultural and Forest Meteorology 120(1-4):249-65.
Rowland FS (1990). Stratospheric ozone depletion by chlorofluorocarbons. Ambio 19(6-7):281-292.
Salama HM, Al Watban AA, Al-Fughom AT (2011). Effect of ultraviolet radiation on chlorophyll, carotenoid, protein and proline contents of some annual desert plants. Saudi Journal of Biological Sciences 18(1):79-86.
Santos A, Almeida JM, Santos I, Salema R (1998). Biochemical and ultrastructural changes in pollen of Zea mays L. grown under enhanced UV-B radiation. Annals of Botany 82(5):641-645.
Sarghein SH, Carapetian J, Khara J (2008). Effects of UV-radiation on photosynthetic pigments and UV absorbing compounds in *Capsicum longum* (L.). International Journal of Botany, pp. 486-490.
Tossi V, Lamattina L, Jenkins GI, Cassia RO (2014). Ultraviolet-B-induced stomatal closure in Arabidopsis is regulated by the UV resistance locus8 photoreceptor in a nitric oxide-dependent mechanism. Plant Physiology 164(4):2220-2230.
Vanhaelewyn L, Prinsen E, Van Der Straeten D, Vandenbussche F (2016). Hormone-controlled UV-B responses in plants. Journal of Experimental Botany 67(15):4469-4482.
Wang S, Xie B, Yin L, Duan L, Li Z, Egrinya Eneji A, Tsuji W, Tsunekawa A (2010). Increased UV-B radiation affects the viability, reactive oxygen species accumulation and antioxidant enzyme activities in maize (*Zea mays* L.) pollen. Photochemistry and Photobiology 86(1):110-116.
Wargent JJ, Jordan BR (2013). From ozone depletion to agriculture: understanding the role of UV radiation in sustainable crop production. New Phytologist 197(4):1058-1076.
Yao X, Chu J, He X, Si C (2014). Grain yield, starch, protein, and nutritional element concentrations of winter wheat exposed to enhanced UV-B during different growth stages. Journal of Cereal Science 60(1):31-36.
Yao X, Liu Q (2006). Changes in morphological, photosynthetic and physiological responses of Mono Maple seedlings to enhanced UV-B and to nitrogen addition. Plant Growth Regulation 50(2-3):165-177.
Yin LN, Wang SW (2012). Modulated increased UV-B radiation affects crop growth and grain yield and quality of maize in the field. Photosynthetica 50(4):595-601.
Zhao D, Reddy KR, Kakani VG, Mohammed AR, Read JJ, Gao W (2004). Leaf and canopy photosynthetic characteristics of cotton (*Gossypium hirsutum*) under elevated CO2 concentration and UV-B radiation. Journal of Plant Physiology 161(5):581-590.
Zheng L, Van Labeke MC (2017). Long-term effects of red-and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants. Frontiers in Plant Science 8:1-12.
Zhu PJ, Yang L (2015). Ambient UV-B radiation inhibits the growth and physiology of *Brassica napus* L. on the Qinghai-Tibetan plateau. Field Crops Research 171:79-85.

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Contribution of parkland agroforestry in supplying fuel wood and its main challenges in Tigray, Northern, Ethiopia

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Agroforestry is an aged practice in the Ethiopian farming systems of which parkland trees comprise the large part of agricultural landscapes. It is also the most dominant agroforestry practice in the semi-arid and sub-humid zones of Ethiopia. However, there is lack of research based evidence that shows the contribution of parkland agroforestry on fulfilling households' fuel wood demand and towards improving the smallholder farmers' livelihood. Hence, the main objective of this study was to assess the role of parkland agroforestry practice on fulfilling households' fuel wood demand, improving livelihood and to identify the main constraint. Primary data was collected from actual field measurement and questionnaire based face to face interview with randomly drawn 138 parkland agroforestry user and non-user. Guided field observations, interview with key informants and focused group discussion were also conducted. About 108.56 ton (79.2%) annual fuel wood consumption was harvested from the parkland trees; whereas the non-parkland agroforestry households were mainly dependent on the surrounding natural forests to meet their fuel wood demand. The Propensity Score Matching model result indicated that there was significant difference ($p<0.05$) among the parkland agroforestry introduced and non-introduced households on the time they spent to collect fuel wood and income. Parkland agroforestry plays a crucial role in the households' livelihood improvement (for example, income) and also to stabilize the pressure on local forests. However, the major challenges faced to improve the parkland agroforestry practice are farmland distance, free grazing, farmland size, general prohibition of fire wood selling, lack of farmers' awareness, lack of extension support and dry climatic condition. Therefore, to enhance the multiple benefits of the parkland agroforestry, the main constraints that hinder the sustainability of the parkland agroforestry should be addressed.

Key words: Agroforestry, fuel wood, livelihood, household, local forest, parkland.

