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Full Length Research Paper

Genetic variability in a quality protein maize (QPM) population under double viral infections

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The type of gene action conferring combined resistance to maize streak virus (MSV) and maize stripe virus (MStV) was investigated in a Quality Protein Maize (QPM) population, ART/98/SW6-OB. Full-sib and half-sib progenies generated using North Carolina Design I mating scheme were evaluated in 2015 and 2016 in two locations endemic to the viral diseases in Nigeria. Serological study was also conducted on the progenies to validate the disease scores on the field. Significant mean square of genotypes was observed for MSV severity, MStV incidence and severity, plant height and days to flowering, while mean square of environment by females in males' interaction was significant for grain yield and MSV incidence. Estimates of additive variances were larger than dominance variance for grain yield and MSV severity with moderate to high narrow-sense heritability estimates. This indicates that there is adequate genetic variability for improving grain yield and resistance to MSV in the maize population. The mean titre values of MSV and MStV for the progenies were significantly higher than the healthy control. MSV and MStV incidence were negatively correlated to days to 50% silking. Titre values were negatively correlated with grain yield but positively correlated with MSV and MStV incidence. Recurrent selection method that capitalizes on both additive and dominance variances would be effective in improving the population for grain yield and resistance to MSV and MStV diseases.

Key words: Correlation, gene action, maize streak virus, maize stripe virus, resistance, North Carolina design.

INTRODUCTION

The development of quality protein maize (QPM) which contains twice the levels of tryptophan and lysine in most normal endosperm maize has brought a great hope for human and animal nutrition (Akande and Lamidi, 2006). Despite this good attribute, QPM varieties are threatened by downy mildew and Maize Streak Virus (MSV) diseases among other constraints (Mariote, 2007).

Maize (*Zea mays* L.) has been reported to be a natural host for more than 30 viruses (Lapierre and Signoret, 2004). Maize stripe virus (MStV) and MSV are two of the biotic factors responsible for reduced yield in maize in Africa (Shepherd et al., 2010). MSV, a geminivirus, indigenous of Africa, is transmitted by at least eleven *Cicadulina* species with C. *mbila* as the main vector. The symptoms are characterized by broken to almost continuous chlorotic stripes centered on tertiary leaf veins

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(Pinner et al., 1988).

Maize plants infected within the first three weeks after emergence become severely stunted, producing abnormal cobs or giving no yield at all (Pinner et al., 1988). MStV is a tenuivirus group transmitted by the maize delphacid, *Peregrinus maidis* (Ashmead) (Homoptera: Delphacidae) in a persistent-propagative manner (Tsai and Brown, 1994). MStV is also transmitted through the egg of the planthopper vector (Tsai and Zitter, 1982). Initial symptoms of MStV are fine chlorotic stripings between leaf veins which later develop into continuous chlorotic stripes of varying width and intensity (Tsai, 1975). Young plants infected at the early growth stage often are stunted with twisted whorl leaves (Tsai, 1975). Both MSV and MStV have been reported to cause up to 30 to 100% reduction in maize yield (Alegbejo et al., 2002; Roca De Doyle et al., 2007).

Although integrated pest management (1PM) has been recommended as a viable option of control, the most appropriate, environmentally safe and economically viable method to minimize damage caused by maize viruses is host plant resistance (Thottappilly et al., 1993; Alegbejo et al., 2002; Lagat et al., 2008).

In 1975, researchers at the International Institute of Tropical Agriculture (IITA), Nigeria detected resistance to MSV in cv. Tropical Zea Yellow (TZY), after it was then improved through mass selection and transferred to the most productive varieties (Soto et al., 1982). Although Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and IITA have worked extensively on breeding for resistance to MSV, global climate change and favorable temperatures for the planthopper vector (*Laodelphax steriatellus*) may have exacerbated the problem.

Disease diagnosis based on symptoms on the plants on the field is unreliable because different viruses may cause similar symptoms and different symptoms may be induced by one virus. Therefore, use of Enzyme-linked Immunosorbent Assay (ELISA), a serological-based technique is important in validating the observations recorded on the field (Kumar, 2009; Sharma and Misra, 2011).

Knowledge of the type of gene action conferring resistance is important in improving crop populations for resistance to the diseases. This enables the breeder to know the type of selection method to adopt and determine the extent of improvement attainable in a breeding program. Estimates of heritability and genetic advance help to predict response from selection (Holland et al., 2003).

Conflicting results have so far been reported on gene actions conferring resistance to MSV. Engelbrecht (1975) found that five dominant genes were involved in resistance to MSV, but Kim et al. (1989) reported that resistance in inbred IB32 was quantitatively inherited through additive gene action of two or three major genes and some modifiers.

DeVries (ISAAA, 1999) pointed out that MSV is known to be controlled by one major gene with several modifiers. Asea (2005) reported mostly dominance gene action for MSV. Lorroki (2009) reported that MSV resistance is controlled by additive gene effects with dominance x dominance epistatic interaction. Dintinger et al. (2005) reported that resistance to MStV is quantitative. This study therefore aims at investigating the type of gene action conferring combined resistance to both MSV and MStV in a QPM population with a view of improving it for dual resistance to both viral diseases.

MATERIALS AND METHODS

Generation of progenies

The maize population used in this study was a QPM population, ART/98/SW6-OB. It is a flint-dent, intermediate maturing, and white-grained maize population adapted to the forest zone. 200 non-inbred (S_0) plants were randomly selected from the maize population and used to produce both full- and half-sib progenies using the North Carolina Design I (NCD I) mating scheme of Comstock and Robinson (1952). Forty individual S_0 plants designated as male parents were each crossed to four different individuals designated as female to produce one hundred and sixty progenies for evaluation. To enhance flower synchronization, male parents were planted 3 days after female parents. To reduce replication size and increase precision of the experiment, the individuals were grouped into sets by males. Five sets of eight males each or 32 full-sib progenies were considered for the experiment.

Field evaluation

The 160 progenies were evaluated under natural infection by MSV and MStV in 2015 and 2016 at Ilora (Lat. 7^o 81'N, Long.3^o 82'E) in the transition zone between rain forest and derived savanna and Ikenne (Lat. 6^o 54'N, Long.03^o 42'E) in the humid forest both in Southwestern zone of Nigeria, a zone well known to be endemic to various maize diseases in Nigeria. The evaluations in both years were carried out towards the late season (July) when viral infections reach the peak (Fajemisin, 2003). A randomized incomplete block design with three replications was used for evaluation in each location. A single row plot of 3m length and plant spacing of 0.75m between rows and 0.25m within rows was used. Two seeds were sown per hole and later thinned to one plant per hill at three weeks after planting (WAP) to get a maximum of 13 plants per plot and a plant density of 53,333 plants ha^{-1}. Other cultural practices carried out at both location included weeding and fertilizer application. Paraquat and primextra herbicide were sprayed a day after planting at the rate of 3l/ha. One hand weeding was done at four weeks after planting, while supplementary herbicide spray was done at flowering using paraquat alone. A basal application of compound fertilizer (NPK 20:10:10) was applied at 2WAP at a rate of 60kg N/ha and Urea at 6WAP at 60kg N/ha for optimum plant growth (Oloyede-Kamiyo, 2013).

Detection of MSV and MStV using ELISA technique

Symptomatic and asymptomatic leaf samples were collected from the 160 maize progenies before tasseling. Double antibody sandwich (DAS) ELISA was used to detect the presence of MSV

Table 1. General form of analysis of variance of North Carolina Design I experiments pooled over sets and over environments.

Source of variation	df	Mean squares	Expected mean squares
Environment (e)	e-1	-	-
Rep/e	e(r-1)	-	-
Set/Rep/e	er(s-1)	-	-
Males/Sets	s(m-1)	M5	σ^2+ rσ^2ef/m + rfσ^2em+ reσ^2 f/m + refσ^2m
Females/Males/Sets	ms(f-1)	M4	σ^2+ rσ^2ef/m + reσ^2 f/m
E x Males/Sets	(e-1)s(m-1)	M3	σ^2 + rσ^2ef/m + rfσ^2 em
E x Females/Males/Sets	(e-1)ms(f-1)	M2	σ^2+ rσ^2ef/m
Pooled error	es(r-1)(mf-1)	M1	σ^2
Total	esrmf-1	-	-

df: degree of freedom r = number of replications; f = number of females per male; s = number of sets; e = number of environments; m = number of males in set; σ^2 = variance due to environment; σ^2m = variance due to male; σ^2f/m= variance due to females within male; σ^2em = variance due to interaction of male with environment; σ^2ef/m = variance due to interaction of females within male with environment.

and MStV in the progenies generated. The antibodies to the two viruses were obtained from Agdia-Bioford Inc, Elkhart, Indiana, USA. For each virus, microtitre wells of ELISA plates were loaded with 100 µl of capture antibody and the plates incubated for 4 h at room temperature (25^{o}C±1.0). Plates were washed three times with 1X phosphate buffer saline-tween 20 (PBST). Approximately, 1 g of leaf sample was grinded in 1 ml of Agdia's general extract buffer (GEB). Extracted samples were coated into the duplicate wells of the microtitre plates at 100 µl per well. Absorbance values were read at 405 nm using a Microtitre Plate Reader (Biotek, ELx800). Samples were considered positive when the values of the test sample were twice the mean value of the sap of healthy plant or negative controls.

Data collection

Data collected at both locations included days to 50% silking estimated as days from planting to the day when 50% of the plants in a plot develop silk. Plant and ear height were measured from five competitive plants per plot as distance from ground level to the base of the tassel, and to the node bearing the first ear respectively. Husk cover rating was on scale of 1 to 9, 1 representing tight and long husk cover, while 9 represents short, very loose and opened husk. Ear aspect was also rated on scale of 1 to 9, 1 representing very clean, well filled ears with well aligned kernel rows, while 9 represents diseased and poorly filled ears. Field weight was determined by weighing harvested ears per plot in kilogram. The moisture content of grains at harvest was determined using a maize moisture meter. Grain yield (t/ha) was obtained as field weight adjusted to 14% moisture content.

Grain yield (t/ha) = (FWT (kg)/Plot size (m^2)) x [(100 – moisture content) x 10,000 x SP]/ (100-14) x 1000.
SP = Shelling percentage (weight of grain expressed as a percentage of ear weight)
FWT = Field weight

Severity of streak and stripe was scored on a scale of 1 to 5 based on the extent of symptoms observed on leaves, 1 represents plants with very few or no streak or stripe symptoms, while 5 represents plants with very severe streaking or striping (75% of leaf area). The incidence of virus was determined by counting the number of infected plants (by streak or stripe virus) and expressed as a percentage of the total plant stand per plot.

$$\text{Incidence (\%)} = \frac{\text{Number of infected plants/plot} \times 100}{\text{Total number of plants/plot}}$$

Data analyses

Means and ranges were estimated. Percentage data were transformed using arcsine transformation before analysis (Steel and Torrie, 1960). Years and locations were pooled as environment. The progenies were referred to as genotypes in the analysis. Analysis of variance for North Carolina Design I (NCD I) was performed using PROC GLM of SAS (Version 9.1). Random model was assumed for the analysis (genotypes and environments were random). The model for NCD I for one environment is:

Yijk = u + mi + fij + rk + eijk (Hallauer et al., 2010)

where *u* is the mean, *m*i is the effect of the *i*th male, *fij* is the effect of the *j*th female mated to the ith male, *r*k is the replication effect, and *eijk* is the experimental error. After analyzing for each season, Bartlett test for homogeneity of variance was carried out to test for significance between the two seasons. Data were then pooled over sets and over environments and analysed. The form of analysis of variance pooled over sets and over environments (seasons and locations) is as shown in Table 1. Mean square (MS) of 'males within sets' was tested with MS due to 'environment x males in sets' interaction and 'females in males in sets'. MS of 'females in male within sets' and MS due to 'environment x males in sets' interaction were tested by MS due to 'environment x females in males in sets' interaction. MS of 'environment x females in males in sets interaction was tested with MS error (Hallauer et al., 2010). Additive variance (σ^2_a), dominance variance (σ^2_d) and their interactions with environments were estimated from mean squares of ANOVA (Table 1) as:

σ^2m = (M5-M4-M3+M2)/ref, σ^2f/m/s = (M4-M2)/re

Table 2. Mean ± S.E and ranges for agronomic and disease traits of progenies of the QPM population ART/98/SW6-OB under MSV and MStV infections at Ilora and Ikenne, Nigeria in 2015 and 2016.

Traits	Mean±S.E	Range
Days to 50% silking	59.79±0.11	51-72
Plant height (cm)	146.92±0.70	80- 280
Ear height (cm)	63.32±0.42	22-115
Husk cover rating (1-9)	2.82±0.05	0-8
Ear aspect (1-9)	2.82±0.05	1-9
Grain yield (t/ha)	1.84±0.04	0-11.16
MSV incidence (%)	2.90±0.28	0-100
MSV severity (1-5)	2.03±0.07	1-5
MStV incidence (%)	37.78±0.61	0-100
MStV severity (1-5)	3.05±0.02	1-5

S.E: Standard error, (1-5): 1 = excellent, 5 = Poor (1-9): 1 = excellent, 9 = Poor.

