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African Journal of Plant Science

Full Length Research Paper

Genotypic screening of tomato's *AREB 1* gene for drought tolerance and computational protein structure prediction

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In this investigation, the coding sequence of the drought-stress inducible gene *AREB1* in tomato derived from cDNA indicated 100% identity with the reference gene in the NCBI PlantEnsembl database. The protein structure of the *AREB1* sequence derived from polymerase chain reaction from tomato DNA template was done using ExPASy and its protein parameter tools ProtParam. The structures of *AREB1* protein showed a MolProbity score of 1.49. Multiple sequence alignment of *AREB1* gene from 20 tomato genotypes revealed a phylogenetic tree with five clusters, each with the same evolutionary trend. The nucleotide sequence analysis showed higher similarities among the selected tomato genotypes. This indicated the conserved nature of the gene among the genotypes.

Key words: Tomato, drought, resistant, *AREB1*gene, *AREB1*protein.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a fruit cultivated and consumed worldwide. Mostly red in colour with different sizes and shapes. Though cultivated worldwide tomato is reported to be originated from Western South America (Acquaah, 2008). Tomato fruit has being classified as berry and consumed in different ways, raw, cooked, sauces, salads, and drinks (Singh et al., 2012). Tomato plants typically grow to 1-3 m (3-10 ft) in height. They are vines that have a weak stem that sprawls and typically needs support (Acquaah, 2008). The tomato sizes vary according to the cultivar, in the range of 0.5-4 inches (1.3-10.2 cm) (IPGRI, 2015). Many water deficit (Drought) stress-inducible genes have been highlighted and found to be activated by abscisic acid ABA. The *AREB-I* gene mutant family are more tolerant to ABA than are the other single and double mutants with respect to primary root growth, and it displays reduced drought tolerance (Takuya et al., 2010). AREB/ABF transcription factors are induced as the result of environmental stresses, only AREB1 is reported to be regulated by ABA-dependent phosphorylation (Fujita et al., 2005). *AREB1* needs to be activated fully for ABA (Fujita et al., 2005), Abscisic acid is a plant hormone that regulates many important processes in plant metabolism, such as seed germination and dormancy, opening and closing of stomata, abscission, and adaptation to water stress (Redenbaugh et al., 1992).

There are reports on drought resistant gene (*AREB*) in

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Table 1. Tomato accessions and their place of collection.

S/N	Accessions name/ number	Species name	Place of collection
1	NG/SA/01/10/002	*Solanum lycopersicum* L.	NACGRAB
2	NGHB/09/120	*Solanum lycopersicum* L.	NACGRAB
3	NG/AA/SEP/09/045	*Solanum lycopersicum* L.	NACGRAB
4	NHGB/09/113	*Solanum lycopersicum* L.	NACGRAB
5	NG/AA/SEP/09/044	*Solanum lycopersicum* L.	NACGRAB
6	L00170	*Solanum lycopersicum* L.	NACGRAB
7	NGHB/09/114	*Solanum lycopersicum* L.	NACGRAB
8	NG/AA/SEP/09/013	*Solanum lycopersicum* L.	FRIN
9	NG/AA/SEP/09/042	*Solanum lycopersicum* L.	FRIN
10	L00169	*Solanum lycopersicum* L.	FRIN
11	VG-004/83	*Solanum lycopersicum* L.	FRIN
12	GRC1936/04	*Solanum lycopersicum* L.	FRIN
13	GRC1925/04	*Solanum lycopersicum* L.	FRIN
14	VE-027/83	*Solanum lycopersicum* L.	FRIN
15	GR279/99	*Solanum lycopersicum* L.	FRIN
16	Mylo	*Solanum lycopersicum* L.	NACGRAB
17	Mylati	*Solanum lycopersicum* L.	NACGRAB
18	GRC1807/04	*Solanum lycopersicum* L.	NACGRAB
19	L00190	*Solanum lycopersicum* L.	NACGRAB
20	Karabola	*Solanum lycopersicum* L.	NACGRAB

tomato; however, to the best of our knowledge, no such report has been published on the *AREB* gene in a wide

range of tomato genotypes, or on the prediction of protein structure and computational protein analysis of the *AREB* gene in tomato genotypes. Plant breeding through a conventional way to improve drought resistance, in many cases, is too slow due to lack of precise molecular and genetic information on drought tolerance associated genes and their regulations. Nigeria is the largest producer of tomato in Sub-Saharan Africa, and ranks 13th in the world. Notwithstanding, the production faces challenges with storage, distribution abiotic and biotic stresses (GAIN, 2018). This is due to climate change such as increase in temperature, evaporation and drought (He et al., 2003).

MATERIALS AND METHODS

Plant materials

The seeds of the selected tomato genotypes were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), North Centre Zone, Badeggi Nigeria, Forestry Research Institute of Nigeria (FRIN), Department of Agricultural Technology, Federal College of Forestry, Jos, Nigeria and National Centre for Genetic Resources and Biotechnology (NACGRAB), Department of Plant Genetic Resources, Ibadan. Seeds of each genotype were germinated on nursery beds. After 14 days of germination fresh leaves of the seedlings were collected for DNA (Table 1).

DNA and RNA isolation

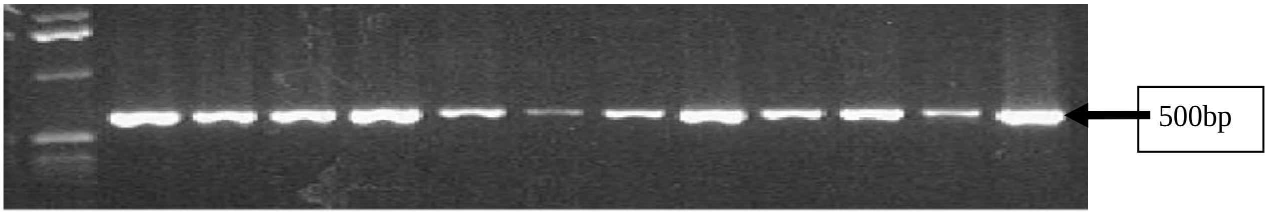
Leaves of the tomato genotype (weight 100 g) were frozen in liquid nitrogen. The leaves were ground into a fine powder in a free chilled mortar. The powder was transferred into a tube of pre-warmed CTAB buffer and the mixture was incubated at 65°C for 20 min. DNA was isolated following the CTAB protocols of DNA extraction (Singh et al., 2012).

Drought resistant gene sequence retrieval and primer design

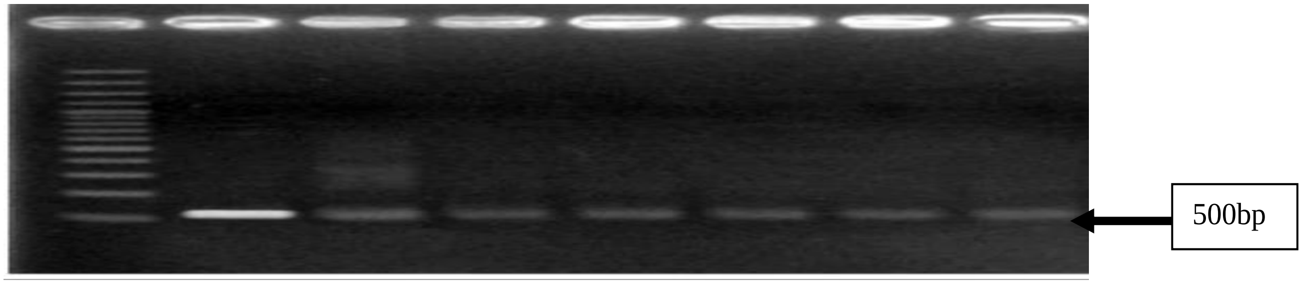
AREB1 also known as *AREB*; *LeAREB*; *SlAREB1* gene sequence (NCBI Reference Sequence: NC_015441.3) was retrieved from the National Center for Biotechnology Information (NCBI) database. The sequence was used to design primers for the *AREB* gene amplification in the selected tomato genotype. The retrieved *AREB* gene sequence was used to design primers with the following parameters: primer length 18-30 bp, melting temperature 50-60°C, GC percentage 40-60 and product size: 160-500 bp, using Vector NTI software (Ja'afar et al., 2018).

Polymerase chain reaction (PCR) Amplification of the *AREB* Gene

Polymerase chain reaction amplifications of *AREB* gene in the selected tomato genotype was carried out using *AREB* gene specific primers in a total volume of 25 µl using a C1000 Thermal Cycler (Bio Rad, USA). Each 25 µl volume of reaction mixture contained 50 ng of genomic DNA as template, 1X Taq polymerase buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mix, 0.4 pM each of the forward and reverse primer, 1 U of Taq polymerase. The optimized condition was initial 5 min incubation at 97°C for complete



a. Ladder:1Kb



b. Ladder:1Kb

Figure 1. Polymerase chain reaction of the *AREB* gene in 19 genotypes of tomato. (a) DNA bands of NG/SA/01/10/002, NGHB/09/120, NG/AA/SEP/09/045, NHGB/09/113, NG/AA/SEP/09/044, L00170, NGHB/09/114, NG/AA/SEP/09/013, L00169, VG-004/83, GRC1936/04 and GRC1925/04, tomato genotypes PCR with *AREB 1*. (b) DNA bands of VE-027/83, Karabola, L00190, GRC1807/04, Mylati, Mylo, GR279/99, VE-027/83 and GRC1936/04 tomato genotypes PCR with *AREB 1*.

denaturation, followed by 38 cycles consisting of 94°C for 1 min, 55- 60°C (varying with the primer pair) for 1 min., 72°C for 2 min, and finally 72°C for 10 min. The experiments were repeated twice (Molla et al., 2015).

Resolving of all PCR products was performed in a vertical non-denaturing 3% Agarose gel electrophoresis system at constant 90 V with 1X TAE (Tris acetate EDTA) buffer (pH = 8.0). The gel was stained with ethidium bromide solution and visualized using a gel documentation system (Protein Simple, USA) adopting the methods of Botstein et al. (1980).

Gene sequencing and sequence submission to gene bank

The PCR products were purified with Gel Extraction Kit, the products were used for sequencing. The forward and reverse contigs was edited and joined to make a complete sequence, which was used for *in silico* analysis (Molla et al., 2015).