INTRODUCTION

The problem of deforestation is much higher in East Africa than other parts of the continent (Kassie, 2015). The increasing populations of smallholder farmers in developing countries are the main driving force for deforestation and land degradation meant for intended benefits such as agricultural expansion, fuel wood and

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fodder (Liman, 2015).

In Ethiopia, the steadily growing population pressure and the need for agricultural expansion and fuel wood consumption increased exploitation of forest resources which can ultimately lead to unsustainability and depletion of the total forest area (Fekadu, 2015). In the country, dependence of urban dwellers on surrounding rural areas for fuel wood consumption for long period of time and the associated population growth has aggravated the level of deforestation and forest degradation especially in recent times (Gebreegziabher et al., 2012). Agroforestry can help to enhance fuel wood availability, sustainably and to mitigate deforestation (Ernstberger, 2017) and climate change.

Parklands are scattered trees in croplands. They are a very common type of agroforestry system in the tropics and characterized by well-known scattered trees on cultivated and recently fallowed lands (Raj and Lal, 2014) .Such a system of integrating tree species into farmlands provide productive, protective and socio-economic as well as cultural roles that can improve the livelihoods of the society, particularly for smallholder farmers in the developing world suffering from hunger, poverty, and malnutrition (Raj and Lal, 2014).

Parkland agroforestry is a system practiced by many local populations, and is very important for food security, microclimate amelioration, income generation and environmental protection. It is found at different corners of the world, primarily in the semi-arid and sub-humid zones of Africa (Boffa, 1999). Kindeya (2004) reported that agroforestry practice is an aged practice in the Ethiopian farming systems, of which parkland trees comprise the large part of agricultural landscapes and it is also the most dominant agroforestry practice in the semi-arid and sub-humid zones of Ethiopia. Parkland trees are used to satisfy the needs and demands of the households. Some of the major roles they play includes: heating, cooking, household utensils, cultural values, provision of pollen and nectar for honey production, construction of houses and handles of farm implements (Negash, 2007), traditional medicines (CIFOR, 2005), economic benefits, fodder values, employment opportunities as well as contribution to regional and national economy (Abebe, 2005). Parkland agroforestry is a major source of fire wood, which contributes significantly to household income and appears to be important for local economies (FAO, 2013).

In the study area, many farmers practiced parkland agroforestry (PLAF), but still there is lack of research based evidence. This investigation shows clear evidence about the contributions of PLF towards improving household's livelihood and its major constraints to sustain such function.

MATERIALS AND METHODS

Description of the study area

The research was conducted in Hawzen district of eastern Tigray Northern Ethiopia. Hawzein district is geographically located at 13° 47 ˈ 30ˈˈ to 14° 9ˈ 25ˈˈ North latitude and 39° 11 ˈ 40 ˈˈ to 39° 33 ˈ 20 ˈˈEast longitudes (Figure 1). From the total 80949.8ha area of the district, about 17687 ha (21.85%) were farmland with approximately 0.53 ha land holding size per household. Varying land forms, ranging from plain and semi plain agricultural areas to steep slope escarpments are dominated .Gheralta Mountains are the main features of steep slope escarpment of the district (HWEPLAU, 2017).

According to the HWFED (2017a, b) total population size of the district is about 127,265 with 2875 household heads, of which 93.4% lives in rural Kebeles. The average family size is about 4 persons per household. The district is the second most densely populated in Eastern zone (about 67.8 people per square kilometere), next to Atsbi-wenberta district, which is above the zone's and the region's rural areas average population density, 61.6 and 55.5 people per square kilometere respectively (Kidanemariam, 2011).

Research approach and design

Fuel wood consumption of the study area was quantified with interviews, combined with precise field measurements (Jensen, 1995). Based on these assumptions and nature of the enquiry, the combinations of both quantitative and qualitative approaches were also used to obtain the required data. By applying quantitative tools, attempt was made to address the existing situations in relation to the amount of fuel wood generated from the parkland agroforestry system. Opinions of the respondents on the benefits and constraints of the parkland agroforestry system were also collected.

Data sources and methods of data collection

The required data was collected from primary and secondary data sources. The primary data were collected through actual field measurement of each household's fuel wood consumption, household survey based on face to face interview using semi structured questionnaires, focus group discussion (FGDs) and key informants interview. Secondary sources of data were also collected from the agricultural office of the district, government documents, and articles of scholarly journals, book chapters, and newspapers.