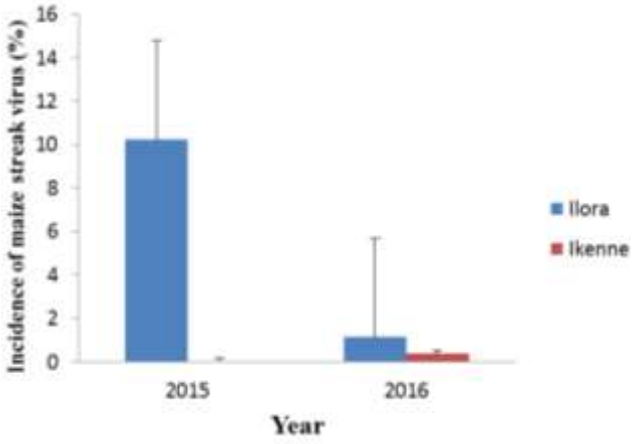

Figure 1. Percentage MSV incidence in Ilora and Ikenne in 2015 and 2016.

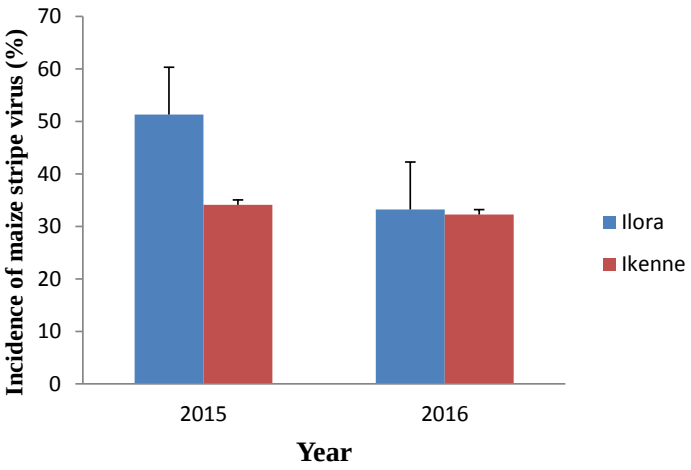

Figure 2. Percentage MStV incidence in Ilora and Ikenne in 2015 and 2016.

Table 3. Bartlett test of homogeniety of variance for the two cropping seasons.

Trait	Pooled variance	χ^2 cal	Diff.
Days to 50% silking	9.97	345.37	*
Plant height (cm)	406.29	209.57	*
Ear height(cm)	182.56	502.66	*
Husk cover rating (1-9)	0.66	159.77	*
MSV incidence (%)	0.05	0	ns
MSV severity (1-5)	0.57	0	ns
MStV incidence (%)	30.36	517.52	*
MStV severity (1-5)	59.53	781.58	*
Ear aspect (1-9)	17.65	420.53	*
Grain yield (t/ha)	228.13	0.28	ns

χ^2 cal: calculated chi-square value; *: significant, ns: not significant. Diff: difference at (k-1)=1 d.f @5% level of significant.

σ2a = 4σ2m;
σ2d = 4(σ2f/m- σ2m)
σ2m = (M5-M4-M3+M2)/ref,
σ2f/m/s = (M4-M2)/re
σ2e/m/s = M3-M2)/rf;
σ2 e/f/m/s =(M2-M1)/r

Narrow-sense heritability (NSH) and its standard error were calculated according to Hallauer and Miranda (1988). Phenotypic and genotypic correlation coefficients were computed using variance-covariance matrix and estimates of genotypic and phenotypic variances as described by Falconer (1996).

RESULTS AND DISCUSSION

Ranges were high for all the traits studied (Table 2). The wide ranges suggested wide variability for these traits in the maize population. MStV incidence was much higher (37.8%) than MSV incidence (2.9%). MSV and MStV incidence were much higher in Ilora than Ikenne in both 2015 and 2016 (Figure 1 and 2). MSV incidence was lower in both locations in both years compared to MStV incidence (Figure 1) indicating that MSV was not as prevalent in the locations and seasons under study as MStV. Taiwo et al. (2006) also reported low incidence of MSV (0 to 20%) and maize mottle/chlorotic stunt virus in and around Lagos state, Nigeria. This suggests that disease incidence and severity will differ from year to year and from location to location. The moderate grain yield (1.84 ton/ha) despite the high MStV incidence suggested that the maize population has some level of tolerance for MStV infection.

Karavina et al. (2014) also identified some hybrids with multiple resistance to turcicum leaf blight and MSV. QPM varieties are known to be more vulnerable to diseases

Table 4. Mean squares from Analysis of Variance for agronomic and disease traits of progenies of ART/98/SW6-OB under MSV and MStV infections at Ilora and Ikenne, Nigeria in 2015 and 2016.

Source	df	Days to 50% silking	Plant height (cm)	Ear height (cm)	Husk cover rating (1-9)	MSV incidence (%)	MSV severity (1-5)	Ear aspect (1-9)	Grain yield (t/ha)	MStV incidence (%)	MStV severity (1-5)
Env	3	2324.71**	43363.07**	4003.92**	109.39**	2.21**	46.09**	48.13**	358.60**	108.23**	144.38**
Rep (Env)	8	12.54**	5206.32**	1013.49**	10.36**	0.08	0.51	3.98**	25.56**	0.65**	10.28
Set(Env*Rep)	48	3.39	301.50	16.95	0.99*	0.07*	0.45	1.79**	17.73**	0.40**	9.18**
Males (Set)	23	9.62**	942.68**	233.54	0.99	0.06	1.42**	1.01	12.90	0.35	7.81
Females (Set*Male)	72	12.90**	626.36**	252.10**	2.55**	0.07	1.04	1.43	12.30	0.41**	11.53**
Env*Males (Set)	69	3.75**	300.99	171.69**	1.47*	0.05	0.50	1.09	13.24	0.33	10.36**
Env*Females (Set*Male)	163	0.00	320.13	95.34	0.96**	0.07**	0.79	1.13**	15.86**	0.26**	6.32
Error	247	3.56	423.23	126.89	0.66	0.05	0.63	0.83	10.91	0.14	5.78

df: degree of freedom, * and **: Significant at P = 0.05 and 0.01 respectively, (1-5): 1 = excellent, 5 = Poor; (1-9): 1 = excellent, 9 = Poor.

because of the soft floury endosperm of the Opaque-2 maize which foster fungal growth (NRC, 1988). Bartlett test for homogeniety of variance showed that some traits

were significant in the two seasons, while some were not (Table 3).

Combined analysis of variance showed significant mean squares for males in set for days to silking, plant height and MSV severity (Table 4). Mean square (MS) for 'females within males in set was significant for MStV incidence and severity and most of the agronomic traits except grain yield and ear aspect. MS of 'environment by female in male in set' interaction was significant for ear aspect, grain yield, husk cover rating, MSV incidence and MStV incidence and severity. MS of 'environment by male in set interaction' was significant for only MStV severity among the disease traits (Table 4).

Ige et al. (2017) reported significant MS for GCA by environment and SCA by genotype x environment interactions for maize streak severity. The significant MS of genotypes for plant height, days to 50% silking and the disease traits indicated that the characters are under genetic control and improvement could be made on them. Similar observation was made by Ininda et al. (2006) and Asea (2005) for MSV score.

Dintinger et al. (2005) also reported significant MS of genotypes for maize stripe incidence and severity. Rodier et al. (1995) reported that resistance to MSV in S_I and S_2 lines derived from population CVR3-C3 was under genetic control with major genes controlling high to complete resistance and minor genes controlling partial resistance. However, Akande and Lamidi (2006) working on three fungal diseases reported on susceptibility of eight QPM varieties to varying degrees with no significant varietal differences. Estimate of dominance variance were larger than additive variance for most of the traits studied except for grain yield and MSV severity (Table 5). This corroborates the finding of Kim et al. (1989) who reported that resistance to MSV in 1B32 inbred line from IITA is controlled quantitatively, mainly by additive gene action, with relatively small (2 to 3) number of genes involved.

Studies conducted by Pixley et al. (1997) showed that additive effects are important for resistance. Lorroki (2009) working on six generations also reported that MSV is controlled by additive gene effect with dominance x dominance epistatic interaction. The number of effective factors was estimated to be between 2 to 7 genes. Ige (2016) reported that additive gene effect was preponderant for MSV severity, while non-additive gene effect was important for grain yield. Sibiya et al. (2013) however reported that additive gene effect was preponderant for grain yield.

Gichuru (2013) stated that varying gene actions controlling grain yield is dependent on the parent and the environment under consideration. Additive genetic variance for husk cover rating and ear aspect were negative and were therefore equated to zero. Dominance variance being larger than additive variance for MSV incidence, MStV incidence and severity with average degree of dominance being above unity has also been reported (Dintinger et al., 2005; Rodier et al.,1995; Asea, 2005).

Dintinger et al. (2005) reported that resistance

Table 5. Components of genetic variances and narrow-sense heritability estimates for agronomic and disease traits of progenies of Quality Protein Maize (QPM) population ART/98/SW6-OB under maize streak virus (MSV) and maize stripe virus (MStV) infections at Ilora and Ikenne, Nigeria in 2015 and 2016.

Parameters	Days to 50% silking	Plant height (cm)	Ear height (cm)	Husk cover rating (1-9)	Ear aspect (1-9)	Grain yield (t/ha)	MSV incidence (%)	MSV severity (1-5)	MStV incidence (%)	MStV severity (1-5)
σ^2a	0.69±1.92	49.89±55.04	9.90±43.46	-0.01±0.28	-0.01±0.21	0.27±0.32	0.00 ±0.01	0.05±0.21	0.01±0.07	0.06±1.63
σ^2d	3.62±5.21	52.19±97.19	42.35±106.88	0.54±0.99	0.11± 0.60	-1.46±5.68	0.00±0.03	0.03±0.47	0.05±0.17	1.68±4.75
σ^2d/σ^2a	5.25	1.05	4.28	-54.00	-11.00	-5.41	-	0.60	5.00	28.00
$h^2\%$ ± S.E	34.75±0.01	51.10±1.24	27.20±0.86	-4.80±0.01	-10.52±0.01	22.13±0.26	12.61±0.00	44.13±0.004	12.13±0.003	4.40±0.08

σ^2a: Additive variance; σ^2d: Dominance variance; σ^2d/σ^2a: average degree of dominance; h^2: Narrow-sense heritability; S.E: Standard error. Negative variances and were equated to zero. (1-5): 1-Excellent, 5- Poor; (1-9): 1- Excellent, 9 – Poor.

to MStV is quantitative and may vary in relation to cumulative number of innoculative planthoppers by hour. Rodier et al. (1995) also reported that dominance was important for resistance to MStV. Asea (2005) reported mostly dominance gene action for MSV. Out of the 10 traits studied, average degree of dominance (σ^2d/σ^2a) was above unity for five of the traits. No dominance was observed for husk cover rating, ear aspect, grain yield and MSV incidence, while partial dominance was observed for MSV severity (Table 5).

The report of Mariote (2007) corroborates the finding in the present study that resistance to MSV is controlled by partial dominance. This result is however surprising because this population has not been selected. According to Hallauer and Miranda (1988), in an unselected maize material, the variance for GCA, an indicator of additive genetic variance was larger than the variance for SCA, related to dominance variance. Average degree of dominance being above unity for MStV incidence and severity and the agronomic traits suggests presence of complete dominance for the genes affecting these traits or possible occurrence of over-dominance at some loci (Hallauer and Miranda, 1988).

The strikingly high degree of dominance and very low heritability for MStV severity suggests presence of epistatic variance and considerable portion of genotype by environment interaction variance. This is revealed in the mean square table with environment by male in set interaction being significant for MStV severity. The build-up of dominance variance component may be due to the fact that when there is overdominance, heterozygote is favoured, and both favourable and unfavourable alleles will be accumulated in the population instead of eliminating the unfavourable alleles. The presence of both additive and dominance variance in the maize population, ART/98/SW6-OB, suggests that breeding scheme that capitalizes on both types of gene action such as S_1 selection, half-sib or test cross could be used to improve the population for resistance to both maize MSV and MStV diseases.

Narrow-sense heritability (NSH) estimate was moderate for most of the traits. It was least in MStV severity (4.4%) and highest in plant height (51.0%) (Table 5). Among the disease traits, MSV severity had the highest NSH of 44.1%. Ige (2016) also reported high NSH (55.3%) for maize MSV severity. Negative NSH were observed for husk cover rating and ear aspect. The moderate to high heritability, and wide ranges observed in the maize population for grain yield, MSV severity, days to silking and plant height, suggests that there is adequate genetic variation in the population for grain yield and resistance to the MSV and that progress would be made from selection. The heritability value obtained for MSV in this study falls within the range obtained by Asea (2005) on MSV. Soto et al. (1982) also found resistance to be simply inherited and fixable rapidly through breeding for MSV.