Sequence and phylogenetic analysis

The *AREB* gene sequences from the DNA of the selected tomato genotypes was aligned with the original reference sequence and edited for SNPs and INDEL detections. The phylogenetic relationship was designed using a molecular evolutionary genetic analysis tool (MEGA).

Computational protein analysis and structure prediction of *AREB1* protein

The open reading frame (ORF) of the AREB nucleotide sequence was translated into amino acid using ExPasy translation tool and aligned to the amino acid residue of other sequences of tomato using a multiple sequence alignment tool. The 3-D structure prediction of AREB protein was performed using SWISSMODEL program and NCBI prediction tool (Botstein et al., 1980).

Data analysis

The data obtained from plant DNA was analyzed using bioinformatics software: MEGA, SWISS model and ExPasy tools.

RESULTS

AREB gene amplification

The primers designed from the *AREB* gene of tomato showed amplification in all the twenty selected genotypes after PCR using genomic DNA of each genotype (Figure 1a and b). The PCR products were extracted and purified using gel extraction kit and then sequenced. The products were check on 1.2% agarose gel electrophoresis to view the respective sizes. All the amplified bands studied correspond to 500 bp size with the aid of 1 kb ladder (Figure 1a and b).

Gene sequencing

The amplified PCR products (Genomic DNA and cDNA derived sequences) were sequenced using next generation contigs (forward and reverse), and edited

Table 2. AREB1 gene sequences of 20 *Solanum lycopersicum* genotypes using the blast approach of the Ensembl plants database.

Name of Genotypes	Length	Score	Orientation	%ID	Chromosome no.	Location	E-value
NG/SA/01/10/002	500	200	Reverse	100.0	1	4:63589828-63590027	1.3e-108
NGHB/09/120	500	500	Reverse	100.0	1	4:63589328-63589827	0.0
NG/AA/SEP/09/045	484	484	Reverse	100.0	1	4:63588827-63589310	1.3
NHGB/09/113	500	500	Reverse	100.0	1	4:63588327-63588826	2.3e-08
NG/AA/SEP/09/044	500	283	Forward	96.0	1	4:63587757-63588039	3.7e-158
L00170	500	500	Reverse	100.0	1	4:63587257-63587756	0.3
NGHB/09/114	500	500	Forward	100.0	1	4:63586757-63587256	0.0
NG/AA/SEP/09/013	500	480	Forward	95.5	1	10:10397596-10397617	5.1
NG/AA/SEP/09/042	500	500	Forward	100.0	1	10:2354125-2354142	0.0
L00169	500	500	Forward	100.0	1	11:32688512-32689011	0.0
VG-004/83	500	500	Forward	100.0	1	11:32689012-32689561	0.0
GRC1936/04	500	500	Forward	100.0	1	11 32689562 to 32690061	0.0
GRC1925/04	500	500	Forward	100.0	1	11 32690062 to 32690561	0.0
VE-027/83	500	500	Forward	100.0	1	11 32690562 to 32691061	0.0
GR279/99	500	496	Forward	99.8	1	11 32691062 to 32691561	0.0
Mylo	500	500	Forward	100.0	1	11 32691562 to 32692061	0.0
Mylati	500	450	Forward	95.0	1	11 32692062 to 32692611	0.0
GRC1807/04	500	500	Forward	100.0	1	11 32692612 to 32693111	0.0
L00190	500	500	Forward	100.0	1	11 32693112 to 32693611	0.0
Karabola	500	482	Reverse	92.5	1	10 21263510 to 21263735	1.5e-83

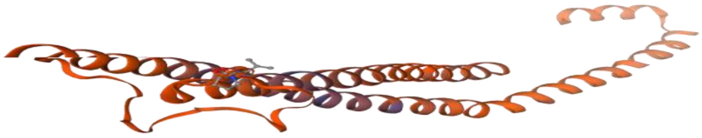


Figure 2. The predicted 3-D structure of the GC box binding domain of the *AREB1* protein of the tomato reference sequence with alpha helical structures of 2.6 amino acid residue and alphatic index of 56.66.

using Vec Screen software. All of the sequence analyses revealed a 500 bp sequence length. The genomic sequences of the *AREB1* gene from 20 tomato genotypes were aligned with a reference sequence of tomato obtained from ensemble plant (Gramene Database), using a multiple sequence alignment method. The aligned sequence revealed the presence of the conserved AREB1 gene throughout the selected genotypes (Table 2).

Computational analysis and structure prediction of tomato *AREB* genes family

The ExPASy bioinformatic tool was used to translate the DNA sequences into amino acid sequences and to construct the 3-D secondary structure of the *AREB1* protein of tomato generated using the SWISS-MODEL program (Figure 2). The structural protein properties predicted by the ProtParam tools had a MolProbity score of 1.49, and Ramachandran favored of 95.83%.

Phylogenetic relationships analysis of the *AREB1* gene among the selected tomato genotypes

The phylogenetic dendrogram (Figure 3) was generated using a Neighbor-Joining method, with MEGA software based on the data set from the tomato sequences generated from the product of PCR with *AREB1* gene primers. The results showed an optimal tree with the sum of branch lengths that equals 68.50928440. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is

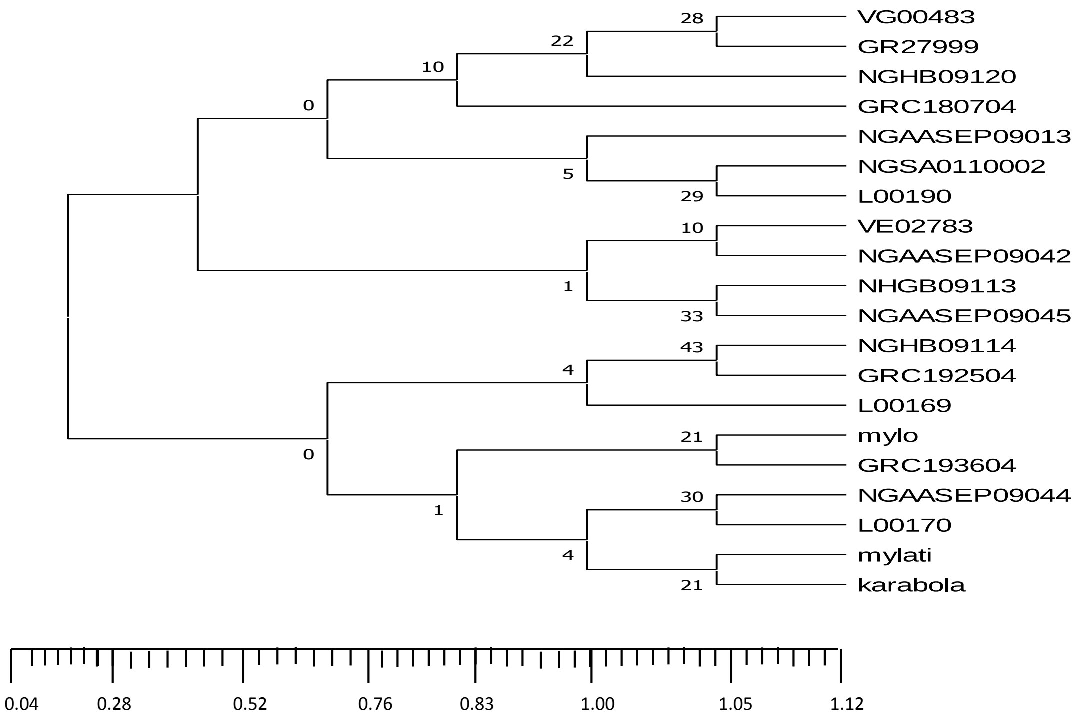


Figure 3. The phylogenetic tree based on nucleotide sequence of tomato genotypes including scale bar for base sequence distances.

shown next to the branches (Figure 3). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding (Figure 3).

DISCUSSION

Sequence alignment of the *AREB1* gene from the genomic DNA of the selected tomato genotypes was successfully done using specific primers derived from the AREB1 gene. The DNA isolated revealed a sequence with a 500-bp length, which is similar to some of the reference genes in the Ensembl Plants data base. Sakshi and Kavita (2020) reported that conventional separation by agarose gel electrophoresis results only in a single DNA band and is largely non-descriptive. However, Zhou et al. (2016) reported that the gene *ABF1* was clearly induced by drought, high salinity and ABA treatments, although its expression levels were low even under stress conditions compared with those of *AREB1, AREB2* and *ABF3*. The *AREB1* gene along with *Dreb* gene were identified as a strong genes in the drought responsive pathways in *Arabidopsis*, tomato, rice and other members of solanaceae (Alves and Setter, 2004). *AREB1* gene is found to have a very poor sensitivity for abscisic acid; and, therefore, this suggested that it could be involved in the ABA independent pathway (Barry, 2001).

The local coordinate system was defined using the main chain atoms of each amino acid, as described previously. This is the foundation of neighborhood analysis for each amino acid. The structure shows a property of free proline and glycine betaine which are the major biochemical parameters of abiotic resistance in plants. Free proline contents and glycine betaine are the signals that the plant shows in response to stress and can be used to measure the level of tolerance to a particular stress in plants (Ja'afar et al., 2018). The sequence-based phylogenetic tree generated (Figure 3) showed five distinct clusters with different bootstrap values. Those genotypes in the same cluster are found to be closely related and share a common evolutionary trend. A similar trend was reported by Molla et al. (2015) for *Oriza sativa* and *Oriza glaberrima* with some of the closely related species occupying the same cluster.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Efficacy of soil solarization on the control of root-knot nematodes infecting eggplant (*Solanum melongena*) in Plateau State

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The efficacy of soil solarization was tested for the control of root-knot nematodes in Foron District of Barkin Ladi Local Government Area of Plateau State using three commercially available cultivars of eggplant (*Solanum melongena*) namely: Yallo Bello, Chida Masoyi and Farin Yallo. Two levels of soil solarization based on time of exposure namely: Four weeks, five weeks and a control were employed. Soil temperature for each bed was taken weekly in the morning and afternoon using soil thermometer at different soil depths. Results revealed that growth and yield parameters of eggplant namely plant height, stem girth, number of leaves, number of fruits, and fresh weight of fruits grown in solarized soil were significantly higher than those of the control (unsolarized soil) at 0.05 level of probability. More galls were seen on the roots of unsolarized plants, followed by the four weeks and the five weeks' solarization had the least. This is indicative of the effectiveness of soil solarization in the control of nematodes, especially for longer periods of solarization. The three cultivars of eggplant did not differ with reference to soil solarization. Soil solarization could be an effective tool for nematode control on the Plateau since it is cheaper, has no phytotoxic effects, and does not constitute environmental and health hazards. The technique can be improved with more investigation's on length of exposure and improvement of the durability of the polyethylene film. Continuous use of these polyethylene films will reduce the cost of buying the polyethylene films repeatedly when it is needed.