Sampling technique and sample size determination

Purposive and Simple Random Sampling (SRS) techniques were employed. In the first stage, the study site (Freweyni Kebele) was selected purposely based on its relative abundance of the parkland trees on farmlands. In the second stage, households were stratified into parkland agroforestry users and non-users; then from 1,192 farmer households and 83 parkland agroforestry user household heads were identified as a sample frame. The simplified formula employed to determine the sample unit households were:

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

n=sample size, N=total population, e=level of precision (0.05)

$$n = 83/1+83(0.05)^2 = 69$$

Then, 69 parkland agroforestry user households were taken, using the SRS technique for sampling. Therefore, 69 parkland agroforestry introduced households were selected randomly using the lottery system. In the same area, another 69 households who have farmlands but without parkland tree was identified and all members of this group were directly taken to use as sampling unit

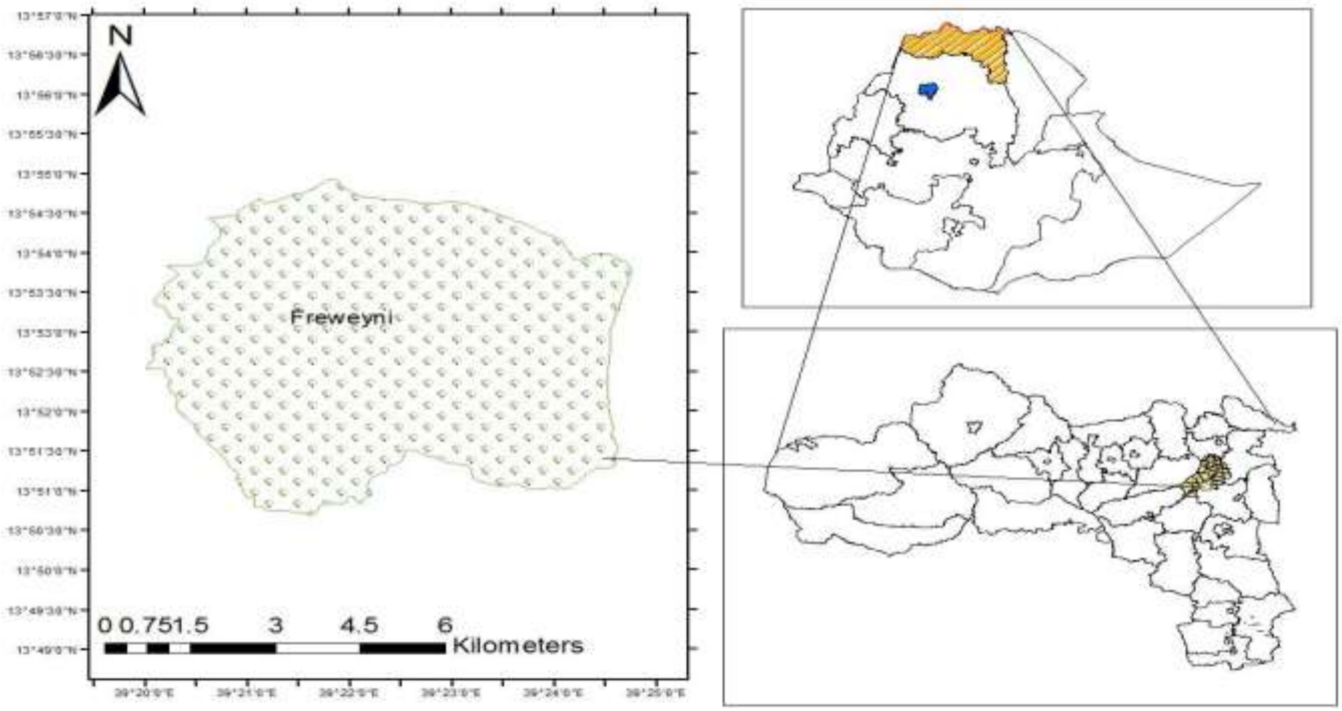

Figure 1. Map of the study area.

as they were limited in number. In this study, equal weight was given for both (parkland agro forestry user and non-user) households in order to see contribution of the parkland agro forestry practice on the farmers livelihood.

Data analysis

Data were organized in Excel spread sheets and analyzed using SPSS version 20 software package. To reduce bias due to confounding variables, Propensity Score Matching (PSM) model was also used to analyze the contribution of PLAF on household's livelihood improvement.

RESULTS AND DISCUSSION

Parkland agro forestry and livelihood of the households

The actual households fuel consumption measurement result showed that from the total parkland agroforestry users, about 137.3 ton (94.9%), was woody biomass and the rest 7.35 ton (5.1%) was non woody biomass; especially cattle dung and crop residues. From the total annual household fuel consumption, 108.65 ton (75.03%) was harvested from the parkland trees found on farmlands. The Propensity Score Matching model (PSM) result showed that the parkland agroforestry user households were spending a mean of 1.56 h per week to collect fuel wood; whereas the households that have not practiced parkland agroforestry spent a mean time of 3.4 hours per week (Table 1). This indicates that each parkland agroforestry user households were required to assign on average of about 74.9 h per year for fuel wood collection; whereas the non- parkland agroforestry user households were required to assign about 163.2 h per year for fuel wood collection, which is more than 2 folds higher than the parkland agroforestry user households. Kassie (2015) reported a similar result that, to collect 30 kg (one bundle) of fuel wood from the natural forests and shrub lands in Maytemeko watershed (in Amhara National Regional State, Ethiopia) required about 4 h; while for the households who used their own farmland trees, it was about 1 h to collect the same amount of fuel wood.