Means of ELISA titre values for MSV and MStV are shown in Table 6. Means of the progenies were significantly higher than the control except for MSV titre in 2015. Ranges of the titre values were moderately wide for both MSV and MStV in each season (Table 6). The mean ELISA titre values being significantly higher than the healthy control revealed the presence of the viruses in the maize population. Some of the progenies tested positive while some tested negative compared with the healthy control (data not shown). The ranges of the titres presented here further indicated that there is variability for resistance to the double viruses in the maize population. ELISA technique has been widely used to detect the presence of viruses in maize with or without disease symptoms (Kumar et al., 2004; Fajinmi et al., 2012).

Estimates of correlation coefficients among

Table 6. Mean of ELISA titre values (A_{405nm}) for the progenies of the QPM population ART/98/SW6-OB under MSV and MStV infections at Ilora and Ikenne, Nigeria in 2015 and 2016.

Titre value	MSV		MStV	
	2015	2016	2015	2016
Progenies titre	1.66	0.4	0.487	0.258
Range	0.69	0.42	0.38	0.24
Control	1.14	0.055	0.165	0.045

Samples are considered positive when the titre values of the test samples are twice the mean value of the control.

Table 7. Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients of agronomic and disease traits of progenies of ART/98/SW6-OB under MSV and MStV infections at Ilora and Ikenne, Nigeria in 2015 and 2016.

Traits	Days to 50% silking	Plant height (cm)	Husk cover rating(1 - 5)	MSV incidence (%)	MSV severity (1 – 5)	Grain yield (t/ha)	MStVincidence (%)	MSV titre	MStV titre
Days to 50% silking	-	-0.24*	0.20*	-0.08	-0.05	-0.21*	0.03	-0.16	-0.10
Plant height (cm)	0.18	-	-0.25**	-0.02	0.09	0.32**	-0.05	0.13	0.12
Husk cover rating (1-5)	0.36	-0.40	-	-0.08	-0.16	-0.28*	-0.14	0.24	0.21
MSV incidence (%)	-0.36	-0.18	-0.19	-	0.69**	0.02	-0.05	0.13	0.13
MSV severity (1-5)	0.19	-0.21	-0.22	0.80	-	0.10	-0.16	-0.22*	-0.08
Grain yield (t/ha)	-0.21	0.35	-0.35	0.24	0.34	-	-0.10	-0.32**	-0.33**
MStV incidence (%)	-0.62	0.63	-0.24	0.28	0.10	-0.02	-	0.09	0.08
MSVtitre	-0.19	0.25	0.58	0.51	-0.42	-0.34	0.78	-	0.89**
MStV titre	-0.36	0.15	0.49	0.54	-0.22	-0.49	0.22	0.84	-

* and **: Significant at P = 0.05 and 0.01 respectively.

traits under MSV and MStV infections are shown in Table 7. Days to 50% silking had negative correlations with plant height (rp= -0.24*) and grain yield (rp = -0.21*, rg = -0.21). Grain yield had positive and significant correlations with plant height (rp = 0.32*, rg = 0.35) but negative correlation with husk cover rating (rp = -0.28*, rg = -0.35). This indicates that tall plants with good husk cover rating tend to have better grain yield. For the relationship among agronomic and disease traits, days to 50% silking had negative genotypic correlations with maize MSV incidence (-0.36) and MStV incidence (-0.62).

Plant height also had negative correlations with disease incidence except with MStV incidence (rg =0.63) although phenotypic correlation was not significant for MSV and MStV incidence. The significant negative phenotypic correlation between plant height and disease incidence was also observed by Mariote (2007) and Asea (2005). Mariote (2007) reported negative phenotypic correlation between MSV and plant height, while Asea (2005) reported negative correlation between MSV and days to silking.

Grain yield was significantly correlated with MSV titre (rp = -0.32*, rg = -0.34) and MStV titre (rp = -0.33**, rg = -0.49). The negative correlation between grain yield and the titre values indicated that the lower the titre values the higher the yield. The positive phenotypic correlation between grain

yield and MSV incidence and severity was also reported by Mariote (2007). Lorroki (2009) reported that MSV disease significantly reduced stover dry matter and grain yield with yield loss ranging between 8 to 48%. Strong relationship existed between MSV incidence and severity (rp =0.69**, rg = 0.80).

MSV and MStV incidence also had strong correlation with MSV and MStV titres. The strong positive relationship between the titre values and MSV and MStV incidence further supports the ability of ELISA technique to detect various viral infections. The significant correlation between MSV incidence and severity indicates that the higher the incidence, the higher the severity. Similar observation was made between MStV incidence and severity (Dintinger et al., 2005). Highly significant correlation also existed between the two titres (rp =0.89**, rg = 0.84). This is an indication of possible interaction between the two viruses and among diseases generally on plants. Karavina et al. (2014) reported that yield of MSV-infected plants was not significantly different from yield of turcicum leaf blight-infected plants.

Conclusion

The larger additive variance estimates and moderate to high heritability for grain yield and MSV severity with wide ranges indicates that improving the maize population for grain yield and MSV resistance would be successful. However, progress in improving the population for MStV resistance would be slow due to the low heritability estimates for MStV scores. Breeding scheme that capitalizes on both additive and dominance gene action such as S_1 selection, half-sib or test cross could be used to improve the population for resistance to both maize MSV and MStV diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Incidence of seed borne fungi in farm saved rice seeds, quality declared seed and certified seed in Morogoro Region in Tanzania

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Rice (*Oryza sativa* L.) is the second most important food crop after maize in Tanzania. It faces many challenges like diseases, pests and physical damages, which reduce the seed quality. This article identified microorganisms and the district where they occurred. 13 fungal species comprising of 11 pathogenic and 2 saprophytic fungi were detected and the incidence level varied among sample. Mvomero District showed the highest incidence recorded (64.5%) for sample collected from Hembeti and Dakawa/Msufini villages. In Kilombero, the fungal specie which detected highest incidence was *Fusarium equiseti* (31%) followed by *Fusarium moniliforme* (28.5%). *Verticillium cinnabarinum* and *Curvularia inaequalis* had the least incidence of 1 and 1%, respectively. The study recommends that before planting season, seeds should be tested to minimize spread of fungal species.

Key words: Pathogens, quality declared seed (QDS), farmer saved seed (FSS), rice seed, seed quality, Kilosa, Kilombero, Mvomero.

INTRODUCTION

Rice (*Oryza sativa* L.) is a globally important staple food for more than half of the world's population (Khush et al., 2013). The crop is increasingly becoming an important staple food and cash crop in Africa (Tanko et al., 2016). In this continent, 15 million tons of rice is produced annually (Ronald et al., 2014). Eastern and Southern Africa contributes 16.1% of rice where the major contributors are Madagascar and Tanzania (Food and Agriculture Organization (FAO), 2013; United Republic of Tanzania (URT), 2012).

In Tanzania, rice is the second most important and popular food crop after maize (URT) The major rice producing regions in Tanzania include Morogoro, Shinyanga, Mbeya, Mwanza, Rukwa and Tabora, respectively. The trend of increase or decline of rice yield in Tanzania has not been clear for the past 20 years, due to numerous factors including emergence and poor management of production. Rice yields may fluctuate

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depending on the effects of climate change, poor agricultural practices, and inadequate standard of harvest and post-harvest techniques deployed.

Seed is one of the three basic elements for crop production and help to increase agricultural productivity as it provides the maximum limit of crop yield for all other production inputs. Unlike fertilizers and pesticides, farmers cannot produce without seed (Miva et al., 2017).

However, seeds carry pathogens such as fungi, bacteria, nematodes and viruses responsible for transmitting seed-borne diseases, which often cause partial or total crop losses (Barret et al., 2015). When seed has good physical, physiological, health and genetic qualities, farmers have greater prospects of producing a good crop (Miva et al., 2017).

The present investigation has been carried out to establish baseline information on quality of farm saved rice seed by smallholder farmers, to enhanced strategy of yield improvement. This is important as, many farmers' uses own stored seeds for the next cropping season, though seed borne diseases can be transferred easily and hence other quality attributes may be sub-standard. Proper assessment of the quality of seed, stored by farmers from their previous crop will establish the broad picture of seed quality of locally produced rice as compared to Quality Declared Seed (QDS) and Certified Seed. Therefore, this paper aims to identify the seed borne fungi and evaluate their incidence in farm saved rice seeds in Morogoro Region in Tanzania.

MATERIALS AND METHODS

Description of the study area and collection of rice samples for laboratory tests

The rice samples were collected from 45 farmers, 15 from each district in the region. Laboratory investigations were carried out from mid-December 2017 to June 2016 at the African Seed Health Centre and Tanzania Official Seed Certification Institute (TOSCI) by Sokoine University of Agriculture (SUA) in Tanzania.

Multi-stage and cluster-sampling techniques were used to identify the village samples, which ensure good representativeness of rice farming population in the study areas. Rice seed samples of 1.5 kg were collected from each farmer packed in a paper bags, labeled and transported to the African Seed Health Laboratory at Sokoine University of Agriculture then stored in the refrigerator at 5°C (to avoid further development of microorganisms), for laboratory test.

Isolation and identification of the fungi

Seedborne fungi collected from rice sample were detected by Blotter method (Yu et al., 2015). Sterile blotter papers with 9 cm diameter were placed on the Petri dishes. 200 seeds were evaluated from each sample. 8 Petri dishes is used per sample. Twenty five seeds placed in each Petri dish in a radial manner on the blotter papers were incubated at 25°C for 24 h, of an alternating cycle light and darkness for 7 days. After 7 days, seeds were examined using a stereomicroscope to determine the presence or absence of fungal growth.

Fungal conidia and conidiomata were detected by light microscope. Various magnifications were used to identify conidia and mycelia produced by each group of fungi. Individual genus was classified to species level, using respective keys (Senbeta and Abdella, 2014).

The referenced two hundred seeds were used to determine the incidence of fungal microorganisms. Fungal species, found growing on the surface of seeds were identified and their incidence was calculated as:

$$\text{Incidence} = \frac{\text{No.of rice seeds on which fungus appear}}{\text{Total No.of seeds examined}} \times 100$$

Data analysis

Difference in the various samples, representing pathogens and types of sources of seeds (Farmer Saved Rice, certified and (QDS)) were established, through mean separation using Turkey's test after significant ANOVA results at P≤ 0.05. Correlation among the incidence of different microorganisms detected during health test was also analyzed using Viera et al. (2016) method.

RESULTS

Fungal contamination

Generally 13 fungal species comprising of 11 pathogenic and 2 saprophytic fungi were detected (Table 1). Mvomero District showed the highest fungal incidence recorded (64.5%), for sample collected from Hembeti and Dakawa/Msufini villages. In Kilombero, the fungal specie which detected the highest incidence was *Fusarium equiseti* (31%) followed by *Fusarium moniliforme* (28.5%). *Verticillium cinnabarinum* and *Curvularia inaequalis* accounted for 1% respectively (Table 1).

Fungal incidence of seeds infestation per district surveyed

Table 2 gives a summary of the fungal incidence of seeds infestation per district surveyed. Sample seeds observed were all infested with fungal at different level. 3000 rice seeds were analyzed from each district for the presence of, seed-borne microorganisms. Mvomero district recorded the highest percentage of contaminated seeds (16.36%), followed by Kilosa District (13.10%) and the lowest in Kilombero District (12.43%). The highest number of fungi species detected was 10, 9 and 8 in Kilosa, Kilombero and Mvomero, respectively.

Conidia of identified fungal species; the most prevalent species

Characteristics of the identified fungal species and conidia of few species are summarized in Table 2 and Plates 1 to 3. The characteristics generally ranged from, appearance of colony (colour or shape) and morphology of species structures under microscope. Plates 1, 2 and 3 further illustrate conidial characteristics detected.

Table 1. Fungi incidence in rice seed samples detected from Morogoro Region.