Key words: Soil, Solarization, *Solanum melongena*, nematodes, efficacy.

INTRODUCTION

Solanum melongena, popularly called "eggplant" (family *Solanaceae*) a plant of enormous importance, ranking third out of five useful vegetables (Choudhary and Gaur, 2009). It is mainly used as a food crop and also has various medicinal uses that make it a valuable addition to the diet (Daunay and Janick, 2007). Despite the importance of this crop, there are various production constraints, which include diseases and pests such as

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nematodes, slugs, snails and caterpillars (Choudhary and Gaur, 2009; Stirling, 2014).

Plant-parasitic nematodes are by nature organisms that impair crop health and reduce their yields and their presence has been found to cause profound metabolic disturbance throughout the plant (Stirling, 2014). Nematodes, apart from being destructive, predispose plants to other pathogens and serve as carriers of other pathogens (Stirling, 2014). This has been a major cause for concern to farmers because they cause a series of losses, particularly in plant yields. Therefore, there is the need to control this "cankerworm" eating deep into the fabrics of crop production. There are various ways of controlling nematode infection, including chemical, cultural, biological, physical, and genetic methods (Stirling, 2014).

Chemical control is reported to be a very effective method for controlling nematodes however, it has its tolls on the environment and can constitute health hazard (Aktar et al., 2009; Stirling, 2014). It is also very expensive and mainly used for crops of very high economic value that can produce more than enough yields to upset the cost of control (Stirling, 2014), thus there is the need to investigate soil solarization as a physical method of controlling nematodes.

Soil solarization is the application of soil mulch (polyethene) to trap solar energy in order to increase soil temperature to levels that are lethal to microorganisms that cause diseases to crops of economic importance (McSorley et al., 2006). The principle of soil solarization is accomplished by manipulating the energy balance of the soil. This energy balance depends on the direction and magnitude of the net heat exchange between the soil and the atmosphere. As the soil is exposed to solar radiation, it accumulated heat throughout the day (Hasing et al., 2004). Soil temperatures are maintained within a range that is determined by conditions such as climate and soil characteristic. It is important to note that the mulch used during solarization also reduces heat loss without significantly interfering with the absorption of solar energy. This results in increased soil temperature.

Soil solarization was initially pioneered in countries of the Middle East where intense solar radiation and high temperatures are appropriate for solar heating (Hasing et al., 2004). It has been known to affect not only soil-borne pathogens but also other organisms and abiotic factors that indirectly affect plant development and growth (Hasing et al., 2004). There is a scarcity of information on the efficacy of soil solarization in the control of plant-parasitic nematodes of crop plants in Nigeria even when Nigeria is a tropical country with abundant sunlight. This is a great potential for effective nematode control to be achieved by this method. It is against this background that the present investigation has been designed to assess the effects of soil solarization for the control of nematodes infecting eggplant and to determine the least time of exposure that is most effective for soil solarization.

MATERIALS AND METHODS

Study area

This research was conducted in Foron District of Barkin Ladi Local Government Area of Plateau State, Nigeria.

Cultivars of eggplant

Three cultivars of eggplant namely, "Yallo Bello", "Chida Masoyi" and "Farin Yallo" were used in this investigation. The seeds of these three cultivars were collected from local farmers.

Research design

A complete randomized block design was used in assigning treatments and cultivars to the various plots and was replicated 72 times. Analysis of variance was done using SPSS version 23.0 (IBM SPSS Statistic, Version 23.0. Armonk, NY: IBM Corp).

Collection of soil samples

Soil samples from root rhizosphere were randomly collected into well-labelled polyethylene bags from different parts of the farm using a hand trowel. These soil samples were brought to the Botany Laboratory, University of Jos and processed for nematode extraction. The modified Baermann funnel method was employed for this extraction (Hamilton et al., 2009; Sato et al., 2009). The nematode populations were estimated per gram of soil and average counts from 1 ml of the homogenized extract of 50 g of the soil sample.

Estimation of nematode population before planting

This was done by counting the number of nematodes in 1 ml of the homogenized suspension under a binocular research light microscope at x40 magnification. An average of two root-knot nematodes was found in 1 ml of the homogenized suspension from 50 g of soil, which approximates 50 nematodes in 250 g of the soil. Therefore, this implies that for every 250 g of soil from the experimental site, there were 50 nematodes.
50 g of soil ----- 1 ml ----- 2 nematodes
250 g of soil ----5 ml ----- 50 nematodes

Nursery preparation

Steam sterilization was done between 70 and 100°C soil and cow dung in the ratio 3:3:1 of cow dung, sharp sand and top soil, respectively. This was to kill soil pests including nematodes and filled into three wooden trays. Seeds of three cultivars of eggplant that were previously soaked for four days and allowed to drain were then broadcasted on the soil and a garden fork was used to mix up the seeds and the soil.

Preparation of the beds for transplanting

Beds were raised to about 10 inches and mulched with a black polyethylene film measuring 1.5 × 1.5 m wide and 38 µm thick. These beds were kept wet and mulched. Wetting of the beds continued once every week to keep the soil moist and aid heat conduction within the soil for the period of solarization. These

Table 1. Root galls rating scale.

Number of galls	Root index	Resistance rating
0	0	immune
1-2	1	Resistant
3-10	2	Moderately resistant
11-30	3	Moderately susceptible
31-100	4	Susceptible
>100	5	Highly susceptible

Taylor and Sasser (1978).

polyethylene films were held firmly in place using stones. Soil temperature was monitored once every week at 10: 00 am and 4: 00 pm at 5, 10 and 20 cm depth for each bed. This was done for both solarized and unsolarized treatments throughout the period of solarization. The minimum and maximum temperatures for each week were recorded.

Soil solarization

Solarization films were installed for four- and five-weeks' treatments. The soil was kept clean and allowed to stand for this period of treatment. The unsolarized soil (control) was also kept clean but not moistened for the period of treatment. After four and five weeks of solarization, seedlings from the nursery were transplanted and solarization was discontinued. Fertilizer was applied at 168/224 kg hectare of Urea and Nitrogen, Phosphorus and Potassium (NPK). Irrigation was done to sustain seedlings for the period when there was no rainfall and discontinued at the start of the rains. Dichlorvos 76% EC, a pesticide, was applied to prevent shoot pests from perching on the leaves since the start of planting was the peak of hot and conductive weather for these pests. Planting was done at 35 × 35 cm between and within rows. Eggplants which were about 80% matured were harvested after three months of planting. These plants were in the field for 13 weeks after transplanting

Growth and yield parameters

Plant growth and yield parameters measured include plant height, stem girth, number of leaves, number of fruits, and fresh weight of fruits.

Estimation of the nematode population

The nematode populations in the soil were estimated at the end of the research and analysed statistically. Fifty soil samples were taken from each of the 36 plots, placed in well- labelled polyethene polyethylene bags, and brought to the laboratory. Each of the plots represented the four and five weeks of treatment, their control (unsolarized) and their replications. The modified Baermann funnel was used after which the set-ups were dislodged and the nematode suspension was homogenized and average counts of nematodes per ml were done for the estimation of the nematode populations in the soil.

Observation of plants for nematode infection

Roots of the 72 eggplants were examined for galls. When galls were seen, they were counted, recorded and the gall indexes were calculated for each treatment. Root galls were rated according to the Taylor and Sasser (1978) scale, as shown in Table 1. Gall indexes were calculated by first placing each plant in a class and the average gall indexes of the plants was calculated by multiplying the class number by the number of plants in each class and the products were summed. The sum was then divided by the total number of plants for an average to determine the gall indexes.

RESULTS

Soil temperature was generally higher in solarized soil compared to unsolarized soil. These temperatures also varied with soil depth with the highest at the 20 cm depth and lowest at the 5 cm depth. Soil temperatures were also higher in the afternoon than in the mornings (Table 2). The results of this investigation revealed that the two levels of solarization, namely four and five weeks, generally resulted in higher mean plant height compared to the control (unsolarized). The plant height of eggplants grown in solarized soil for five weeks was significantly higher than those grown in four weeks solarized soil ($p<0.05$). The average plant height of plants grown on four weeks solarized soils were higher than those grown in unsolarized soil (control) but did not differ significantly ($p<0.05$) (Table 3).

Table 4 shows that the mean stem girths of plants grown in solarized soil were significantly higher than those grown in unsolarized soil at five and four weeks ($p<0.05$). The mean number of leaves per plant was generally higher for plants grown in solarized soil as compared to those grown in unsolarized soil (control). Plants grown in soil solarized for five weeks had a significantly higher number of leaves compared to those of plants grown in the control treatment. Although the number of leaves of plants grown in soil solarized for four weeks averaged higher than those grown in the control treatment, there were significant differences in the number of leaves per plant. The mean number of leaves per plant did not vary between the cultivars at $p<0.05$ (Table 5).

Table 6 shows the mean number of fruits per plant was generally higher in plants grown in solarized soil compared to those grown in unsolarized (control) soil.

Table 2. Average temperatures of solarized and unsolarized soil at three soil depths for morning and afternoon.

Time	Unsolarized			Solarized		
	5 cm	10 cm	20 cm	5 cm	10 cm	20cm
WK 1. 10.00am	26.62	28.22	28.32	34.62	35.83	35.89
4.00pm	28.81	29.33	29.64	38.57	39.65	39.75
WK 2. 10.00am	28.01	27.72	29.91	35.53	36.61	36.69
4.00pm	29.02	29.97	31.00	39.83	40.88	40.81
WK 3. 10.00am	28.32	29.99	30.00	34.55	35.65	35.70
4.00pm	31.62	31.32	33.91	38.54	40.67	40.77
WK 4. 10.00am	28.72	29.67	30.07	36.55	37.41	37.32
4.00pm	32.33	31.66	33.99	39.59	41.59	41.61
WK 5. 10.00am	29.91	30.79	30.11	38.32	39.51	39.61
4.00pm	33.07	33.33	33.00	41.27	42.07	42.11

Table 3. Mean plant height per plant of three cultivars of eggplant grown in solarized and unsolarized soil (control).