The time required to collect fuel wood from the natural forest and shrub lands may increase with deforestation, since the forest cover will be pushed up to the marginal areas. This showed that the tasks of fuel wood collection from the surrounding common areas are time consuming and it is proportionally correlated with distance of the site in which the fire wood is found. In line with the present result, Palmer (2009) reported that fuel wood scarcity has a positive effect on labor inputs to fuel wood collection from common areas.

The annual mean income of the parkland agroforestry introduced household in the year 2016/2017 was about 25915 birr (equivalent to 863 USD) and this was greater than the mean annual income of 21684.4 Birr (equivalent to 722 USD) earned in the same year by the households who was not introduced parkland agroforestry (Table 1)

Table 1. Contribution of parkland agroforestry inhouseholds' time saving and income diversification in the year 2016/2017 in Hawzeien district, Northern Ethiopia.

Variable	HHs time spent to collect fire wood (Hours/week)		
	Mean ±SD	**Minimum**	**Maximum**
PLAF users	1.56, 0.69	1	3
Non users	3.4, 1.38	1	5
Total	4.96, 2.07	2	8
	HHs Income (Birr/year)		
PLAF users	25915, 15785.77	1700	66338
Non users	21684.4, 13812.65	2888	55480
Total	47599.4, 29598.24	4588	121818

PLAF is parkland agroforestry; SD is standard deviation, 1USD= 30 Ethiopian Birr.

Table 2. Propensity score matching regression result.

Outcome variable: Hours spent	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
Treated	-1.84058	0.1867261	-9.86	0.000	-2.209842	-1.471318
_cons	3.405797	0.1320353	25.79	0.000	3.144689	3.666905
Outcome variable:INC	**Coef.**	**Std.Err.**	**t**	**P>|t|**	**[95% Conf.**	**Interval]**
Treated	4230.565	2525.176	1.68	0.096	-763.1243	9224.255
_cons	21684.41	1785.569	12.14	0.000	18153.33	25215.48

Hours spent= hours spent, INC=income.

though there were some uncontrolled factors that can influence the income of each households.

The PSM model result showed that there is statistically significant difference (P< 0.05) among the parkland agroforestry user and non-user households on the time they were spent to collect fuel wood from the different sources. The parkland agroforestry user households saved 1.84 h per week than the non-parkland agroforestry households (Table 2). This implies that the parkland agroforestry introduced households have more additional time (88.3 h) per year to assign to other income generating activities and attending regular schools than the non-user households.

Regarding households' total annual income, variations were observed among the annual income of the parkland agroforestry introduced and non-introduced households. The total annual income of the parkland agroforestry user households was higher than the non-parkland agroforestry user households; however, the variation was not significantly (P > 0.05) different (Table 2).

Parkland agroforestry and fuel wood collection

From the parkland agro forestry introduced households (n=69), the responsibility of harvesting and transporting fuel wood for the whole family, only male and only female were 62.3, 29 and 8.7% respectively (Figure 2). Majority of the household heads are of the opinion that the big trees require participation from all family members, initially to prune some selected branches of a tree which is commonly and traditionally performed by the male family members and transporting task also left mostly for all family members after the foliages and smaller part of branches are consumed by livestock. This indicates that the trees grown in the farm lands were important not only to provide fire wood and other products but also to minimize the work load of women and children by creating opportunities for labor division among all family members to harvest fuel wood and this in turn could have impact on the families' socio-economic developments. FAO (2013) pointed out similar result that combines agricultural crop and fuel wood production through agroforestry to save woodland trees and frees up labor, especially for women, who traditionally collect fire wood. On the other hand, the survey result derived from the non-parkland agroforestry users showed that fuel wood collection responsibility in these households was inclined to same particular family members rather than distributing it to all of the family members. 71% of the non-parkland