Sample no.	Variety	Seed category	Village	Fungal spp	No. of seed infested	Incidence (%)
Kilombero District						
N.1	TXD 306	QDS	Michanga	*Curvularia lunata*	31	15.5
N.2	TXD 306	FSS		*Fusarium moniliforme*	57	28.5
N.3	Mbawambili	FSS		*Fusarium pallidoroseum*	24	12
N.4	Mbawambili	FSS		*Verticillium cinnabarinum*	2	1
N.5	TXD 306	Certified 1		*Curvularia. inaequalis*	2	1
N.6	Mdomo wa fisi	FSS	Mbasa	*Alternaria. padwickii*	7	3.5
N.7	Dai pesa	FSS		*Alternaria alternate*	5	2.5
N.8	Mkia wa Fisi	FSS		*Fusarium equiseti*	62	31
N.9	Ngome	FSS		*Fusarium pallidoroseum*	29	14.5
N.10	Supa india	FSS		*Curvularia lunata*	41	20.5
N.11	Supa india	FSS	Idandu	*Fusarium pallidoroseum*	12	6
N.12	Supa Malolo	FSS		*Bipolaris oryzae*	26	13
N.13	Masamtula	FSS		*Curvularia lunata*	46	23
N.14	Supa india	FSS		*Exserohilum rostratum*	24	12
N.15	Kisegese	FSS		*Bipolaris oryzae*	24	12
				Total % sum		13.07
Kilosa District						
N.16	Supa india	FSS	Rudewa	*Fusarium moniliforme*	10	5
N.17	Mdomo wa fisi	FSS		*Bipolaris oryzae*	7	3.5
N.18	Supa india	FSS		*Curvularia inaequalis*	7	3.5
N.19	Mbawambili	FSS		*Alternaria alternate*	12	6
N. 20	Lawama	FSS		*Curvularia lunata*	17	8.5
N.21	Lawama	FSS	Kimamba- B	*Fusarium pallidoroseum*	15	7.5
N.22	TXD 306	FSS		*Alternaria alternata*	20	10
N.23	TXD 306	FSS		*Fusarium moniliforme*	13	6.5
N.24	TXD 306	QDS		*Alternaria padwickii*	12	6
N.25	Kabangala	FSS		*Fusarium moniliforme*	51	25.5
N.26	Masamtula	FSS	Chanzuru	*Aspergillus* spp.	12	6
N.27	Supa india	FSS		*Alternaria alternata*	4	2
N.28	Supa india	FSS		*Bipolaris oryzae*	2	1
N.29	Muunguja	FSS		*Exserohilum rostratum*	20	10
N.30	TXD 306	Certified 1		*Aspergillus* spp.	24	12
				Total % Sum		7.53
Mvomero District						
N.31	Shingoyamwaline	FSS	Wami	*Phoma* spp.	10	5
N.32	TXD 306	QDS		*Fusarium pallidoroseum*	22	11
N.33	Mbawambili	FSS		*Fusarium moniliforme*	22	11
N.34	Supa India	FSS		*Exserohilum rostratum*	10	5
N.35	Muunguja	FSS		*Curvularia lunata*	33	16.5
N.36	TXD 306	FSS		*Phoma* spp.	15	7.5
N.37	Supa Malolo	FSS	Hembeti	*Bipolaris oryzae*	6	3
N.38	Masamtula	FSS		*Alternaria padwickii*	12	6
N.39	TXD 306	FSS		*Curvularia lunata*	15	7.5
N.40	TXD 306	FSS		*Fusarium pallidoroseum*	25	12.5
N.41	TXD 306	FSS		*Fusarium moniliforme*	129	64.5
N.42	626	FSS	Dakawa/Msufini	*Curvularia lunata*	45	22.5
N.43	Domola-fisi	FSS		*Alternaria padwickii*	16	8
N.44	Shingoyamwalinene	FSS		*Exserohilum rostratum*	4	2
N.45	TXD 306	Certified 1		*F. pallidoroseum*	6	3
				Total % Sum		12.33

FSS= Farmer saved –seed, QDS = Quality declared seed.

Table 2. Summary of fungal species and their incidence on farmer saved rice seed collected from rice growing districts in Morogoro Region*.

District	No. of contamined seeds	Seed contamination (%)	No. of detected species	Most prevalent species
Kilombero	373	12.43	9	*F. moniforme* (1) *C. lunata* (2) *F. pallidoroseum* (3)
Kilosa	393	13.10	10	*F. moniliforme* (1) *C. lunata* (2) *F. pallidoroseum* (3)
Mvomero	491	16.36	8	*F. moniliforme* (1) *C. lunata* (2) *F. pallidoroseum* (3)
Mean	419	13.96	9	

*The number of samples were 15 and the number of tested seeds by District were 3,000 in Kilombero, Kilosa and Mvomero. Note: Number in bracket means the first (1), second (2) or third (3) most prevalent among samples from each districts.

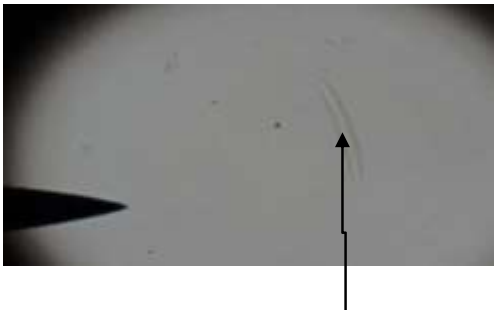

Plate 1. Conidia of Bipolaris oryzae under microscopy (× 750 mignification).

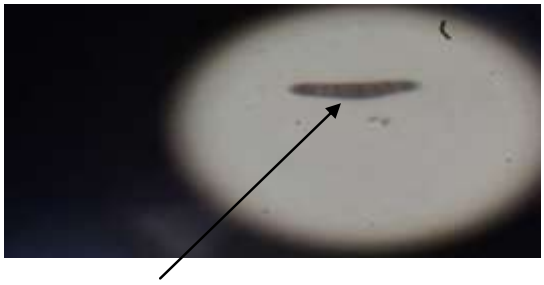

Plate 2. conidia of *Fusarium monliforme* under microscopy (× 40 magnification).

Correlation analysis

Correlation analysis coefficients among physical quality attributes and seedborne fungi incidences, were observed between *Curvularia lunata* and *F. moniliforme* (0.518**), *Fusarium pallidoroseum* (0.545**), *Alternaria padwickii* (0.526**) and *Exserohilum rostratum* (0.515**). Significant (0.01) correlation was observed between *Aspergillus* and *Phom*a (0.0498**), *Penicillium* (0.948**), and *Bipolaris* and *Exserohilum* (0.695**); *Phoma* and *Penicillium* were also positively correlated (0.532**). *F. pallidoroseum* positively correlated with *A. padwickii*

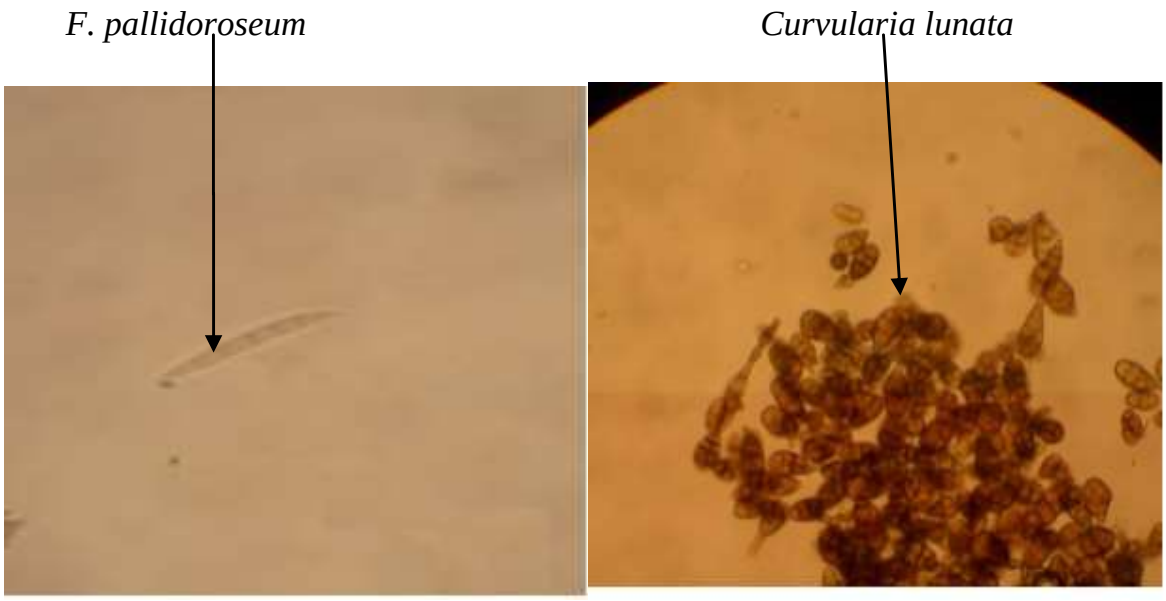

Plate 3. Conidia of *Fusarium pallidoroseum* and *Curvularia lunata* under microscopy (40 x magnifications).

(0.312**), *Alternaria alternata* (0.286**), *Fusarium equisti* (0.363**), *Bipolaris* (0.491**) and *Exserohilum* (0.297**).

Results from the current study also revealed significant correlation between seed physical quality and fungi incidences (Table 3). There was a positive correlation between germination and *C. lunata* (0.665**), *F. moniliforme* (0.468**), *F. pallidoroseum* (0.568**), *Aspergillus* (0.382**), *A. alternatka* (0.348**), *F. equisti* (0.188*), *Bipolaris* (0.287**), *Exserohilum* (0.299**) and *Penicillium* (0.198*).

Moisture content was also correlated with *F. pallidoroseum* (0.347*), *C. inaequalis* (0.353**), *Aspergillus* (0.360*) and *Penicillium* (0.35). Conversely, fungi incidence did not affect seed purity, with exception of *A. alternata*.

DISCUSSION

Seedborne fungi observed in this study are usually found in rice seeds, as compared to study conducted by Tokpah et al. (2017). In additional, Azam et al. (2012) reported a rate of 13 to 20% for *A. alternate*, 10 to 17% for *F. moniliforme* and 8.4% for *Curvularia* spp. in rice seeds. Sharma and Kapoor (2016) obtained similar results. In this study, there were many seedborne fungi in rice seeds, as compared to report of Wang et al. (2012).

The highest rate of incidences of seedborne fungi in rice seeds was found in Mvomero, followed by Kilosa. These districts have a wide range of seed varieties compared to Kilombero. Other comparable reasons might be storage practices and seed handing, which might lead to high incidence level. High incidences include *F. moniliforme* (65%) and *F. equiseti* (31%) with *C. lunata* (16.5, 20.5, and 23%) which may have justifications. It is also important that, these fungal species are commonly found in rice seeds worldwide despite the locations and climatic conditions (Azam et al., 2012).

Frequent occurrences of seedborne fungi in farmer's saved seeds were due to the fact that, most seeds are stored in the rooms which result to difficulty in temperature and humidity control. The environmental factors such as temperature, moisture and relative humidity affect the growth of seed-borne. Generally, high temperature of 15 to 30°C is required for growth and survival of many fungi in the seeds and high relative humidity of more than 65% is required for spore germination (Singh et al., 2012).

Seed-borne fungi such as *A. alternata, Bipolaris oryzae, F. moniliforme, A. padwickii, C. inaequalis, C. lunata, F. pallidoroseum, E. rostratum, F. equiseti, V. cinnabarinum, Phoma* species*, Aspergillus flavus, Penicillium* spp. and *F. moniliforme* have also been identified on rice seeds in other studies. In this research, *F. moniliforme*, *F. pallidoroseum* and *C. lunata* were the most important pathogens frequently detected in the rice seeds.

F. moniliforme, which causes Bakanae disease and Brown spot on rice seeds have negative impact on the quality of rice (Singh et al., 2012) and are commonly detected. Since farmers in the study area did not report these diseases as constraints, the species do not cause much yield loss in rice. It was reported that, Brown spot disease in rice cause losses of 3 to 15% of yield which is lower than loss caused by rodents and birds.

Owolade et al. (2011) determined that, cereal crops including rice seeds colonized by fungal species during storage were responsible for low germination and

Table 3. Correlation among physical quality attributes between these attributes and fungal incidences and among the fungi species.

Variables	CL	FM	FP	VL	CI	AP	AA	FE	BP	EX	AG	PH	PL	Germ	MC P
C.lunata															
F.moniliforme	0.518**														
F.pallidoroseum	0.545**	0.358**													
V.cinnabarinum	0.133	0.106	0.182												
A.padwickii	0.526**	0.929**	0.312**	0.149	0.023										
A.altenata	0.213*	0.057	0.286**	-0.073	-0.091	-0.133									
F.quiseti	0.271**	0.227*	0.363**	0.610**	0.281**	0.281**	-0.072								
Bipolaris	0.469**	0.156	0.491**	-0.059	-0.073	0.173	0.126	-0.058							
Exserohilum	0.515**	0.200**	0.297**	-0.073	-0.092	0.081	0.042	-0.072	0.695**						
Aspergillus	-0.100	-0.031	-0.074	-0.048	-0.060	-0.087	-0.007	0.047	-0.050	0.160					
Phoma	0.135	-0.024	-0.161	-0.054	-0.068	-0.099	-0.010	-0.054	-0.081	0.213	0.0498**				
Penicillium	-0.130	-0.091	-0.115	-0.039	-0.048	-0.070	-0.072	-0.038	-0.058	-0.007	0.948**	0.532**			
Germination	0. 665**	-0.468**	0.568**	-0.040	-0.129	0.382**	0.348**	0.188*	0.287**	0.299**	-0.015	0.040	0.198*		
Moisture (mc)	-0.209*	0.161	0.347*	0.045	0.353**	-0.062	0.020	-0.004	-0.023	-0.201*	0.360**	0.113	0.354*	0.263**	
Purity	0.033	0.007	0.110	-0.046	0.079	0.090	0.217*	0.103	-0.074	-0.180	0.096	-0.035	-0.073	0.099	0.140

*P ≥ 0.05 ** P ≥ 0.01 N = 108, CL = *Curvularia lunata,* FM = *Fusarium moniliforme,* FP = *Fusarium pallidoroseum,* VL = *Verticillium cinnabarinum,* CI = *Curvularia inaequalis,* AP = *Alternaria padwickii,* AA = *Alternaria alternata,* FE = *Fusarium equiseti,* BP = *Bipolaris oryzae,*EX = *Exserohilum rostratum,* AG = *Aspergillus niger,* PH = *Phoma* spp., PL = *Penicillium*, Germ = Germination, MC = Moisture content.

reduction of plant population by 42% in the field. The consequence of such infestation is not only limited to yield losses, but also accounts for the build-up of mycotoxins in infected grains, which had negative impact on seed quality.