Solarization	Cultivars		
	Yallo Bello	Chida Masoyi	Farin Yallo
Four weeks	53.25^{b}	52.26^{ab}	44.08^{b}
Five weeks	67.21^{a}	63.10^{a}	63.59^{a}
Unsolarized control	42.14^{b}	41.66^{b}	35.53^{b}

Means with the different superscripts within the same column are significantly different at $p<0.05$ (LSD 11.29).

Table 4. Mean stem girth per plant of three cultivars of eggplant grown in solarized and unsolarized soil (control).

Solarization	Cultivars		
	Yallo Bello	Chida Masoyi	Farin Yallo
Four Weeks	3.21^{b}	3.20^{b}	3.10^{b}
Five Weeks	4.05^{a}	4.04^{a}	4.04^{a}
Unsolarized Control	1.41^{c}	1.31^{c}	1.20^{c}

Means with the different superscripts within the same column are significantly different at $p<0.05$ (LSD 0.33).

Plants grown for five weeks in solarized soil had significantly higher ($p<0.05$) mean number of fruits than the control treatment. The number of fruits on plants grown in soil solarized for four weeks did not differ significantly from the control treatment ($p<0.05$). The values for mean fresh weight of fruits are presented in Table 7. The mean fresh weight of fruits per plant was generally higher in plants grown in solarized soil compared to those grown in unsolarized (control) soil. Plants grown in solarized soil for five weeks had significantly higher mean dry weight of fruits than the control treatment ($p<0.05$). The mean fresh weight of fruits grown in soil solarized for four weeks did not differ significantly from the control treatment at $p<0.05$.

Table 8 shows the estimated number of nematodes per 50 g of soil after harvest at the different time intervals of solarization and the unsolarized (control). This was to ascertain if the population of nematodes reduced with solarization or the increase in growth and yield parameters just favoured solarized soil than the unsolarized control. Nematode populations were seen to be significantly higher and different for the control

Table 5. Mean number of leaves per plant of three cultivars of eggplant grown in solarized and unsolarized soil (control).

Solarization	Cultivars		
	Yallo Bello	Chida Masoyi	Farin Yallo
Four weeks	89.67^{ab}	84.33^{a}	75.67^{ab}
Five weeks	104.00^{a}	91.00^{a}	98.00^{a}
Unsolarized control	58.00^{b}	68.33^{a}	52.67^{b}

Means with the different superscripts within the same column are significantly different at $p<0.05$ (LSD 35.09).

Table 6. Mean number of fruits per plant of three cultivars of eggplant grown in solarized and unsolarized soil (control).

Solarization	Cultivars		
	Yallo Bello	Chida Masoyi	Farin Yallo
Four weeks	14.00^{ab}	15.67^{ab}	12.67^{a}
Five weeks	19.00^{a}	20.33^{a}	15.00^{a}
Unsolarized control	11.67^{b}	10.33^{b}	9.67^{a}

Means with the different superscripts within the same column are significantly different at $p<0.05$ (LSD 6.94).

(unsolarized) and the four weeks' treatment at $p<0.05$. Root gall indices differed amongst plants grown in solarized soil as compared to those grown in the unsolarized soil. The root gall index was 1.29≈1.3.

DISCUSSION

The results of this investigation revealed that there was an increase in temperature of solarized soil compared to the unsolarized soil. This agrees with the earlier report of Hasing et al. (2004) who observed a marked increase in the temperatures of solarized soil as compared to the unsolarized soil. This increase in the temperature may be attributed to the fact that the polyethylene films used as mulch during soil solarization trapped heat. The increase in soil temperature can also be associated with limited air circulation that consequently led to limited energy lost through evaporation, which is recovered as water condenses on the mulch thereby increasing the soil temperature via modification of optical characteristics of the water (Stirling, 2014).

Soil temperature also increased in the afternoon (4:00 pm) compared to the morning (10:00 am). This is in line with reports from Hasing et al. (2004) and Stirling (2014), that as the soil is mulched and exposed to solar radiation, it accumulates heat throughout the day because the mulch reduced heat loss. The temperature was also seen to increase with soil depth in the afternoon. Hasing et al. (2004) reported that peak temperature is normally experienced in the afternoon as soil depth increase.

Nematode populations were seen to be significantly higher and different for the control (unsolarized) compared to the four weeks' treatment. The estimated number of nematodes per 50 g of soil after harvest at the different time intervals of solarization and unsolarized (control) was carried out to ascertain if the population of nematode reduced with solarization or the increase in growth and yield parameters just favoured solarized soil than the unsolarized. Nematode population has been reported to reduce with increase time of solarization (Bacha et al., 2007).

The mean plant height per plant for plants grown in the three levels of solarization averaged higher than those on unsolarized soil (control). The five weeks' soil solarization treatment was more significant in nematode control than the four weeks ($p<0.05$) indicative by stem girth, number of fruits, number of leaves, fresh weight of fruits. Bacha et al. (2007) reported a reduced nematode population with increase time of solarization, which might have favoured the growth and yield parameters of eggplant. Soil solarization is also reported to improve soil characteristics that can influence crop performance such as nutrient concentration (Mauromicale et al., 2010).

Nematode populations were seen to have reduced with increased time of solarization. Soil solarization has been reported to reduce the nematode population between 37-100% (Candido et al., 2008). There is also a report that soil solarization reduced soil pest including nematodes, weed presence, and enhance soil chemical and physical properties, which in turn increases yield (Bacha et al., 2007).

Table 7. Mean fresh weight of fruits per plant of three cultivars of eggplant grown in solarized and unsolarized soil (control) at two different time intervals.

Solarization	Cultivars		
	Yallo Bello	Chida Masoyi	Farin Yallo
Four weeks	9.42^{ab}	8.78^{b}	8.61^{ab}
Five weeks	13.51^{a}	17.33^{a}	12.72^{a}
Unsolarized control	7.28^{b}	6.62^{b}	6.70^{b}

Means with the different superscripts within the same column are significantly different at $p<0.05$ (LSD 4.74).

Table 8. Estimation of nematode population eggplant grown in solarized and unsolarized soil (control) at two different time intervals.

Solarization	Cultivars		
	Yallo Bello	Chida Masoyi	Farin Yallo
Four weeks	3.33^{b}	3.67^{b}	2.33^{b}
Five weeks	2.00^{b}	1.67^{c}	1.67^{b}
Unsolarized control	5.33^{a}	4.00^{a}	4.33^{a}

Means with the different superscripts within the same column are significantly different at $p<0.05$ (LSD 1.37).

Root gall indices differed amongst plants grown on solarized soil as compared to those in the unsolarized soil. The root gall index of the plants was 1.29≈1.3 meaning the gall indices are greater than one but less than two which could imply that the plant cultivars used might be resistant.

In conclusion, solarization led to lower nematode population with consequently better plant performance than non-solarized treatment and the cultivars under investigations did not respond differently to solarization. Therefore, soil solarization could be an effective means of nematode control in the soil. It is cheaper and less stressful compared to other methods of nematode control. Besides, it is scientific and its adoption does not require much expertise. Solarization has no phytotoxic effects and does not constitute any environmental and health hazards.

Soil solarization can be improved with more investigations. Since mulch pigmentation plays an important role in the efficacy of the mulch, it is therefore recommended that varying thickness of the polyethylene films should be investigated for efficacy in soil solarization for the control of nematodes to detect the best, or more effective, polyethylene films. Also, checking and improving the durability of this polyethylene is essential to ensure the continuous use of these polyethylene films because these plastic films degrade actively when exposed to ultraviolet radiation. Ultraviolet radiation is a component of natural light and thereby reduces the cost of buying the plastic films repeatedly when needed. Research should also be done on the economics of the technology to compare the cost of solarization to the market value of the crops produced. Lastly, solarization can also be integrated with fumigation in nematode control to get the best results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Viral synergism and its role in management of maize lethal necrosis disease

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Maize lethal necrosis disease (MLND) in Kenya has been reported to be caused by a coinfection between a non-potyvirus *Maize chlorotic mottle virus* (MCMV) and a potyvirus *Sugarcane mosaic virus* (SCMV). The control of the disease in Kenya has been a challenge owing to the synergistic interactions that exist between the two viruses. This study, sought to determine the stage of synergism between the two viruses and its role in influencing the severity of the disease. Three maize hybrids were grown in a greenhouse and were mechanically inoculated with MCMV, SCMV and MCMV+SCMV at the vegetative stage, V4-5. The synergism was studied for a period of 90-days and double-antibody sandwich (DAS)-ELISA was used to estimate the viral titer of MCMV and SCMV under individual and co-infection states of maize plants. The results showed that the viral titers of the two viruses in both single and double infection followed a normal curve. Synergistic effect was observed between the 21- and 28-days post-inoculation (dpi). A significant increase in the titers of MCMV was observed at this time in days, while that of SCMV was more or less constant. Also, the study revealed that viral titers of SCMV in both individual and co- infected maize plants remained constant; while the viral titers of MCMV in co-infected maize increased significantly as compared to the individual infections. Furthermore, there was a positive correlation between increased symptom severity and synergism. Based on these results, SCMV plays a major role in the severity and spread of MLN disease in the South-Rift region.

Key words: Maize lethal necrosis, maize chlorotic mottle virus, Sugarcane mosaic virus, maize virus diseases.

INTRODUCTION

Studies on synergistic interactions between two pathogenic viruses have been reported to be common among plants (Mahuku et al., 2015). Potyvirus-associated synergisms are the most common type of synergism, in which one of the viruses is a member of the potyvirus group and the other is not (Mbega et al., 2016). Maize lethal necrosis (MLN) disease in maize is a classic example of potyvirus-associated synergism because it involves a synergistic relationship between *Maize chlorotic mottle virus* (MCMV) and any potyvirus that has been confirmed to cause the disease, such as *Sugarcane mosaic virus* (SCMV), *Maize dwarf mosaic virus* (MDMV),

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or *Wheat streak mosaic virus* (WSMV) (Isabirye and Rwomushana, 2016). SCMV has been reported as the most common potyvirus in synergy with MCMV causing MLN in Kenya and other countries of East Africa (Adams et al., 2014). Leitich et al. (2020) also reported that SCMV was the primary potyvirus causing MLN disease in Kenya's South-Rift region, in synergy with MCMV. Furthermore, Mbega et al. (2016) reported MCMV as a primary disease-causing virus of MLN with the potential to establish itself alone in warm, arid, semi-arid, and sub-humid tropics in his review (Isabirye and Rwomushana, 2016). Synergism has been reported to increase symptom severity such as; chlorotic mottling, leaf necrosis from the margins to the midrib, stunted growth, premature death or ageing, male sterility and failure to tassel, rotten or small cobs with little or no grain (Xia et al., 2016; Wangai et al., 2012). Such symptoms make MLN a devastating maize disease. The synergistic interactions are more pronounced (Mbega et al., 2016) and result in serious damage that usually kills the infected plant (Makone et al., 2014). The disease has been reported to have caused an estimated loss of $187 million equivalent to $364/ton in Kenya since it was first reported in 2011 (De Groote et al., 2016). This is a direct loss to farmers especially those who rely on the crop for food production and income (Bulegeya, 2016).