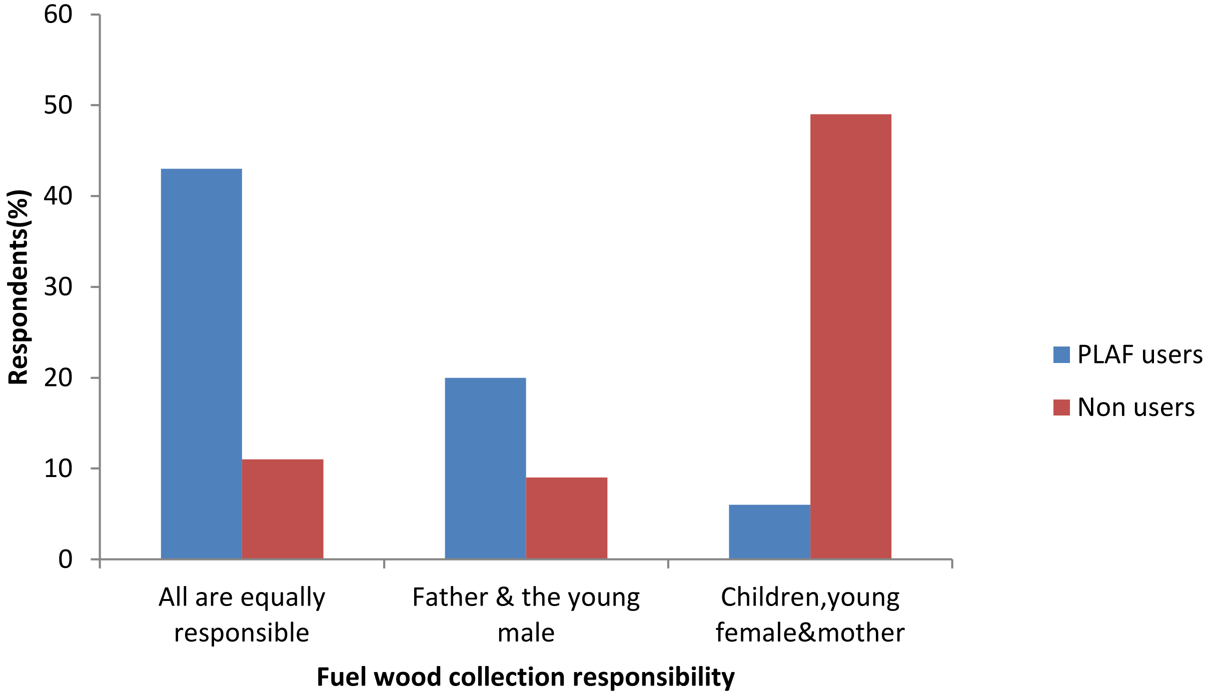

Figure 2. Fuel wood collection responsibility among family members of the PLAF introduced and non-introduced households in Hawezien district, Northern Ethiopia.

Table 3. Parkland trees found on each household farm plots and species composition in Hawezien district, northern Ethiopia.

Tree species	No. of trees	Percent	No. of HHs planted
F.albida	652	80.8	69
A. abyssinica	118	14.6	53
C. africana	19	2.35	16
E. camaldulensis	12	1.5	10
Others	6	0.74	5
Total	807	100	

agroforestry introduced households (n=69) affirmed that the fire wood collection responsibility in their family mostly rest on the shoulder of children, young female and the mothers. About 15.94% of the respondents also replied that all family members had equal responsibility on fuel wood gathering activities and only 13% opined that the father and the young male were the most responsible to collect fuel wood from the surrounding forest and non-forest areas (Figure 2).

This shows that the children and women found in the households who have no parkland trees on their farmlands took the responsibility of fuel wood collection from the local forest and shrub lands. Kassie (2015) reported similar result that fuel wood collection responsibility from the nearby forest and shrub lands is performed by mothers and daughters.

Common parkland tree species on the farmlands of the study area

All the mature parkland trees found in the farm plots of the parkland agroforestry introduced households (n=69) was counted and a total of 807 mature scattered trees were recorded in the Fireweini village. Thus, households had owned different number of trees with a minimum of 2 trees (in 2 farmers) to a maximum of 27 trees (in 1 farmer) and on average, there was about 11.7 trees per household heads and 16.3 trees per ha.

It was also shown that *Faidherbia albida* was the most dominant parkland tree and it was the only tree species found under all of the parkland agroforestry practice households accounting to about 80.8% (Table 3). The main purpose of keeping this tree species by all of the