Generally, seedborne diseases affect the seed quality of any cultivar (Naqvi and Zeeshan, 2013). The findings of this study are therefore important as, they highlight the need for effective measures aimed at establishing the status of microbes in farm saved rice seed, outfits for quality assurance.

The significant relationship by rice seeds germination capacity with some fungal species such as *C. lunata, F. moniliforme, F. pallidoroseum, A. alternata, E. rostratum, F.equiseti* and *Penicillium* spp is differ. In previous research these fungal species have been observed to cause poor germination in rice, also in contrary, significant correlation between some fungi incidence and moisture content of seed were not the same.

Seed moisture content enhances fungal growth, which increases seed moisture content through microbial respiration. Ok et al. (2014) observed similar result in samples where; moisture content was high, the number of fungal species increased, and the increased number of fungal species was reciprocally responsible for increased moisture.

In this study, *C. lunata* and *E. rostratum* were not significant with moisture content which means that, fungi have more incidences of low moisture content. Perhaps, this implies the inability to compete with other fungi that would grow more where moisture content is higher. Ironically, negative correlation between incidences of different fungal species, though existed abundantly, was never statistically significant. This indicates insignificance of direct antagonistic effects of the different fungal species against each other.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Phenotypic and serological evaluation of cowpea (*Vigna unguiculata* L.Walp) genotypes for resistance to viral infection under field conditions

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Viral infections are a major challenge to sustainable cowpea production in sub-Saharan Africa (SSA). Nine cowpea (*Vigna unguiculata* L. Walp) genotypes were evaluated for resistance against viral infection in a field trial involving randomized complete block design with 4 replications. Viral disease severity was assessed at 2, 4 and 6 weeks after planting (WAP) based on disease symptoms. Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) using antisera raised against cucumber mosaic virus (CMV), cowpea severe mottle virus (CPSMV), cowpea mosaic virus (CPMV) and southern bean mosaic virus (SBMV) was used to detect the viruses associated with diseased leaf samples collected from the field. Biometric and yield data were also taken. The mean disease incidence and area under disease progress curve (AUDPC) varied significantly ($P < 0.05$) among the cowpea genotypes, with 7 (Apagbaala, UCC-366, UCC-489, UCC-490, UCC-497, UCC-514 and UCC-523) out of 9 cowpea genotypes showing field resistance whilst the other two (UCC-473 and UCC-484) exhibited moderate resistance. ELISA showed that all the 9 cowpea genotypes were infected with at least one of the three viruses namely CMV, CPMV and CPSMV, whereas SBMV was not detected. Co-infection by CMV, CPMV and CPSMV was observed in UCC-366. Mean plant height, canopy diameter and seed yield differed significantly ($P < 0.05$) among the cowpeas. UCC-473, UCC-316, and UCC-523 had high mean seed yields of 6.690, 4.922 and 4.144 t ha^{-1} respectively, above the overall mean seed yield of 3.63 t ha^{-1}, emphasizing their resilient to viruses.

Key words: *Vigna unguiculata,* viral infection, disease incidence and severity, resistance screening.

INTRODUCTION

Legumes play important roles in provision of food security, generation of income, and maintenance of environment in most smallholder farming systems in sub-Saharan Africa (SSA) (Odendo et al., 2011). Africa produces about 8 million tonnes of grain legumes estimated at about 70% of the total global production, from 17.7 million hectares of land (IITA, 2007).Cowpea (*Vigna unguiculata* L.Walp) is a major staple food crop in

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sub-Saharan Africa (SSA), especially in the dry savanna regions of West Africa (Asare et al., 2010; Dugje et al., 2009).The seeds are a major source of plant proteins and vitamins for man, feed for animals, and also a source of cash income. Cowpea leaves and green pods are consumed as vegetable and the dried grain is used in many different food preparations (Dugje et al., 2009; Kyei-Boahen et al., 2017).

Cowpea is an essential component of the cropping systems because it fixes atmospheric nitrogen and contributes to soil fertility improvement particularly in smallholder farming systems where little or no fertilizer is used (Kyei-Boahen et al., 2017). The crop is drought tolerant and adapted to stressful environments (Bisikwa et al., 2014; Ddamulira et al., 2015) such as the prevailing conditions of the dry savannah regions in Ghana. Cowpea is an important food security crop and a major source of income especially in the Northern and Volta regions of Ghana where the bulk of the crop is produced.

Viruses are a major biotic constraint to cowpea production, reported to bring about yield losses ranging from 10 to 100% (Dhanasekar and Reddy, 2015). Over 140 viruses worldwide have been reported to attack cowpea and at least 11 of these occur in Africa (Hughes and Odu, 2003). Viruses considered most damaging to cowpea in Africa are bean common mosaic virus-black eye cowpea mosaic (BCMV-BICM), cowpea aphid-borne mosaic virus (CABMV), cucumber mosaic virus (CMV), cowpea mosaic virus (CPMV), cowpea severe mosaic virus (CPSMV), southern bean mosaic virus (SBMV) and cowpea mottle virus (CPMoV) (Hampton et al., 1997). Others are cowpea mild mottle virus (CPMMV) (Jeyanandarajah and Brunt, 1993), cowpea golden mosaic geminivirus (CGMV) and cowpea chlorotic mottle virus (CCMV) (Hampton et al., 1997). Viruses so far reported to be infecting cowpea in Ghana include SBMV, CABMV, BICMV and cowpea mild mottle virus (CPMMV) (Jeyanandarajah and Brunt, 1993; Lamptey and Hamilton, 1974; Zettler and Evans, 1972). The seed-borne nature of these viruses renders them very destructive to emerging seedlings and insect vectors can spread these further (Ndiaye et al., 1993; Bashir et al., 2002).

Effective management of these viruses is important to improve yields and quality of cowpea. Managing these viruses with insecticides is not effective because they are transmitted by several insect species in a non-persistent manner (Umaharan et al., 1997). The most economical, practicable and effective method of reducing crop losses due to viral infection is through the use of resistant varieties (Taiwo, 2003; Mbeyagala et al., 2014). Development of resistant varieties against different type and strain of viruses entails screening of germplasm in a particular agro-climate for identification of resistance to the particular strain prevailing in that region. However, field screening for virus resistance based solely on symptoms is not reliable (Shoyinka et al., 1997), as different viruses display overlapping symptoms (Dhanasekar and Reddy, 2015). Moreover, plants can also exhibit virus-like symptoms when exposed to adverse weather conditions, soil nutrient imbalances, pest infestations and non-viral infections (Hughes and Odu, 2003). Nine out of thirty-two (32) cowpea genotypes that were screened against viral infection under natural conditions in Ghana during 2015 major cropping season (Essandoh et al., 2017) were observed to exhibit mild symptoms of viral infections, indicating field resistance. Viruses associated with these symptoms are unknown and the performance of these resistant genotypes under different environment is also unknown. This study was therefore conducted to evaluate the performance of nine cowpea genotypes and to identify the associated viruses.

MATERIALS AND METHODS

Study area

Field experiment was conducted at the Teaching and Research farm of the School of Agriculture, University of Cape Coast (UCC), which falls within the coastal savannah agro-ecological zone of Ghana, from July to October 2016 during the major cropping season. This location (5°10'N, 1.2°50'W) has Acrisol soil type and a distinct bimodal rainfall behaviour, with a major rainy season (May-June) and a minor rainy season (August - October) with an annual rainfall ranging from 750 to 1000 mm (Parker et al., 2010). Temperatures of the area range from 23.2 to 33.2ºC with an annual mean of 27.6°C (Owusu-Sekyere et al., 2011).

Plant material

A total of nine cowpea genotypes were used for the study. These included eight recombinant inbred lines (F1, F2 or F3 generations) from University of Cape Coast (UCC-366, UCC-473, UCC-484, UCC-489, UCC-490, UCC-497, UCC-514, UCC-523) and the genotype Apagbaala from Savannah Agricultural Research Institute (SARI), Nyankpala, Ghana.

Experimental design and field layout

The randomized complete block design with nine treatments (cowpea genotypes) and four replications was used. A total land area of 2880 m^2 measuring 40 m x 72 m was ploughed and harrowed to render the soil loose. It was then divided into four blocks, spaced 1.5 m apart, and each block was further divided into 9 plots, spaced 1 m apart, and a plot size of 4 m x 4 m. Three seeds were sown per hill with an inter row spacing of 40 cm and intra-row spacing of 60 cm and later thinned to two plants per hill. There were four rows per plot, with 17 plants in a row, making 68 plants per plot.

Cultural practices

The study was under rain-fed conditions. Weeding was done manually using a machete and a hoe, as well as pulling out weeds by hands, when necessary. Fertilizer (NPK 15:15:15) was applied at a rate of 250 kg ha^{-1}.

Table 1. Visual scale for assessing severity of cowpea viral disease.

Scale	Symptom description
1	No symptoms on all leaves
2	Slight symptoms (1 to 25% of the leaves infected)
3	Moderate symptoms (26 to 50% of leaves infected)
4	Prominent symptoms with stunting (51 to 75% of leaves infected)
5	Highly severe symptoms with stunting (> 75% of leaves infected)

Data collection

Data was collected on disease incidence and severity, plant height, canopy diameter and seed yield. In each case data was taken from 10 inner rows of each plot and the mean per plant determined. Data on disease incidence and severity were assessed at 2, 4 and 6 weeks after planting (WAP), based on disease symptoms described by Gumedzoe et al. (1998).

Incidence of virus disease for the various fields was calculated using the formula:

$$\text{Disease incidence} = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants} \times 100$$

The severity of viral disease in each field was assessed based on the 1 to 5 symptom severity scale developed by Gumedzoe et al. (1998) as shown in Table 1.

Detection of viruses from cowpea samples

Symptomatic leaf samples were collected from six weeks old plants of each of the eight cowpea genotypes from the field in order to identify viruses associated with them. Between three and five young leaves were taken from each plant sampled. Virus identification was done using standard double antibody sandwiched enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (1977) using polyclonal antisera raised against CPMV, CPSMV, CMV and SBMV (DSMZ, Braunschweig, Germany).

The leaf samples were pulverized with mortar and pestle in an extraction buffer (8.0 g NaCl, 0.2 g KH_2PO_4, 1.1 g Na_2HPO_4, 0.2 g KCl/L, pH 7.4) containing 0.05% v/v Tween 20, and 2% w/v polyvinylpyrolidone at a 1:10 ratio (tissue weight: extraction buffer volume)**.** The microtitre plates (96 wells Nunc, Maxisorp, Roskide, Dernmark) were coated with primary (coating) antibody (IgG, 1/200 in coating buffer, Na_2CO_3, $NaHCO_3$, NaN_2) and incubated at 37°C for 4 h. After washing the plates four times with phosphate-buffer saline pH 7 with Tween 20 (PBS-T), the wells were loaded with the leaf extracts at 200 µL extract per well and incubated overnight (for 18 h) at 4°C.The plates were then washed four times with PBS-T, and incubated with enzyme conjugate (alkaline phosphatase conjugate (Sigma-Aldrich, Inc.), diluted at 1/200 in PBS-T+BSA+NaN_2) at 37°C for 2 h. After washing the plates four times with PBS-T, they were incubated for 1 hour at room temperature with freshly prepared phosphate substrate solution (100 µL per well) composed of p-nitrophenyl phosphate tablet (ADGEN Phytodiagnostics) at 1.0 mg mL^{-1} in 9.87% diethanolamine, pH 9.8. Healthy leaf sample and purified individual virus samples from DSMZ were included as negative and positive controls respectively in order to test the specificity and efficiency of the various polyclonal antibodies used in the assay.

The absorbance values at 405 nm (A_{405}) were recorded using Anthos microplate reader (Biochrum Ltd, Cambridge, UK). Absorbance values of three uninfected leaf samples were also measured. A test sample was deemed to be positive when the A_{405} was higher than 3 times the mean absorbance of the uninfected leaf samples (threshold value).

Data analysis

Data on mean viral disease severity scores were used to calculate area under the disease progress curve (AUDPC) for each of the cowpea genotypes according to Shaner and Finney (1977):

$$AUDPC = \sum_{i}^{n} [(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]$$

where: Y_i = disease severity at the i^{th} observation; X_i = time (weeks) at the i^{th} observation, and n = total number of observations

The AUDPC, which is a quantitative summary of disease intensity over a period 10 weeks was used to measure disease resistance in each cowpea genotype. Data on disease incidence was arcsine-transformed in order to homogenize the variances before subjecting to analyses of variance (ANOVA). The other data (disease incidence and severity, AUDPC, plant height, canopy diameter, and yield) were also subjected to ANOVA and the mean separated by least significance difference (LSD) method at 5% level of probability. Pearson's correlation coefficients were calculated for the relationships between disease, biometric and seeds yield data. All statistical analyses were performed using GenStat Release version 12 (VSN International).