In Kenya, the disease has since spread to other maize-growing areas since its first report in 2011. The areas include; Central, Nyanza, Western, South and North-Rift regions of Kenya (Karanja et al., 2018). Although there were efforts to contain the spread of the disease in the country, the farmers in MLN hotspot areas have continued experiencing significant yield losses as high as 100% due to the severity of the disease (Kagoda et al., 2016). The huge losses of the crop yield have been linked to virus synergism (Mbega et al., 2016). Therefore, these huge losses could be attributed to limited information on the effect of synergism between the two viruses in the severity of MLN and management. It has been reported that understanding the mechanism behind synergism and the time it occurs can contribute to more effective management through resistance breeding targeted at the components, specifically potyvirus leading to reduction in yield losses (Mahuku et al., 2015). Therefore, this study sought to establish the stage at which synergism is at the peak when the two viruses (MCMV + SCMV) co-infect the maize plants at the same time. The results will be useful to maize breeders in breeding for tolerant/resistant against the MLN disease.

MATERIALS AND METHODS

Site of the experiment

A screen house experiment was carried out between June and October of 2017 at the CIMMYT-MLN screening site in Naivasha (latitude 0°43′S, longitude 36°26′E, 1896 m.a.s.l.) to assess the stage of synergistic interaction between MCMV and SCMV.

Experimental design

The experiment was carried out in a netted screenhouse where insect vectors were controlled. The trial was a two-level factorial experiment (3×4) in a complete randomized design with three replicates. The factors studied were; hybrids at three levels namely; (Tolerant to MCMV-CKMLN150078, CKH12603 and Susceptible to MCMV-DUMA43) and inoculation combinations at four levels namely; (MCMV, SCMV, MCMV+SCMV and uninoculated control).

Planting, inoculum preparation and inoculations

To check virus purity, inoculation, and disease evaluation for MCMV and SCMV, the serological assay ELISA was used. Prior to planting, the seeds were tested in the laboratory using DAS-ELISA to ensure that they were virus-free. Three clean seeds from each entry (tolerant and susceptible hybrids) were planted in a pot of sterile soil mixture of red soil, pit moss, and compost manure at a ratio of 3:1:1 respectively. The MLN inoculum was achieved by harvesting the leaves of plants that had been artificially inoculated with two viruses at 3-4 leaf stage. The infected MCMV and SCMV leaves were harvested from the greenhouse in a 1:4 ratio (MCMV: SCMV). The infected MCMV and SCMV leaves were blended separately in a cold 0.1 M phosphate extraction buffer using a dilution ratio of 1:10 (leaf material: buffer). The extract was then sieved through folded cheesecloth to remove any debris. For the double inoculations, the inoculum from MCMV and SCMV was mixed in a bucket, and 1 g of celite, abrasive agent was added per litre and stirred thoroughly to ensure even distribution of the celite. All the plants were mechanically inoculated at the 3-4 leaf stage by rubbing the two youngest leaves together (Karanja et al., 2018). The inoculum was then kept cool during the inoculations using ice cubes.

Synergistic interactions between MCMV and SCMV

To determine the stage of synergism between MCMV and SCMV, the plants were mechanically inoculated with MCMV, SCMV, and (MCMV+SCMV) at the 3-4 leaf stage using equal volumes of 5 ml of inoculum per plant. Seven plants from each treatment were randomly selected and tagged for sampling one week after inoculation. For a span of 90 days, severity ratings on a scale of 1 to 5 were done at weekly intervals. The leaf sample was collected using sampling bags, labelled, and placed in a cool box. The collected samples were taken to the laboratory for virus detection using a double-antibody sandwich (DAS)-ELISA.

Symptom’s identification/rating

Plants were assessed for virus symptoms starting at 7 days post-inoculation (dpi) and then every 1 week for the next 90 days, using a 1 to 5 scale, with 1 indicating no symptoms, 3 indicating moderate symptoms, and 5 indicating severe chlorosis. The diagnoses included whether the signs were local lesions on inoculated leaves or systemic infections with mosaics, mottles, flecks or mottles that were limited or general.

Virus detection using DAS-ELISA

Relative amounts of MCMV and SCMV in leaf samples were determined using DAS-ELISA as described by Dijksta and De Jager (1998). Samples were taken weekly for a period of ninety days to understand the disease development and interaction between the two viruses. The leaf material (5 g) of the infected plant was

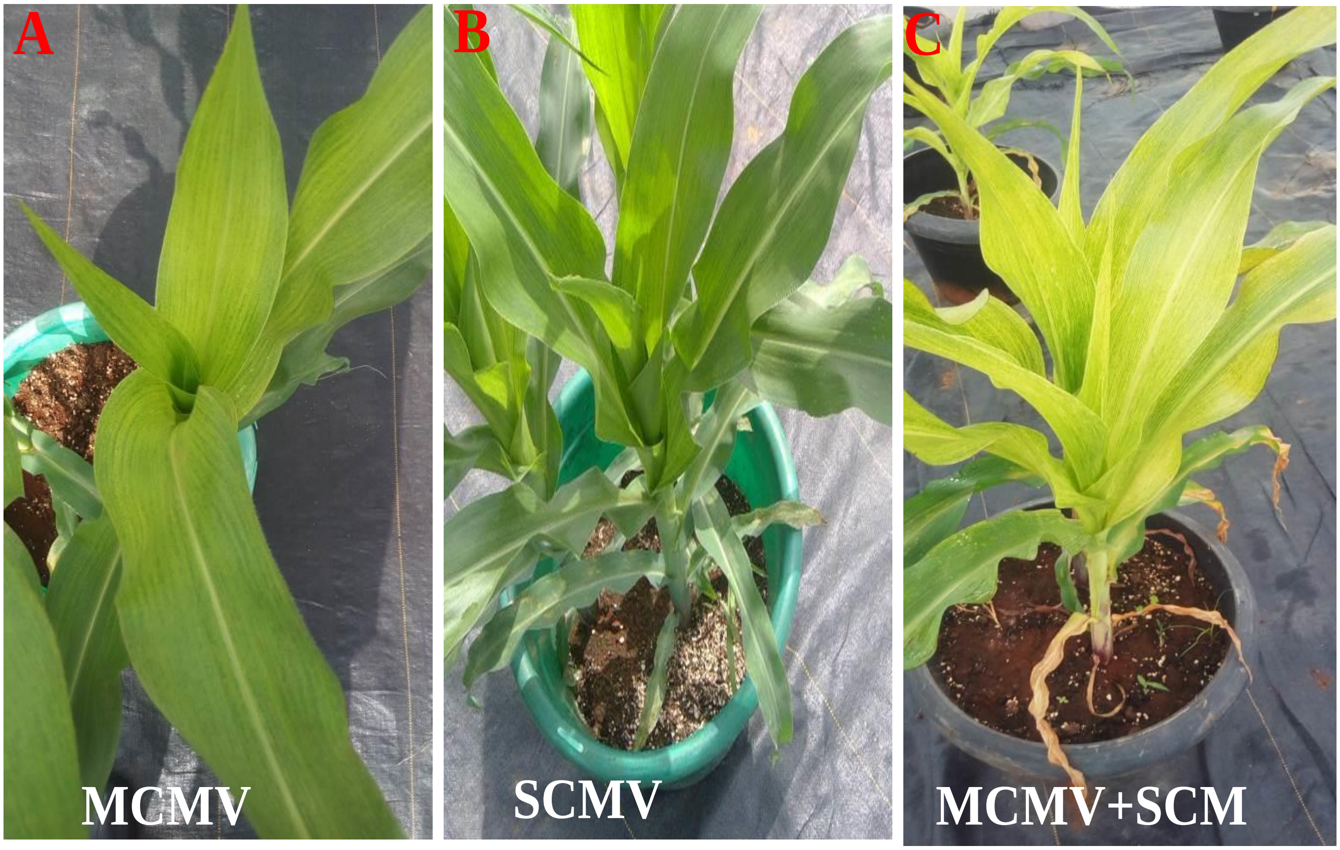

Figure 1. Symptoms of DUMA 43 at 28 days post-inoculation. A, Maize plant singly infected with MCMV showing chlorosis and mottling. B, Maize plant infected singly with SCMV showing mild mosaic and mottling. C, Maize plant infected with both MCMV+SCMV showing severe chlorotic mottle.

extracted using phosphate-buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing Tween 20 at 5 ml/L and polyvinyl pyrrolidone at 20 g/L, using a mixer mill (Retsch, Germany). Each sample was tested in duplicate wells in microtiter plates and commercial MCMV and SCMV antiserum (DSMZ, Braunschweig) were included in paired wells as controls. The substrate used was p-nitrophenyl phosphate at 0.6 mg/ml in diethanolamine at 100 ml/L, pH 9.8. The plates were measured at an absorbance of 405 nm in a microplate reader (Bio-Rad Laboratories).

RESULTS

Symptom expression on maize plants

Symptom expression between single and double infections was compared. The single infections of MCMV and SCMV alone, induced relatively mild symptoms comprising of chlorosis and mottling for MCMV (Figure 1A) and mild mosaic and mottling for SCMV (Figure 1B). As for the double infection with MCMV+SCMV, it resulted in the enhancement of symptoms comprising of severe chlorotic mottling and yellow streaks parallel to leaf veins (Figure 1C).