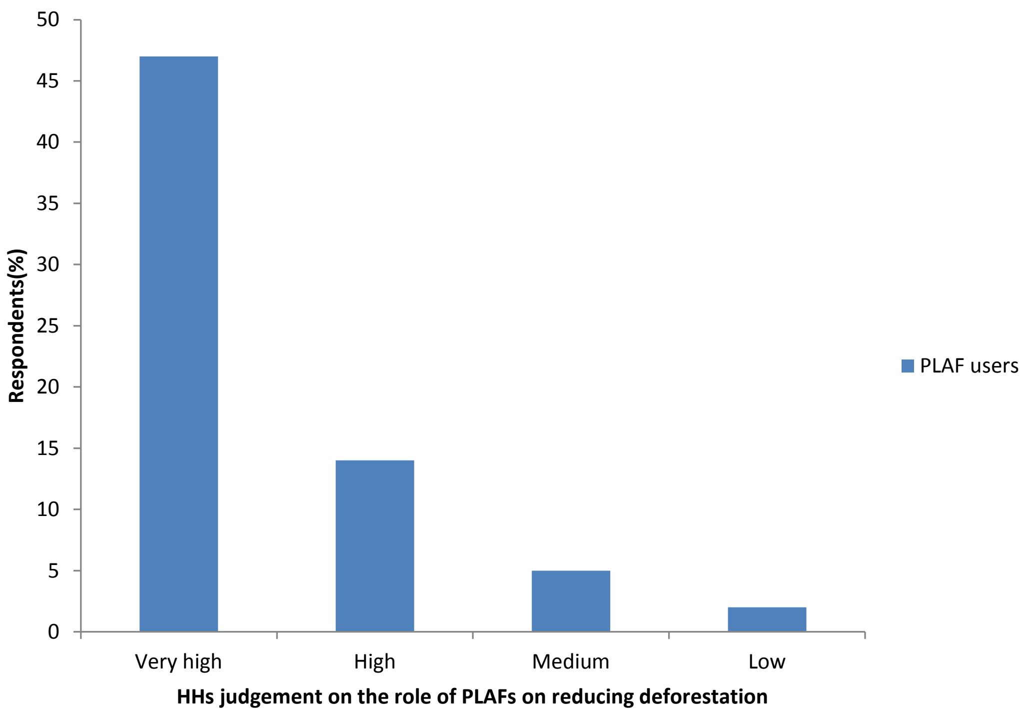

Figure 3. The PLAF user households' judgment on the role of PLAF on reducing deforestation in Hawzen district, northern Ethiopia.

households and in a dominant number was mainly for its better fodder value, complementary nature of the tree with growing annual food crops, fencing service and fuel wood production. Due to this reason, *F. albida* had been the most dominant parkland tree species; followed by *Ampelocissus abyssinica*, 14.6%; *C. Africana* 2.35%; *E. camaldulensis*1.5%; and 0.74% was covered by other tree species like *Oleaeuropaea* (Table 3).

Farmers' judgment on the role of PLAF to minimize pressure on local forests

From parkland agroforestry user households (n= 69), majority (68.1%) of the household heads responded that the parkland trees found in their village had very high contribution in stabilizing the pressure on the local natural forest and non-forest areas by providing fuel wood/charcoal, fodder and other demands. These were even better than any other available technologies provided in the study area. Such practices are mainly introduced to minimize deforestation. Similarly, 20.3% of the respondents also replied that contribution of the parkland agroforestry in reducing the pressure on the local forests was high, 7.2% also said it was medium while the rest 3% of the respondents said low (Figure 3).
However, no respondent believed that parkland agroforestry had very low/ no contribution on reducing the pressure on the surrounding common forests. This idea was supported by Duguma (2010) who reported that agroforestry practice could be a promising option to solve environmental problems such as deforestation and to improve household food security by diversifying farm products and reducing vulnerability for seasonal food and fodder shortages.

Challenges of parkland agroforestry

The result indicated that 45.16% of the household heads (n=63), pointed out that the distance from home to farm plots, free grazing, shortage of farmland, prohibition of fire wood/charcoal selling and lack of awareness were considered as major limiting factors to have improved parkland agroforestry. About 24.2% of the respondents believed that farmland distance and the limited farmland size were identified as the major limiting factors. About 9.7% of the respondents also replied that small size of farmland were the only constraint to improve the parkland agroforestry practice, 8% said that free grazing and lack of awareness was the primary limiting factors for them and 4.8% believed that labor demanding nature of the parkland trees, lack of government support and shortage of farmland were main constraints; whereas 3.2% said that farmland distance, free grazing, general prohibition of fire wood/charcoal selling and weak local forest protection performance were major constraints (Figure 3). Some respondents (1.6%) also believe that lack of