RESULTS

Viral symptoms observed on the field

Varying degrees of symptoms were observed on the field (Figures 1a to e). These include stunted growth, yellow mosaic, vein clearing, mottling of leaves and necrotic lesions**.**

Incidence and severity of cowpea viruses on selected cowpea genotypes

Viral disease incidence showed significant differences among the cowpea genotypes at 2 WAP ($F_{8, 24} = 43.08$; $P<0.001$), 4 WAP ($F_{8, 24} = 6.91$; $P<0.001$) and 6 WAP ($F_{8, 24} = 7.02$; $P< 0.001$) (Table 2). At 2 WAP, the genotype UCC-484 had the highest mean disease incidence (90%) which was significantly different ($P<0.05$) from all the other cowpea genotypes. On the other hand, the genotypes, ApagbaalaUCC-514 and UCC-489 had

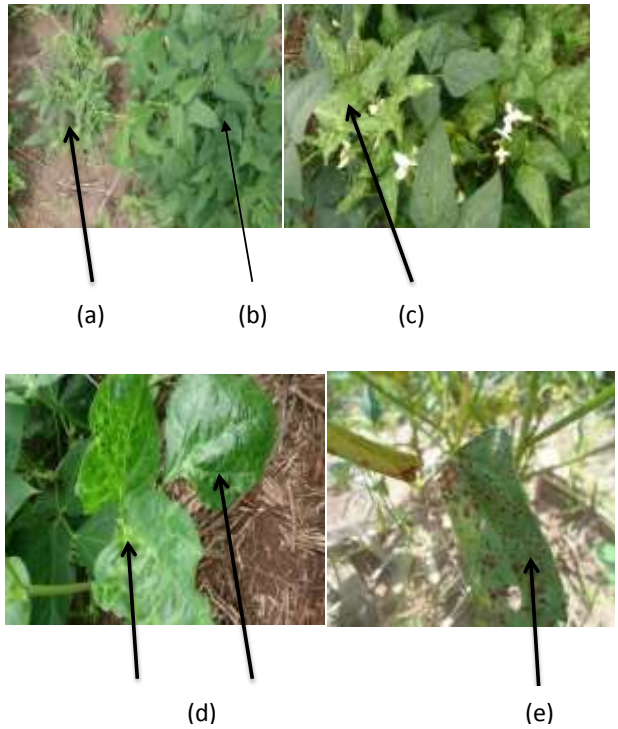

Figure 1. Viral disease symptoms observed on the field; **a** = stunted growth, **b** = healthy plant, **c** = mosaic, **d** = vein clearing and mottling of the leaves, and **e** = necrotic lesions (Photographed by Carlos Tettey).

Table 2. Mean viral disease incidence at 2, 4 and 6 weeks after planting, final severity and AUDPC for eight cowpea genotypes.

Genotype	Incidence			Final severity	HR	AUDPC
	2WAP	4WAP	6WAP			
Apagbaala	0.00^{e}	7.24^{b}	0.00^{b}	1.00^{e}	R	4.07^{f}
UCC-366	7.24d^{e}	12.96^{b}	21.21^{b}	1.13^{c}	R	4.40^{cd}
UCC-473	50.83^{b}	68.58^{a}	68.58^{a}	1.37^{b}	MR	5.40^{b}
UCC-484	90.00^{a}	53.57^{a}	58.68^{a}	2.00^{a}	MR	7.87^{a}
UCC-489	0.00^{e}	12.96^{b}	7.24^{b}	1.07^{cde}	R	4.20^{def}
UCC-490	18.43^{cd}	12.96^{b}	18.43^{b}	1.13^{c}	R	4.37^{cde}
UCC-497	26.57^{c}	18.27^{b}	12.96^{b}	1.10^{cd}	R	4.57^{c}
UCC-514	0.00^{e}	7.24^{b}	12.96^{b}	1.03^{de}	R	4.10^{ef}
UCC-523	18.43^{cd}	12.96^{b}	12.96^{b}	1.13^{c}	R	4.50^{c}
Mean	23.54	25.0	23.7	1.21		4.83
LSD	13.30	24.63	25.99	0.09		0.22

Means followed by the same letter in the column are not significantly different from each other (P>0.05). Host reaction (HR) 1 = no symptoms on all leaves (NS), 2 = slight symptoms (SS), 3 = moderate symptoms (MS), 4 = prominent symptoms with stunting (PSS), 5 = highly severe symptoms with stunting (HSS).

mean viral incidence of 0% each which was not significantly different (P > 0.05) from the mean incidence of 7.24% recorded for the genotype UCC-366 (Table 2).

At 4 WAP, the genotype UCC-473 had the highest mean disease incidence of 68.58% while Apagbaala had the lowest mean disease incidence of 7.24% (Table 2). At 6 WAP, the genotype UC-96-473 had the highest mean disease incidence (68.58%) while Apagbaala had 0%. Mean disease incidence declined between 4 and 6 WAP in Apagbaala, UCC-489 and UCC-497 (Table 2).

Table 3. Mean plant height, mean canopy diameter and mean seed yield of selected cowpea genotypes.

Genotype	Plant height (cm)	Canopy diameter (cm)	Seed yield (t ha^{-1})
Apagbaala	14.7^{cd}	88.5^{cd}	3.38^{bc}
UCC-366	41.8^{a}	150.1^{a}	4.92^{ab}
UCC-473	13.0^{d}	67.1^{d}	6.69^{a}
UCC-484	37.0^{ab}	127.6^{ab}	2.48^{c}
UCC-489	32.9^{b}	97.4^{bcd}	2.87^{bc}
UCC-490	17.8^{cd}	122.7^{abc}	2.44^{c}
UCC-497	20.4^{c}	128.3^{ab}	2.50^{c}
UCC-514	32.2^{b}	102.6^{bcd}	3.21^{bc}
UCC-523	32.9^{b}	133.4^{ab}	4.14^{bc}
Mean	27.0	113.1	3.63
$LSD_{(0.05)}$	6.74	36.29	2.4

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

The result also revealed that the mean final disease severity scores recorded for the cowpea genotypes varied significantly (P < 0.05) among them (F8, 24 = 102.72; P<0.001) (Table 2). Apagbaala displayed the lowest mean disease severity score (1.00) which was not significantly different (P > 0.05) from the genotypes UCC-514 and UCC-489 with mean severity scores of 1.03 and 1.07 respectively but was significantly different (P < 0.05) from the remaining 6 genotypes. Conversely, genotype UCC-484 had the highest mean final severity score of 2.00, which was significantly different (P<0.05) from the other genotypes.

The area under disease progress curve (AUDPC) showed significant difference among the cowpea genotypes (F8, 24=168.16; P<0.001) as shown in Table 2. Genotype UCC-484 had the highest AUDPC of 7.87 which was significantly different from all the other genotypes, indicating that UCCC-484 accumulated the highest disease pressure during the entire growing period. On the other hand, the genotype Apagbaala had the lowest AUDPC score of 4.07 which was not significantly different (P > 0.05) from the genotypes UCC-489 and UCC-514 with AUDPC of 4.20 and 4.10 respectively, indicating that they experienced the lowest disease pressure during the entire growing period.

Biometric characters and seeds yield

ANOVA showed significant differences among the cowpea genotypes in respect of their mean plant heights (F8, 24 = 20.86; P<0.001), mean canopy diameters (F8, 24 = 4.36; P<0.002) and mean seed yields (F8, 24 = 2.98; P<0.018) as shown in Table 3. The highest mean plant height (41.84 cm) was recorded for the genotype UCC-366 which was not significantly different (P > 0.05) from the genotype UCC-484 with a mean plant height of 36.96 cm. Genotype UCC-473 had the lowest plant height (13.03 cm) which was not significantly different (P > 0.05) from that of the genotypes, UCC-490 and Apagbaala with a mean plant height of 17.77 and 14.71 cm, respectively. Similarly, the highest canopy diameter (150.1 cm) was recorded for genotype UCC-366 which was not significantly different (P > 0.05) from the genotypes UCC-484, UCC-490, UCC-497 and UCC-523 with mean canopy diameters of 127.6, 122.7, 128.3 and 133.4 cm, respectively. Also, genotype UCC-473 had the lowest canopy diameter (67.1 cm) which was not significantly different (P > 0.05) from the genotypes, Apagbaala, UCC-489 and UCC-514 with mean canopy diameters of 88.5, 97.4 and 102.6 cm, respectively (Table 3).

Genotype UCC-473 had the highest seed yield (6.690 t ha^{-1}) which was not significantly different (P>0.05) from the genotype UCC-366 with a mean seed yield of 4.922 t ha^{-1} but significantly higher (P<0.05) from the other genotypes. The seed yield recorded for UCC-366 was not significantly different (P<0.05) from the genotypes UCC-523, Apagbaala, UCC-514 and UCC-489 with mean seed yields of 4.144, 3.379, 3.208 and 2.87 t ha^{-1}, respectively (Table 3).

Correlations among the variables

Correlation coefficients among the variables studied are shown in Table 4. AUDPC is correlated significantly positive with initial incidence (r=0.9123; P<0.01) and initial severity score (r=0.9708; P<0.01) (Table 4).There was also a highly significant positive correlation between initial incidence and initial severity (r=0.9040; P<0.01) (Table 4). Further, there was a significant positive correlation between plant height and canopy diameter (r=0.4317; P<0.05).

Viral detection by double antibody sandwiched enzyme-linked immunosorbent assay (DAS-ELISA)

Three virus species namely CPMV, CPSMV and CMV

Table 4. Correlation between disease incidence and severity and plant height, canopy diameter and seed yields.

Initial incidence	Initial incidence	Initial severity	AUDPC	PH	CD	Seed yield
Initial severity	0.9040**	-				
AUDPC	0.9123**	0.9708**	-			
PH	0.0027	0.1538	0.1580	-		
CD	0.0776	0.0971	0.0581	0.4317*	-	
Seed yield	0.1418	-0.1601	-0.0349	-0.0460	-0.1644	-

*' ** Significant at P<0.05 and P<0.01; PH, Plant height; CD, Canopy diameter; AUDPC, Area under disease progress curve.

were detected in the cowpea genotypes using DAS-ELISA and each sample was infected with at least one of the three viruses (Table 5). It was observed that some viruses were associated with single or multiple infection(s) in the plant samples. The leaf samples had a high prevalence of single virus infection compared with multiple virus infections. In single virus-infected leaf samples, CMV was the most prevalent, infecting 87.7% of the samples tested, followed by CPSMV which infected 25% whereas CPMV was the least prevalent, infecting only 12.5%. Co-infection by CMV + CPSMV + CMV was observed in one cowpea genotype UCC-366. SBMV was not detected in any of the plant samples tested.

DISCUSSION

The virus symptoms observed on cowpea on the field

Symptoms observed in the field (mosaic, leaf mottling, vein clearing and necrotic lesion) were similar to symptoms reported elsewhere on legumes affected by viral diseases (Akinjogunla, 2005; Aliyu et al., 2012). The varying symptoms observed in the study are suggestive of different viruses which infect cowpeas in the study area (Aliyu et al., 2012)**.** The variation in symptoms observed in the study could be attributed to the type of viral strains infecting the cowpea, cowpea cultivar, the time of infection, light intensity, environmental temperature, and mixed infections (Jones et al., 1991; Aliyu et al., 2012).

Incidence and severity of viruses on selected cowpea genotypes

The study to evaluate nine cowpea genotypes for resistance to viral infections under natural conditions showed variation in the disease incidence and severity among the cowpea genotypes at various sampling dates (WAP). Variation in the level of viral infection among the cowpea genotypes in the current study could be related to their genetic variability. This finding is in agreement with the work done by Orawu (2007) in his study on the occurrence of CABMV and prospects of improving resistance in local cowpea landraces in Uganda. Ojuederie et al. (2009) also reported that the reaction of various cowpea accessions to viral disease is genotype dependent. Variation in the incidence and severity of viral disease among the cowpea genotypes could also be due to the age of the plants at the time of infection. According to Picó et al. (1996), plants infected or inoculated at older age produce milder symptoms which may be wrongly considered as manifestation of genetic resistance. This corroborates the report of Ehinmore and Kareem (2010) which states that infection at a later stage results in reduced effects because at that stage, the plants are more mature and the virus has little or no deleterious effects on them. Lapidot (2007) also reported that the success of TYLCV infection of beans is highly dependent on the bean plant age.