Correlation between symptom severity and synergism

As the days passed, the magnitude of the crop's symptoms increased significantly, as seen in Figure 1. The severity score for the three hybrids was low during the first 10 days post inoculation (dpi), but increased substantially as the days progressed. The highest severity scores were recorded between 63-84 dpi, with DUMA 43 exhibiting the highest severity scores (6.5), followed by CKH12603 (4.5) and CKMLN150078 (4.0), respectively (Figure 2).

Titer of MCMV and SCMV in singly inoculated maize plants

The MCMV titer in the three hybrids was found to follow a normal curve (Figure 3). During the first 10 dpi, the viral titer was low; however, as the days passed, the viral titer increased dramatically. At different dpi levels, the three hybrids had higher viral titers, with Duma 43 having the highest viral titer (0.98) compared to the other two

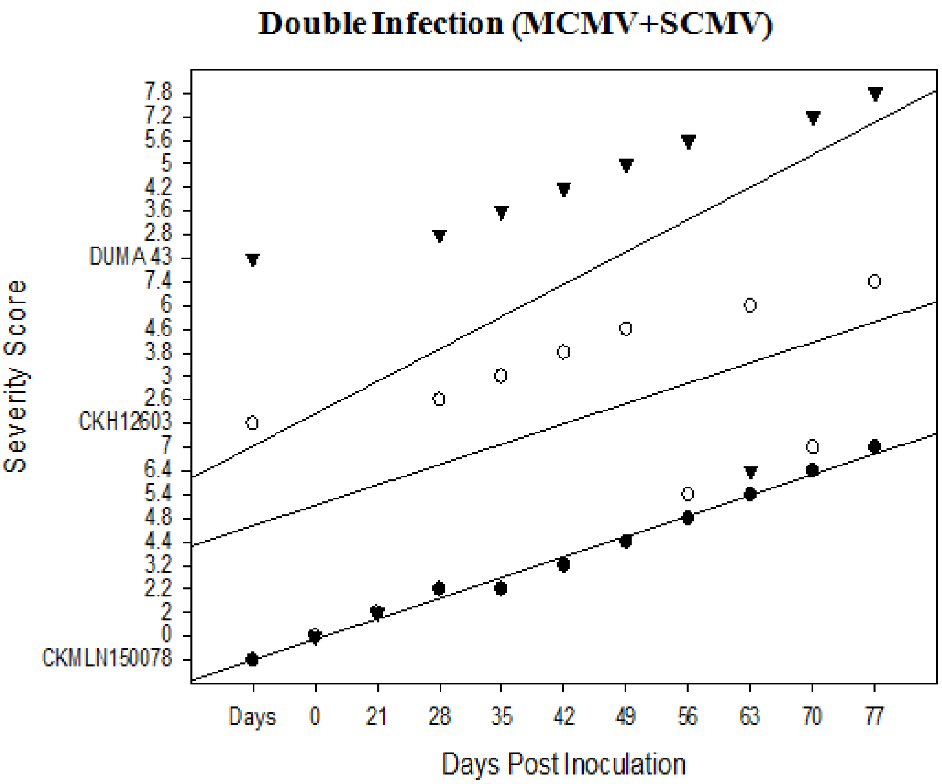

Figure 2. Correlation between symptom severity and synergism.

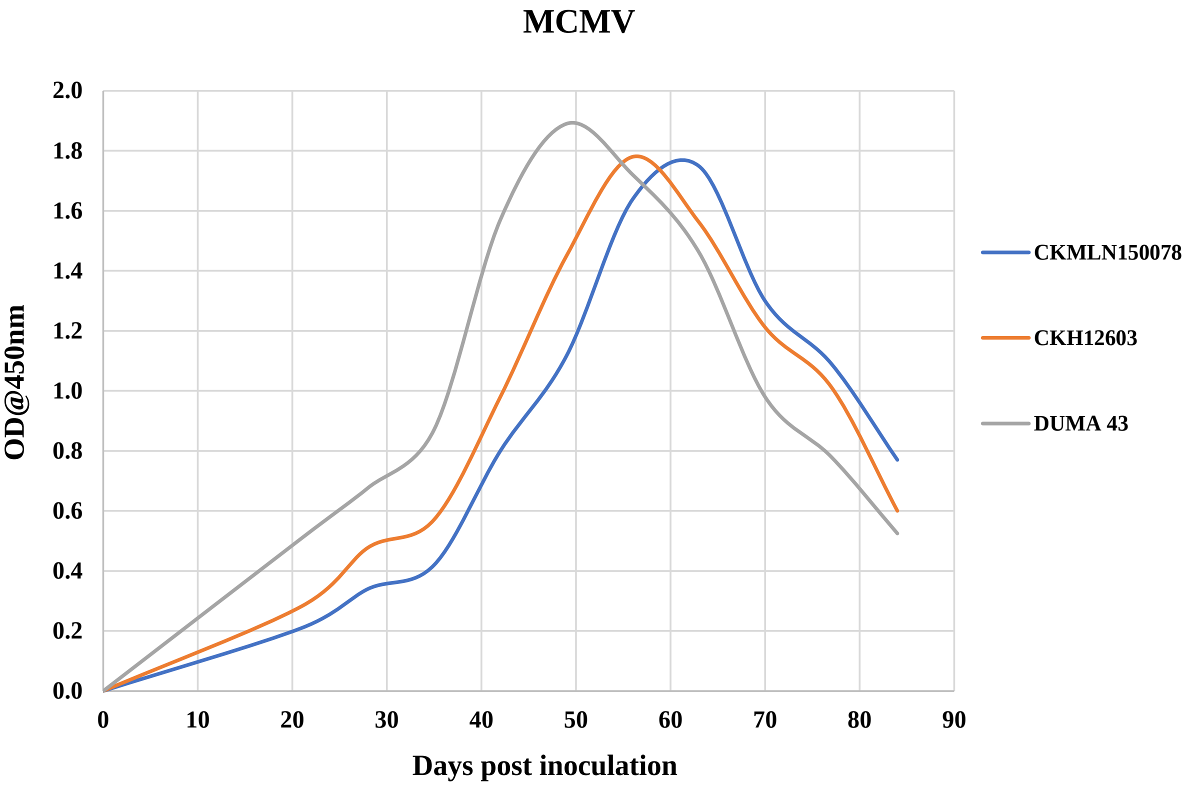

Figure 3. Time course of the titer of MCMV in leaves of maize plants. OD = Optical density.

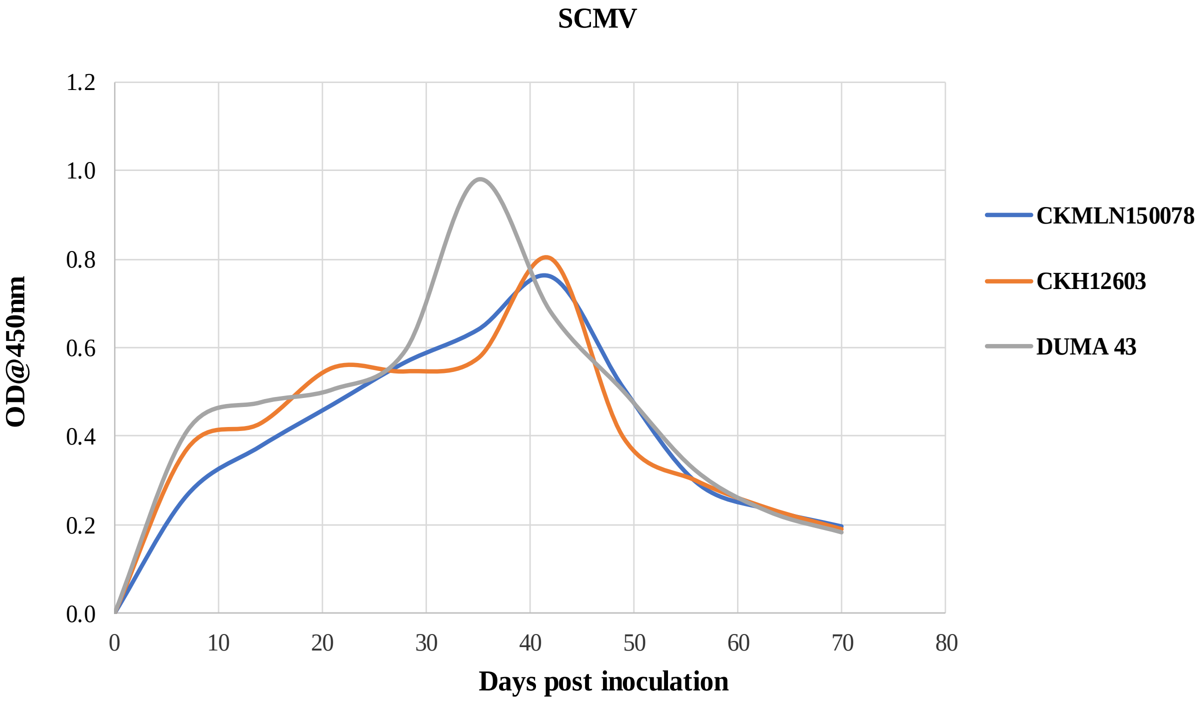

Figure 4. Time course of the titer of MCMV in leaves of maize plants. OD = Optical density.

hybrids. However, there was a decrease in viral titers among the three hybrids between 50, 56, and 64 dpi (Figure 3). The viral titer for the three hybrids of SCMV inoculated maize plants followed a normal curve, similar to the MCMV inoculated maize plants described above (Figure 3). SCMV inoculated alone hybrids, on the other hand, had lower viral titers than MCMV inoculated alone hybrids, which had higher OD values (Figure 4). Between 0 and 35 dpi, the viral titers of the three hybrids increased significantly, with Duma 43 recording a higher viral titer at 35 dpi than the other two hybrids at 42 dpi (Figure 4). The SCMV titer in the three hybrids gradually decreased after that.

Titers of MCMV and SCMV in co-infected maize plants

There was a slight variation in the titers of the two viruses during the first 10 dpi in the doubly infected maize plants with MCMV + SCMV. However, as the days passed, there was a significant increase in MCMV titers compared to SCMV titers (Figure 5). Consequently, MCMV titer values in co-infected maize plants were higher than MCMV titer values in singly infected maize plants, whereas SCMV titer values were less similar. Moreover, the titers of the Duma 43 hybrid were higher than the titers of the other two hybrids (Figure 5). Furthermore, it was observed that Duma 43 took a shorter period of 21 dpi for its titer to reach its maximum, as opposed to the singly infected, which required 35 dpi (Figure 5).