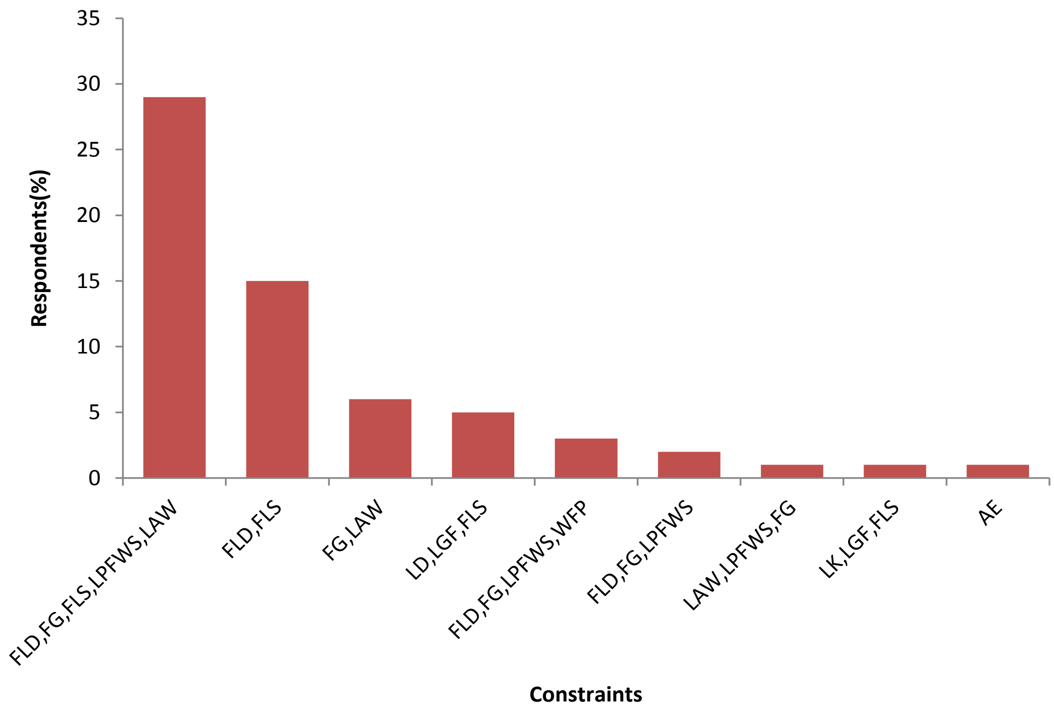

Figure 4. Major challenges pointed out by the Parkland Agroforestry user HHs to maximize beneficial trees on their farmlands in Hawezien, Northern Ethiopia. FLD= farmland distance, FG= free grazing, FLS= farmland size, = LPFWS=legal prohibition of fuel wood selling, LAW= lack of awareness, LD= labour demand, LGF= lack of government focus, WFP= weak forest protection performance, LK= lack of knowledge for better tree species selection mechanism, AE= agronomic effect.

awareness, general prohibition of fire wood/charcoal selling and uncontrolled grazing were main limiting factors. Some others (1.6%) also believe that lack of better tree species identification mechanism, absence of government support and small farmland size was primary constraints. Also some others (1.6%) are of the opinion that the negative effect of trees on growing field crops and being obstacle for farming activities were the main challenging factors to improve the parkland agroforestry (Figure 3).

The result from FGD (Focal Group Discussion) and from the key informants' interview also indicated that, free grazing, lack of government focus on the farmland trees improvement, farmland distance and lack of farmers' awareness were primary constraints to the PLAF improvement in the study area. The uncontrolled grazing of animals on farmlands after the field crops are harvested (dry season) was one of the most limiting factor in the study area, including destruction of the protected and new planted areas. In line with this idea, Mekonnen and Kohlin (2008) was reported that free grazing on agricultural landscape was the major constraint for tree planting and maintenance in central Ethiopia. In general, the distance of farm plots from home and fragmented farmland size is among the main constraints to maximize multipurpose trees (MPTs) on farmlands; to which majority of the PLAF user households were agreed upon. This implies that as distance of farm plots are increasing, farmers' willingness to plant and protect trees are being decreased. This is mainly due to management problems on the farm, since trees require continuous care and close management efforts. It was due to this reason that more trees are observed on the farm plots found near the residential areas than the distant plots in the study area. Therefore, farmland distance and free grazing are strongly interrelated factors that have been major challenges to plant trees on distant farm plots. In the nearby plots, it was easier to grow and manage trees relatively since household members can prohibit animals from browsing after the field crops were harvested. In agreement with this result, Predo and Francisco (2012) have reported that the relative distance from home was negatively affect farmers interest to grow trees in Philippines.

The result from the focus group discussion also indicated that there is widespread problem of theft of tree products, especially animal fodder and fire wood when planted far away from living home (Figure 4).

Non-parkland agroforestry households

Despite most farmers in the study area was integrated selective trees with their farmlands especially on the plots found near their home, there were also some households who had no trees in their farmlands. Though these households had not integrated trees into their farmlands, majority of them believe that integrating selective trees on farmlands is advantageous. As a result, 91.3% (n=69) opines that integrating selective perennial trees in farmlands are beneficial alternative for them; while the rest 8.7% believed that though the parkland trees can contribute to farmers, the negative effect of the trees on the growing annual food crops and farming activities are out weighted than its benefits, hence they were reluctant to integrate trees on their farmlands.

According to the respondents introducing perennial trees into farm lands can damage annual food crops in different forms, including shading effect and becomes harbor for field crop attacking birds, weeds and becomes obstacle for agronomic activities. In agreement with this result, FAO (2013) identified a general problem of farmers' perception on trees as incompatible with their farming activities and may not benefit from planting and managing trees as well as shrubs on their farm plots. This can also influence the introduction and implementation of agroforestry practice in wider areas.

There were some relevant questions provided to the non-parkland introduced households (n= 63) to know the main reasons they remained without introducing trees in their farmlands if they were aware of the advantages of integrating perennial trees on farmlands.

Response from most household heads mainly revolved on a single factor that limits them to grow valuable trees on their farmlands. About 61.9% of the respondents pointed out that, the dry condition of the area was the most limiting factor for them to retain beneficial trees in their farmlands and 15.9% replied that dry condition, availability of firewood in near areas until the near past years and lack of farmers awareness were the main constraints on trees growth on farmlands. However, 14.3% believed that the dry condition and availability of firewood in nearby areas were the main limiting factors to grow trees on farmlands. From the respondents, about 3.2% responded that dry condition and absence of better tree species are main the constraints. The rest, 3.2%, of the household heads said that the dry condition, the negative effect of trees on the growing annual food crops, easily accessibility of fire wood until and lack of farmers awareness are the main reasons farmers do not introduce MPTs on their farmlands.