With the exception of UCC-473 and UCC-484, which exhibited moderate resistance, the other cowpea genotypes exhibited mild symptoms of viral infection (Table 2). In this study, genotypes Apagbaala, UCC-366, UCC-489, UCC-490, UCC-497, UCC-514 and UCC-523 with low disease severity in terms of AUDPC and low final severity scores may offer single or multiple virus resistance, which is comparable to previous work (Essandoh et al., 2017), where these nine cowpea genotypes were found to exhibit field resistance. This finding also corroborates the report by Mbeyagala et al. (2014) when they screened 105 cowpea genotypes for resistance against viral infection under natural condition in Uganda. The ELISA serology revealed that genotype UCC-366 that also exhibited field resistance was co-infected with CMV, CPSMV and CMV (Table 5), demonstrating multiple field resistance against these three viral species. On the other hand, the ELISA detected single viral species from the other genotypes (UCC-489, UCC-490, UCC-497, UCC-514 and UCC-523) suggesting that they offered single virus resistance.

The differences in the levels of prevalence of CMV, CPSMV and CMV in the cowpea genotypes could be explained on the basis of antagonism, inoculum level, the age of the plant, climatic conditions and cultivar type (Orawu et al., 2015). The varying reactions of the cowpea genotypes to different cowpea viruses observed in the present study is quite valuable because it will enable breeders to breed for resistance against viruses that prevail at a particular location, as reported by

Table 5. Viral detection by double antibody sandwiched enzyme-linked immunosorbent assay (DAS-ELISA).

Genotype	CPMV	CPSMV	CMV	SBMV
Apagbaala	-	-	+	-
UCC-366	+	+	+	-
UCC-473	-	-	+	-
UCC-484	-	-	+	-
UCC-489	-	+	-	-
UCC-490	-	-	+	-
UCC-497	-	-	+	-
UCC-514	-	-	+	-
UCC-523	-	-	+	-
Control (+ve)	+	+	+	+

+ indicates the presence of the virus in the sample, - indicates the absence of the virus in the sample.

Dhanasekar and Reddy (2015).

The presence of viruses in a combination may result in synergism or antagonism effects within the infected plants. For instance, viruses acting in a synergistic manner enhance their infection rate, thus leading to the development of complexes of diseases (Syller and Grupa, 2016; Syller, 2012; Fondong et al., 2000). When viruses are antagonised when in a combination with other viruses, their rate of infection may be affected compared with single virus infection (Syller and Grupa, 2016; Syller, 2012). In the present study, genotype UC-366 was co-infected with CMV, CPSMV and CMV yet it exhibited mild symptom severity and low AUDPC compared to genotypes UCC-473 and UCC-484 which were infected with only CMV. This suggests that the three viruses (CMV, CPSMV and CMV) which infected UC-366 exhibited antagonism among them, in contrast with synergism as reported (Syller, 2012; Fondong et al., 2000). Nonetheless, it has been reported (Syller, 2012) that multiple infections may result in the generation of variants showing novel genetic features, and thus change the genetic structure of the viral population. Hence, understanding the interactions between CMV, CPSMV and CMV in cowpea may be of crucial significance for the understanding of viral pathogenesis and evolution, and consequently for the development of efficient and stable management strategies, as suggested by Syller (2012).

There were variations in the prevalence of the three viral species (CMV, CPSMV and CMV) detected by ELISA from the cowpea genotypes. CMV was found to be the most prevalent virus species in the study area, infecting 88.9% of the cowpea genotypes compared to CPSMV and CPMV and which infected only 22.2 and 11.1% of the cowpea genotypes respectively. The high infection rate of CMV observed in the current study could be due to the fact that it is highly seed borne in many cowpea varieties as reported by Thottappilly and Rossel (1987). The finding of this study also agrees with the report of Van Regenmortel et al. (2000) which states that *Bromoviridae* including CMV, is one of the most important widespread viruses in the world infecting the largest number (approximately 1000) of plant species. The higher infection rate of the cowpea genotypes by CMV compared to CPMV and CPSMV could suggest its relative persistence under adverse environmental conditions over the other viruses.

Leaf samples for all the accessions tested negative for SBMV, although the samples were symptomatic. Similar result was obtained by Ojuederie et al. (2009) in their study of serological detection of seed borne viruses in cowpea regenerated germplasm using protein A sandwich-ELISA. This could be due to low virus concentrations in the leaf samples or the presence of serologically variable strains of the viruses and the non-availability of antibodies specific to them (Aliyu et al., 2012).

Mean disease incidence declined between 4 and 6WAP in Apagbaala, UCC-489 and UCC-497, suggest symptom recovery (Table 2). In the case of Apagbaala, there was total recovery with the emergence of asymptomatic leaves following a systemic infection. According to Goshal and Sanfacon (2015), symptom is generally accompanied with reduced virus titres and sequence specific resistance to secondary infection. Jovel et al. (2007) had earlier argued that induction of recovery does not require a reduction of virus titre, and suggest that viral proteins RNAs or virus derived siRNA function to counteract host defense responses.

Average growth and yield performance of selected cowpea genotypes

Significant variations in the growth and yield among the cowpea genotypes (Table 3) could be due to different host-virus interactions (Anneke et al., 2013), age of plants at which plants were infected (Taiwo et al., 2007; Sastry and Singh, 1974) and genetic constitution of the

cowpea genotypes. Taiwo et al. (2007) reported that viral infection of cowpea at early age results in more severe symptoms, sometimes resulting in death of the affected plants. This may explain why the genotype UCC-484 which experienced early and 90% disease incidence had the highest severity score), highest AUDPC and had a low seed yield (2.482 t ha^{-1}) (Table 3). Significant negative correlation between AUDPC and seed yield in the present study (Table 4) further indicates that as the disease pressure increases the yield of the plant reduces. Similar result was obtained by Orawu (2007) when he evaluated cowpea genotypes for resistance to CABMV infection in Uganda. This observation could be due to the fact that the cowpea plants infected by viruses activate sophiscated defense pathways which operate at different levels, often at significant fitness costs, resulting in yield reduction as reported by Syller and Grupa (2016). Similarly, in maize-maize dwarf mosaic virus (MDMV) pathosystem, it was reported that the virus infection in maize induce necrotic disease which results in up to 91% yield loss and death of many plants especially when infection occur early(Uyemoto et al., 1981).

Although genotype UCC-473 that displayed early viral symptom, and had moderately severe infection and high AUDPC, it had a seed yield of 6.69 t ha^{-1} which was higher than the seed yield for all the cowpea genotypes (3.63 t ha^{-1}). This observation could be due to the plant's ability to tolerate viral infection or recover from the damage by the disease (Kessler and Baldwin, 2002; Teetes, 1996) or an infection by mild strain of the virus.

Generally, genotypes UCC-473, followed by UCC-316, and UCC-523 had mean seed yields of 6.690, 4.922 and 4.144 t ha^{-1} respectively, above the mean seed yield of 3.63 t ha^{-1} for all the nine cowpea genotypes.

Conclusions

The study identified 7 (Apagbaala, UCC-366, UCC-489, UCC-490, UCC-497, UCC-514 and UCC-523) out of 9 cowpea genotypes showing field resistance whilst the other two (UCC-473 and UCC-484) exhibited moderate resistance, in respect of their AUDPC values and final severity scores. ELISA serology revealed that each of the nine cowpea genotypes was infected with at least one of the three viral species namely CMV, CPSMV and CPMV, suggesting none was immune to virus infection. CMV was found to be the most prevalent virus species in the study area, infecting eight (8) out of nine (9) cowpea genotypes compared to CPSMV and CPMV which infected two and one cowpea genotypes, respectively. Genotype UCC-473, followed by genotypes UCC-316 and UCC-523 had mean seed yields of 6.690, 4.922 and 4.144 t ha^{-1}, respectively, above the mean seed yield of 3.63 t ha^{-1} for all the nine cowpea genotypes. Multi-locational evaluation of these three cowpea genotypes could be carried out prior to their release as varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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On farm demonstration of improved lettuce variety (*Lactuca Sativa*) in Southeastern zone of Tigray, Ethiopia

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Improved slow bolting lettuce variety is one of the important traits of lettuce for farmers in the study area to increase their production and income. Therefore a field experiment was conducted in Enderta district of South eastern zone of Tigray with the objective to evaluate and demonstrate the slow bolting lettuce variety, enhance the income of farmers by increasing production and productivity, and participate farmers research group in evaluation and demonstration of varieties. Improved (Tesfa Mekelle) and local (Paris Island) lettuce varieties were used as treatment for demonstration. 20 farmers were selected which have access to irrigation and willingness to participate. Both qualitative and quantitative data were collected. To measure the attitude of farmers towards the improved technology, a five-point Likert scale method was used. The data was analyzed using appropriate soft ware. To calculate the gap analysis; technology gap and technology index were used. The results revealed that farmers obtained an average of 412.89 and 283.83 qt ha-1 biomass yield of lettuce from improved and local varieties, respectively. This can clearly show that farmers can increase their lettuce yield and income by 45.5and 79.5% using the improved variety, respectively. Based on the farmers perception, the study also showed that farmers were satisfied in all parameters of improved lettuce like adaptability, slow bolting, marketability, tasty, softness of leaves and productivity. Therefore, research center and the offices of agriculture and rural development of the respective districts should take the lead to further popularization the variety for their respective mandate areas to boost production and productivity of lettuce.

Keywords: On farm demonstration, improved lettuce, yield, farmers perception.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) an annual leafy herb belongs to the family compositae is one of the most popular salad crops and occupies the largest production area among salad crops in the world. It is popular for its delicate,

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crispy texture and slightly bitter taste with milky juice as fresh condition. It is the most popular amongst the salad vegetable crops (Squire et al, 1987). Lettuce is one of the most important vegetable crops in temperate regions (Lebeda et al., 2007).

Lettuce is produced commercially in many countries and is also widely grown as a vegetable in home gardens (Kristkova et al., 2008). It is extremely sensitive to drought due to shallow root system. Lettuce can grow in a variety of soil types and climatic conditions. Lettuce, in general, is a crop with distinct temperature requirements. Optimal growing temperatures range between 23°C during the day and 7°C at night. It grows within an altitude of 1800 2100m.a.s.l. It is best grown in silt loams and sandy soils as these soil types provide better drainage and warm up more readily during the day, which is especially important during cooler periods of the growing season (Kristkova et al., 2008). There are a range of lettuce varieties available allowing gardeners to select those that are easy to grow, highly productive in limited space, and virtually pest and disease free. Lettuce is definitely one of the more "care-free" crops (Kiros et al., 2012). Lettuce is usually used as salad with tomato, carrot, cucumber or other salad vegetable and often served alone or with dressing. Likewise, in Tigray, Lettuce is an important cash and food security crop for small holder farmers and fresh salad retailers (Beyenesh et al., 2017).

Lettuce cultivation is common in many parts of Tigray, Ethiopia. The demand for lettuce production and consumption has increased; few varieties are available with only 'Paris Island' widely grown by farmers. This variety is an erect type and low in biomass yield. Moreover, early bolting is a major problem for the varieties being grown. Such varieties limit the time farmers have to harvest the crop to supply their production to the market on time. Thus, new lettuce varieties have been introduced and tested in different mandate areas. One promising variety, Tesfa Mekelle, showed slow-bolting and greater adaptability. However, demonstration of this lettuce variety in farmers' fields was not done widely in order to promote this variety in other areas. It is also vital to demonstrate this slow-bolting variety in comparison with local lettuce variety under farmer's condition. Therefore, this study was initiated with the objective of demonstrate the improved slow bolting lettuce variety on production, productivity with the local variety.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Enderta which is one of the six districts in the Southeastern administrative zone of Tigray (Figure 1). It is geographically located at a longitude of 13°:15`:00`` N and a latitude of 39°:30`:30`` E with an altitude ranging from 1500 to 2300 m.a.s.l. It shares borders with Kilte`awlaelo district in the north, Hintalo Wajirat in the south, Afar regional state in the east and the district of Degu'a Tembien in the west (Almaz, 2008). The district covers a total area of 89,812 km^2 of which 30,062 hectares are agricultural land. The total population size is 129,886 (CSA, 2011). It constitutes 17 sub-districts and 67 villages. The capital city of the region, Mekelle, is encircled within Enderta making it more advantageous to the district from market proximity point of view. The agro-climatic is mainly (96%) warm mild with the remaining 3 and 1% hot low land climate and temperate climate, respectively. Annual average rainfall ranges from 450 to 550 mm. Smallholder mixed farming remains the single largest contributor to the livelihoods of the population. Major crops grown in the district include teff, wheat, barley, sorghum, millet, oil seeds, pulse seeds, horticultural crops including vegetables especially leaf vegetables (Figure 1).

Sampling techniques and experimental design

The demonstration was conducted in Enderta district tabia Arato under irrigation. Twenty farmers were selected based on their interest in the technology, access of irrigation, willingness to manage and allocate field trial for the activity with collaboration of extension agents. The lettuce varieties were sown in the nursery and the seedlings were transplanted at 5-6 leaf stage to well prepared beds in the field. The improved lettuce variety called Tesfa Mekelle and the popular local variety Paris Island were planted in each farmer's field. Each variety was planted in 100 m^2 demonstration plot in 20 farmers field and each farmer consider as replication. Rows were spaced at 60 cm and40 cm spacing between plants. Date of planting was the same for both varieties. The experiment followed a mother trial approach in which researchers designed and farmers implemented (Witcombe et al., 2002). All necessary crop husbandry practices such as fertilization, weeding and hoeing were implemented as recommended. The field was irrigated every week to meet the water requirement of the crop.