DISCUSSION

The findings of this study have revealed that the viral titers of the two viruses in both singly and co-infection, followed a normal curve. The normal virus titer curve could be ascribed to the plant virus infection cycle, in which the virus must initially overcome pre-existing chemical and physical barriers in plants (Pallas and Garci, 2011). Therefore, the low titer of the two viruses during the first 10dpi could be attributed to low virus replication, cell-to-cell movement, as well as the long-distance movement of the virus through the vascular tissues of the plant as the virus is overcoming the defensive mechanism of the maize plant (Syller and Grupa, 2016). On other hand, the rapid increase in viral titer after the 21dpi is as result of a high rate of virus multiplication and translocation of virus particles throughout the plant cells through production of RNA-silencing suppressors, which interfere with the maize plant physiological processes which depend on RNA-silencing; hence enhancing the pathogenicity of the virus (Roth et al., 2004). Furthermore, it was noted that the viral titers of the two viruses declined after attaining the maximum concentration/symptom development. The decline in viral titer was linked to increased virulence of the viruses which resulted in cells death of the plant which in turn resulted in a decline in virus particles as viruses reside within the cells of the plants (Santi et al., 2006).

Also, it was observed that the viral titers of DUMA 43 in

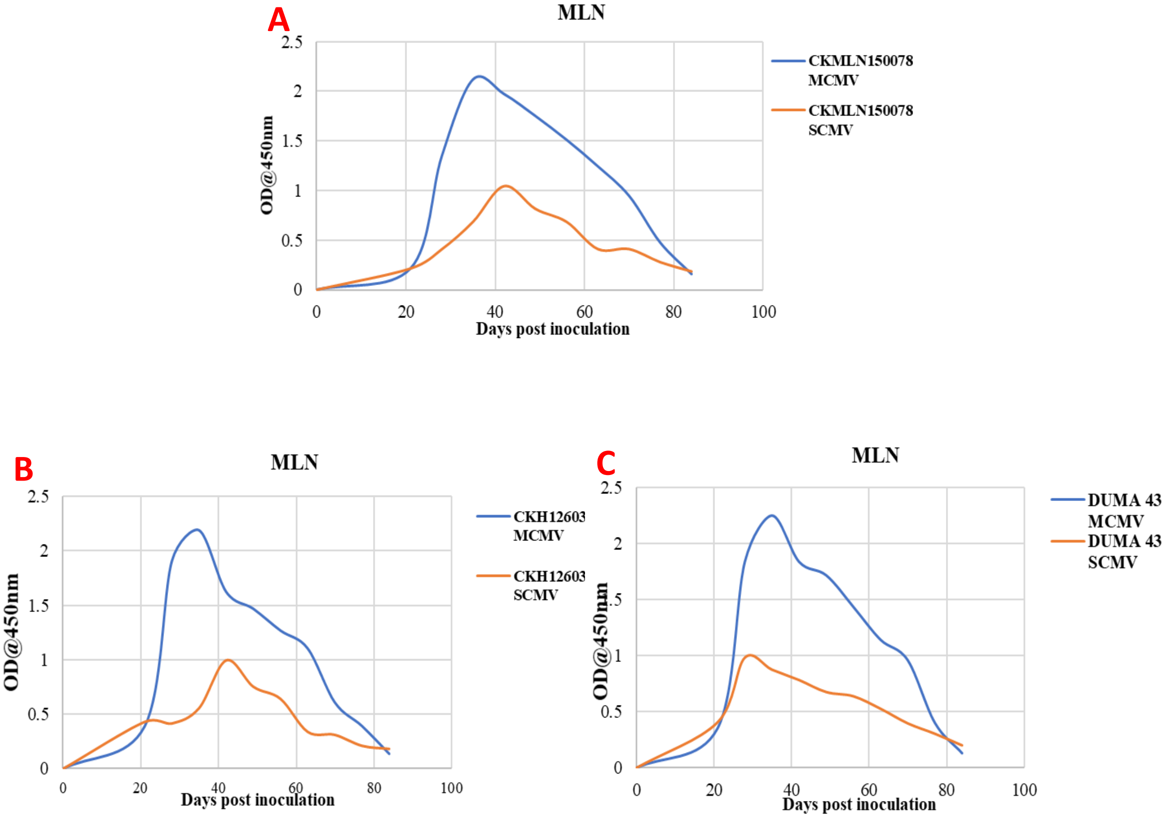

Figure 5. Time course of the titer of MCMV & SCMV:(A) CKMLN150078, (B) CKH12603 and (C) DUMA 43. OD = Optical density.

both singly and co-infections were higher as compared to the other two hybrids. The high viral titer could be due to the susceptibility nature of the variety to MLN disease as previously reported by Mbega et al. (2016) in a review paper. They reported that in a susceptible host the virus particles can move between the cells through the plasmodesmata and the whole plant through the phloem, thus colonizing the plant and eventual expression of the MLN symptoms; while in a resistant host, the virus colonization is only sparingly possible hence no expression of symptoms.

Furthermore, it was also observed that the viral titers of SCMV in both singly and co-infected maize plants remained constant amongst the three hybrids; while the viral titers of MCMV in co-infected maize (MCMV+SCMV) increased significantly as compared to the singly infected plants. Such findings are consistent with previous reports by Awata et al. (2019) who reported that concentration of SCMV in the mixed infections remained constant. The constant concentration of SCMV in a mixed infection could be associated to viral proteins such as P1 and VPg, which are not strong enhancers of replication and movement (Awata et al., 2019). On the other hand, the observed increase in the concentration of MCMV in co-infected maize plants (as compared to the singly infected) could be traced to the ability of SCMV to suppress regulatory systems of the maize plant that would normally limit concentrations of MCMV in a cell; thus, allowing easy transmission of the MCMV, and hence increased symptom severity (Xia et al., 2016). Rajamäki and Valkonen (2009), in their report, found that potyviruses contain two important genes namely: helper component gene (HC-pro) and nuclear inclusions protein gene. These two genes reduce the capacity of the maize plant to inhibit the replication of MCMV. Moreover, it has been reported that SCMV VPg enhances cell to cell movement and long-distance movement of its virus particles as well as those of MCMV (Scheets, 1998). Furthermore, VPg also has been reported to suppress the post-transcriptional gene (PTGS) of the host plant thereby allowing successful colonization of the plant by the virus (Mbega et al., 2016). Such mechanisms, therefore, explain why there were increased titers of MCMV in a mixed infection, especially between 35-42dpi's while the titers of SCMV in both infections remained more or less the same.

Also, it's worth noting that, between 35-42 dpi, there was a significant increase in the viral titers of MCMV while that of SCMV was less constant; and thereafter, there was a decline in viral titers of the two viruses. The observed increase in the viral titers of MCMV in co-infection with SCMV as compared to single infection by MCMV alone is hypothesized to be due to the ability of the SCMV to suppress regulatory systems that would normally limit MCMV concentrations in a cell allowing easy transmission of the MCMV and increasing the symptom severity (Mbega et al., 2016). SCMV has been reported to promote its multiplication and movement, as well as suppression of the host plant defense mechanisms (Mbega et al., 2016). The significant increase in the viral titers of MCMV is termed unilateral synergism (Scheets, 1998). The period is a signal of cumulative effects of the synergistic interaction between the two viruses and resulting in the overrunning of the host plant defense barriers (Awata. et al., 2019). At such a stage, there is increased symptom severity in the plant (Xia et al., 2016), which eventually results in plant death. The period serves as a pointer that synergism between the two viruses was at its peak and therefore it is an indication that the synergism between the viruses had started as early as 21dpi, since there was a rapid increase in the viral titers of the two viruses. This is an important observation for recommendation to maize breeders to take cognizance of such a period in breeding for tolerance/resistance against MLN. The findings also serve as a significant recommendation to seed companies that specialize in seed multiplication, as well as small-scale and commercial farmers, to use recommended insecticides to control aphid and thrip species between 7 and 14 days after emergence, before synergism develops. This practice has been shown to reduce MLN incidence and severity by reducing the population of thrip species responsible for MCMV transmission (Ngala et al., 2018).

Conclusion

This study has clearly elucidated that SCMV plays a significant role in the multiplication and movement of MCMV particles in the plant. It has also been observed that the synergistic interaction between the two viruses in the maize plant is at its peak between 35-42dpi. The determination of the synergistic period is critical for plant pathologists and plant breeders in their effort to develop maize varieties that can avoid this period, overcoming synergism establishment and increasing yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Conservation agriculture-based *Zea mays* (maize)-*Phaseolus vulgaris* (common bean) cropping systems in South Central Ethiopia

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Conservation agriculture (CA) is defined as sustainable agriculture production system comprising a set of farming practices. The experiment was conducted at three districts from 2011 to 2016 at five farmers' field they considered as replicate. The experiment consisted of five treatments (continuous sole maize, maize bean rotation, maize-bean inter-cropping, bean rotation under CA and farmer practice). Maize yield and yield related traits and soil water data were collected from each site. Soil moisture content under CA practices was higher than the farmer practice. At East-Badawacho and Meskan grain yield was higher by 4 and 8% in CA compared with farmer practice, respectively. Maize bean rotation and sole maize under CA out yielded the farmer practice by 13 and 4%, respectively but inter-cropping had 5% lower grain yield. At Hawassa-Zuriya, CA maize bean rotation had higher yield than farmer practice in 2011 and 2013. Maize-bean inter-cropping, maize bean rotation and sole maize under CA had 10, 8 and 6% higher grain yield than farmer practice, respectively. Common bean grain yield from bean rotation under CA had 2799, 2908, and 3226 kg ha^{-1}, from inter cropping bean grain yield of 817, 1065 and 927 kg ha^{-1} obtained at East-Badawacho, Hawassa-Zuriya and Meskan districts, respectively. Generally, CA cropping systems had drought stress reduction potential and greater yields compared with farmer practice.

Key words: Farmer-practice, sole-maize, rotation, inter-cropping, rift-valley.