Conclusion

Parkland agroforestry (PLAF) is major source of fuel wood for households and rely mainly on their own farmland trees rather than going to collect fuel wood from the local forests and shrub lands. PLAF played an important role in fulfilling households' fuel wood demand and thereby reducing the pressure on the local forests and shrub lands. Furthermore, the PLAF introduced households earns multi-faced benefits and services drawn from the parkland trees. Significantly reducing the time that would be required to collect fuel wood from outside farmlands, helping to share the fuel wood collection responsibility among all household members more evenly and improving household income are among the major benefits of the PLAF in the area. The household heads also perceived that the practice of PLAF based on indigenous trees species is the most preferred type of agroforestry mainly for its relative high biomass production per tree, high survival capacity and no required to assign particular area (land use efficiency). Despite its potential to deliver socio-economic and environmental benefits, farm plot distance from home, free grazing, farmland size, the general prohibition of fire wood/charcoal selling, lack of extension support and low level of farmers' awareness are among the major constraints influencing households to improve the existing PLAF practices. The dry /unfavorable condition, accessibility of fire wood from nearby areas and lack of farmers' awareness were the critical limiting factors for the non-PLAF introduced households to integrate beneficial trees on their farmlands.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abebe T (2005). Diversity in Homegraden Agroforestry Systems of Southern Ethiopia. https://edepot.wur.nl/116419

Boffa JM (1999). Agroforestry Parklands in Sub-Saharan Africa. FAO Conservation.

Center for International Forestry Research (CIFOR) (2005). Workshop (p. 26). Nairobi: ICRAF Headquarters.

Duguma LA 2010). Agroforestry as a tool for integrated land resources management: improving farmers' livelihood, providing wood products and minimizing forest encroachment: University of Natural Resource and Life Sciences, Vienna, Austria.

Ernstberger J (2017). Perceived multifunctionality of agroforestry trees in Northern Ethiopia 1(1):82-94.

FAO (2013). Advancing Agroforestry on the Policy Agenda. A guide for decision-makers, by Buttoud G, in collaboration with Ajayi O, Detlefsen G, Place F, Torquebiau E, Agroforestry Working Paper no. 1. Food and Agriculture Organization of the United Nations (FAO), Rome.37 pp.

Fekadu G (2015). Review of Forest loss and climate change in Ethiopia. Research Journal of Agriculture and Environmental Management 4(5):216-224.

Gebreegziabher Z, Mekonnen A, Kassie M, Kohlin G (2012). Urban energy transition and technology adoption: The case of Tigrai, Northern Ethiopia. Energy Economics *34*(2):410-418.

Hawzen Wereda Environmental Protection, Land Administration and Use office (HWEPLAU) (2017). Profile of farmed and non farmland areas of the District.

Hawzen Wereda Finance and Economic Development office (HWFED) (2017). Profile of Hawzen district annual population projection document.

Jensen AM (1995). Wood fuel productivity of agroforestry systems in Asia. Regional Wood Energy Development Programmers in Asia, Bangkok, Thailand.

Kassie MD (2015). Land Use/Cover Changes and the Role of

Agroforestry Practices in Reducing Deforestation and Improving Livelihoods of Smallholders in Maytemeko Watershed, Northwest Ethiopia. A PhD Thesis, University of Natural Resources and Life Sciences, Vienna, Austria.

Kidanemariam A (2011). Poverty and farmers' attitude towards risk: evidence from Hawzenwereda, Tigray, Ethiopia. Doctoral dissertation, Addis Ababa University

Kindeya G (2004). Dryland Agroforestry Strategy for Ethiopia. In The Drylands Agroforestry Workshop 1st-3rd September 2004. ICRAF Headquarters, Nairobi-Kenya (2004).

Liman IH (2015). Agroforestry Parklands of the Sudan Savanna in the Context of Climate Change. Firewood Energy in North-western Benin, West Africa. An M. Sc Thesis Presented to the Department of Geography. P. 63.

Mekonnen A, Kohlin G (2008). Biomass Fuel Consumption and Dung Use as Manure: evidence from rural households in Amhara region of Ethiopia. Environment for Development Discussion Paper, pp. 8-17.

Negash M (2007). Trees Management and Livelihoods in Gedeo's Agroforests, Ethiopia. Forests, Trees and Livelihoods 17(2):157-168. https://doi.org/10.1080/14728028.2007.9752591

Palmer C (2009). Fuel wood scarcity, energy substitution, and rural livelihoods in Namibia. Environment and Development Economics 14(6):343-347.

Predo C, Francisco H (2012). Tree growing objectives of smallholder farmers in Claveria, Northern Mindanao, Philippines. Journal of Environmental Science and Management15 (1):72-85.

Raj JA, Lal BS (2014). Agroforestry Theory and Practices. Jodhpur: Scientific Publishers.

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