Data collection and analysis method

Biomass yield that was determined by Fresh Leaf Weight: The total fresh biomass yield from both cultivars was measured by uprooting them from the ground and removes the soil from the root part of plant loose soil and weigh immediately. The height of the main plant was determined by measuring from the border of the soil to the top of the main plant stem. Number of Leaf per Plant was determined by counting the healthy leaf by selecting ten plants randomly from each plot and Leaves diameter(width) was measured by selecting ten plants randomly from each plot by measuring the width at the middle part of the leaves (at widest part of the leaves).

Farmers' point of view on the attributes of Tesfa Mekelle based on the composite indicators of yield and yield components was collected using Likert scale method, a format that is preferred by Derrick and White (2017). Group discussion (2 groups) was undertaken to collect data on farmer's opinion on the variety. Checklists were used for interviewing the participants to assess their interest in the technology for promotion. Production costs and benefits were collected to see the profitability difference of the treatments.

After data collection, coding and entering the data into SPSS version 20.0 computer program were done for the analysis. Data (quantitative data) were analyzed using t-test and descriptive statistics such as mean, standard deviation, percentage and frequency distribution to compare mean of varieties while qualitative data were analyzed through qualitative interpretation and summarization. ***G***ap analysis was conducted for technology gap, extension gap and technology index using the formulae of Samui

Table 1. Biomass yield of improved lettuce variety Tesfa Mekelle and local variety Paris Island.

Commodity	Yield (qt ha^{-1})						
	Min	Max	Mean	Standard deviation	t	SEM*	P-value
Tesfa Mekelle	350	456.28	412.89	34.49	7.4	14.5	0.000
Paris Island	236.58	312.56	283.83	25.22		9.6	

*SEM- standard error difference.

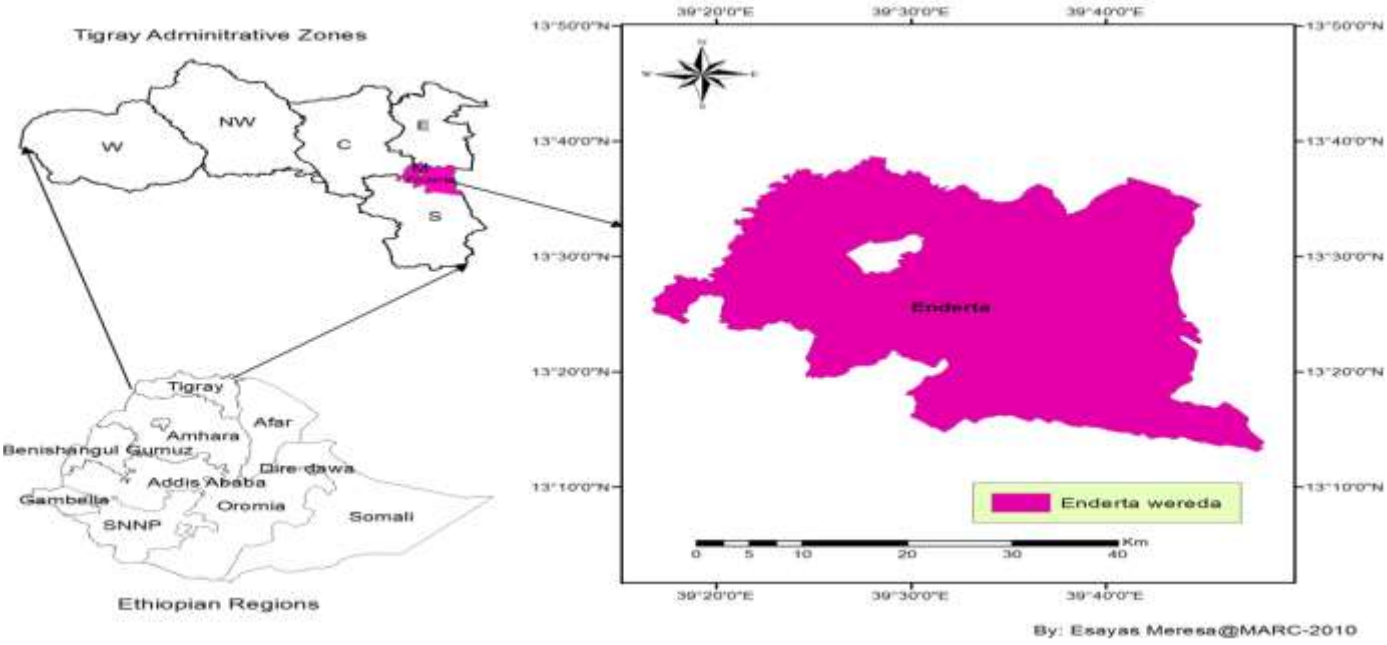

Figure 1. Map of the study area.

et al., (2000) as follows:

Technology gap = Potential Yield – Demonstration Yield
Extension gap = Demonstration Yield – Yield under farmers practice
Technology index (%) = ((Potential Yield – Demonstration Yield) / Potential Yield)) ×100

RESULTS AND DISCUSSION

Comparisons of biomass yield

The improved variety Tesfa Mekelle showed higher biomass yield than local variety Paris Island at the 20 locations. Moreover, the minimum yield of Tesfa meklle variety was higher than maximum of local variety (Table 1). An independent sample t-test was conducted to compare the mean difference between improved variety and local with respect to biomass yield. The t-test result also showed that there is statistically significant mean difference between the two groups at less than 1% probability level (t=7.4) (Table 1). Similar yield enhancement in different crops in demonstration have been documented by Hiremath et al. (2009), Mishra et al. (2009), Kumar et al**.** (2010) and Dhaka et al., (2010***).*** Soto-Cerda et al., (2014) revealed that one important way to increase agricultural productivity is through the introduction of improved agricultural technologies and management systems. This may lead to increase the production efficiency of crop commodity at smallholders' level that is an important step to attain food self-sufficiency for the country (Figure 1). The observed technology gap may be due dissimilarity in the soil fertility status, weather condition and other management practices (Mitra et al., 2010; Katare et al., 2011; Tiwari et al., 2013). Variety-wise location specific recommendation with full package of practices and other pre-requisite appears to be necessary to minimize the technology gap for yield level under different situations. Such steps would boost up the production and bring more prosperity to the farming community. The technology index shows that the feasibility of the evolved technology at the farmers' fields,

Table 2. Yield, technology gap and technology index of demonstration.

Variety	Yield (qt ha^{-1})	Yield increment (%)	Technology gap (qt ha^{-1})	Technology index%
Tesfa Mekelle	412.89	45.5	87.11	17.4
Paris Island	283.83	-	-	-

Figure 2. Illustration of demonstration plots showing bolting characteristics of the varieties

as lower the value of technology index more is the feasibility of the technology (Jeengar et al., 2006) (Table 2).

Agronomic performance

Tesfa Mekelle showed good performance for some agronomic parameters that contributed for biomass yield over Paris Island (Figure 2). The mean of stand count and leaf number of Tesfa Mekelle was 43 and 45.6 respectively which is higher than the local variety. While the average stands count and leaf number of Paris Island was 37 and 38.6 respectively. Moreover, the average plant height and leaf diameter of Tesfa Mekelle was 28cm and 49.6 cm. whereas Paris Island was 22 and 36 cm. This indicates that Tesfa mekelle has a good Agronomic performance , these contributes to harvest more yield (Figure 3).

Cost benefit analysis of improved lettuce verses local

Data from cost benefit analysis were presented in Table 3. This implied that the net benefit gained from the improved lettuce variety is higher by 79.5% than that of the local cultivars. Therefore, higher productivity and profitability made improved lettuce production more competitive implying that the need for encouragement of improved variety in the study area from economic as well as food security perspective.

Perception of farmers towards the improved versus local variety

The percentage scores of farmers' response to the perception statements of each attributes that relate to perceived technological characteristics are given in the Table 4. In general, the host communities have positive perception on the below mentioned attributes of the variety.

Focus group discussion result

The farmers' feedbacks on the use of the improved variety are as follows.

(1) All farmers in the groups agreed that improved variety (Tesfa Mekelle) was really adaptable and real slow bolting
(2) They said that the improved variety highly preferable and tasty
(3) In comparison with local one the improved variety have high market quality demand which they maintain the

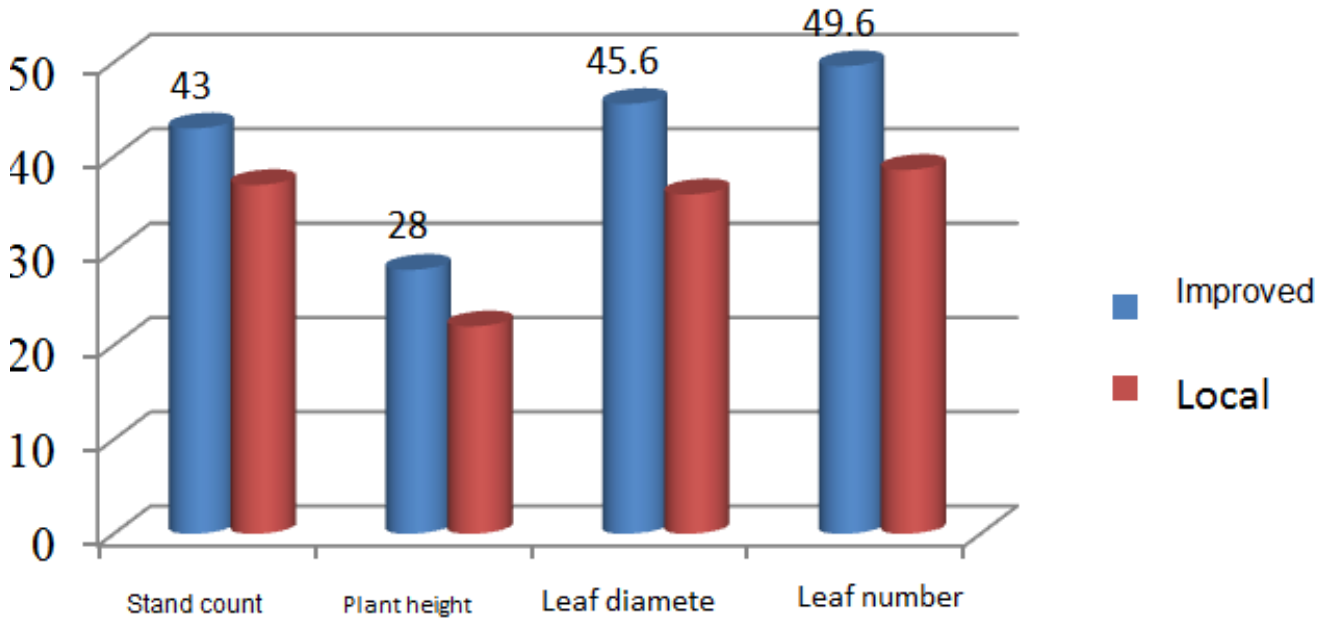

Figure 3. Aveage plant height, average leaf diameter (width), and leaf number.

Table 3. Cost benefit analysis and profitability of sample farmers (Birr[1]per hectare).

Variety	Average cost of production (Birr)	Average qt produced	Average price/qt	Total revenue	Net revenue (Birr)
Improved	15000	412.89	650	268378.5	253378.5
Local	15000	283.83	550	156106.5	141106.5

Table 4. Farmers' perceptions on characteristic of lettuce variety, 2014 (N=20).

Attributes	Perception level (%)				
	Very poor	Poor	Neutral	Good	Very good
Early maturity	-	-	-	-	100
Germination ability/growth habit	-	-	-	25	75
Bolting	-	-	-	-	100
Plant size	-	-	-	-	100
Leaf width	-	-	-	-	100
Plant height	-	-	-	-	100
Insect resistance	-	-	-	-	100
Disease resistance	-	-	-	-	100
Drought tolerance	-	-	-	-	100
Leaf no per plant	-	-	-	-	100
Length of maturity	-	-	-	-	100
softness of the leaf	-	-	-	-	100
Marketability	-	-	-	-	100
Taste	-	-	-	-	100

[1]Ethiopian Birr is equivalent of 0.0365 US Dollar (National Bank of Ethiopia accessed on March 25, 2018)

color preference and plant size.
(4) The improved variety (Tesfa Mekelle) has soft leaves as compare with existing one.

Conclusions

The results of the present study revealed that improved Tesfa Mekelle lettuce variety had good acceptance by farmers with its quality, yield, late flowering or slow bolting and high market acceptability. It should be bear in mind that any technology or variety can perform as expected if and only if all the recommended management practices are properly applied. Therefore, the improved lettuce should be popularized to large areas and number of farmers of their respective mandate area there to boost production and productivity of lettuce through contribute to food security of farming households.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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