INTRODUCTION

In Africa, the agriculture sector dominated by small-scale farmers who use traditional methods and tools of production (Musa, 2015). Agricultural production in the semi-arid regions of Sub-Saharan Africa (SSA) is

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challenged by many risk factors and high vulnerability of poorly resourced farmers (Solomon, 2018). Key sources of risk in agriculture include climate, socio-economic factors, soil degradation, and poorly developed markets (Kassie et al., 2013). Agriculture continues to be the major sector in Ethiopia's economy, with cereals playing a critical role. Maize is Ethiopia's largest cereal commodity in terms of total production, acreage, and the number of farm holdings (Rashid et al., 2010). Rainfall in Ethiopia is seasonal with high spatial and temporal variability. In the Central and Southern Rift Valley of Ethiopia rainfall pattern is bimodal and starts with the spring rains or Belg during the months of March to May and the summer rain or Kiremt extends from June to September (Solomon, 2018). Under conventional practice, soil erosion is one of the principal environmental problems in Ethiopia resulting in decreasing productivity of farmlands (Hurni, 1987). About 2 million hectares of land in Ethiopia have been severely degraded (Shiferaw, 2005). In Ethiopia the major causes of low productivity of the systems were lack of inputs and draft power and equipment, soil nutrient depletion, natural resources degradation, soil erosion, floods uncertain (drought), post-harvest management problems, unsustainable cropping systems, emerging new insect pest and diseases (Ellis-Jones et al., 2013; FAO, 2017; Lunt et al., 2018; MoANRD, 2018).

Conservation agriculture (CA) aims to conserve, improve and make more efficient use of natural resources through integrated management of available soil, water and biological resources combined with external inputs. It contributes to environmental conservation as well as to enhanced and sustained agricultural production. Conservation agriculture is a set of practices that leave crop residues on the surface which increases water infiltration and reduces erosion (Hobbs et al., 2008). Thus, residue levels alone do not adequately describe all CA practices. The importance of conservation agriculture is to conserve time and fuel; moreover, it improves earthworms, soil water, soil structure and increases soil nutrient contents as well as increasing water infiltration (Hobbs et al., 2008). It contributes to environmental conservation as well as to enhanced and sustained agricultural production. No-tillage practice minimizes soil organic matter losses and is a promising strategy yield to maintain or even increase soil carbon and nitrogen stocks (Bayer et al., 2000). Surface mulch helps reduce water losses from the soil by evaporation and also helps moderate soil temperature and promote biological activity and enhance nitrogen mineralization, especially in the surface layers (Hatfield and Pruegar, 1996; Hobbs et al., 2008). Infiltration of water under long-term (8-10 years) conservation tillage with residue retention was higher compared to conventional tillage on a grey cracking clay and a sandy loam soil in South-Eastern Australia (Bissett and O’Leary, 1996).

Rotation is cultural control of plant diseases from an historical view (Howard, 1996). The rotation of different crops with different rooting patterns combined with minimal soil disturbance in zero-till systems promotes a more extensive network of root channels and macrospores in the soil, and this helps in water infiltration to deeper depths (Hobbs et al., 2008). Rotations increase microbial diversity, and the risk of pests and disease outbreaks from pathogenic organisms is reduced (Leake, 2003). The benefits of CA especially when cereals are rotated with leguminous crops increase over time, suggesting that there are improvements in soil structure and fertility (Thierfelder et al., 2012).

Inter-cropping is a type of mixed cropping and defined as the agricultural practice of cultivating two or more crops in the same space at the same time. It increases in productivity per unit of land via better utilization of resources, minimizes the production risks, and stabilizes the yield (Ananthi et al., 2017). Inter-cropping of cereals with legumes has been practiced in tropics (Tsubo et al., 2005) and rain-fed areas of the world (Agegnehu et al., 2006; Dhima et al., 2007). Its benefits include soil conservation (Ananthi et al., 2017), weed control (Ananthi et al., 2017; Banik et al., 2006), and yield increment (Chen et al., 2004). In the southern part of Ethiopia, maize-common bean intercropping is an integral part of the cropping system as small-holder farmers expect better yield and weed suppression (Getahun and Tenaw, 1990), and provides balanced diet compared to the predominant cereal monoculture and gives high total productivity compared to sole crops of bean and maize (Walelign, 2014; Workayehu, 2014). There is a higher performance of maize bean rotation and maize bean inter-cropping under CA compared with continuous sole maize under CA and farmer practice (Liben et al., 2017). Similarly, higher maize grain yield from maize soybean rotation and maize soybean intercropping compared with sole maize under CA was reported (Liben et al., 2018). Better performance of relay cropping using maize and legumes under CA compared with the control sole maize and other inter cropping practices has also been reported (Daniel, 2019). Legumes, such as common vetch, common bean and cowpea are extensively used in inter-cropping with cereals (Daniel, 2019; Liben et al., 2017; Yilmaz et al., 2008), finger millet with maize (Nath, 2016), wheat with soybean (Sandler and Kelly, 2016), and maize with Soybean (Liben et al., 2018).

Under this study, the research questions were (1) which cropping systems performed best under CA compared to conventional practice and (2) which tillage practices conserves more soil water? The study was undertaken to (1) evaluate and compare maize bean cropping systems under CA and with sole maize under conventional practice, (2) to assess soil moisture content of different cropping systems and (3) assess the advantage of cropping systems under CA for reduction to risks from crop failure compared with conventional practice.

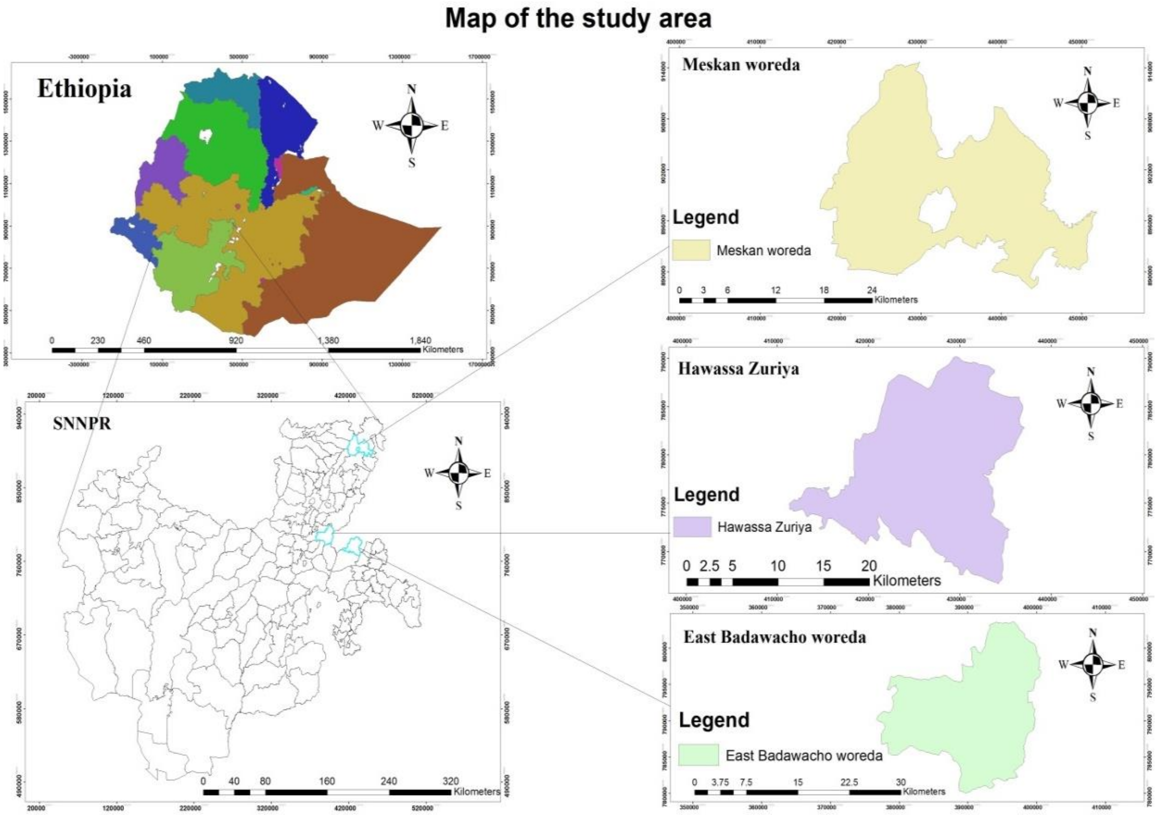

Figure 1. Map of the study area.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at East-Badawacho (1788 masl, 037° 41' 02 E, 07° 05' 34" N), Meskan (1839 masl, 038° 29' 22" E, 08° 04' 53" N) and Hawassa-Zuriya (1696 masl, 038° 23' 22" E, 07° 02' 43" N) districts farmers' fields during the period between 2011 and 2016 cropping seasons under rain-fed in the Southern Ethiopia (Figure 1). The common soil types at east Badawacho, Meskan and Hawassa-Zuriya are black basaltic soils (Vertisols), eutric Cambisols and vitric Andosols, respectively (Addise, 2014; Getahun et al., 2014; Lemma et al., 2015). These areas are characterized by bimodal rainfall received between March and September. The cumulative annual rainfall ranges between 872 and 1322 mm at East-Badawacho, 815 and 1346 mm at Meskan, and 900 and 1400 mm at Hawassa-Zuriya (TAMSAT). These areas are characterized by erratic rainfall distribution. The daily and cumulative monthly rainfall for sites is as shown in Figures 2 to 4.

Treatments

A trial comprising four cropping systems: continuous maize (CSM), maize-bean rotation RMB), bean-maize rotation (RBM), and maize-bean intercropping (MBI); all under conservation agriculture (CA) and continuous maize (FP) under farmers' practice were established at five farmers' field at each site.

For treatments under CA, narrow rows were opened with a hand-hoe to a depth of about 10 cm to place seeds and basal fertilizer application without prior tillage of the soil and retention of all the maize and bean crop residue produced the previous season as surface mulch. The conventional tillage practice or farmer practice was cultivated similar to the traditional farmers' land preparation practice for maize at each district. Land was prepared by conventional ploughing with an ox-drawn traditional plough called Maresha (ploughed the land 2 - 4 times depending on the soil types) before planting (Temesgen et al., 2009). The depth of the first ploughing ranges from 5 to 8 cm while with the last pass up to 20 cm depth could be attained.

Crop husbandry

Maize was planted at a spacing of 0.75 m between rows and 0.30 m between hills, and common bean was planted at a spacing of 0.40 m between rows and 0.1 m between hills. Each plot consisted of 13 rows of 10 m long (100 m^2 area). Two seeds were planted per hill and later thinned to one seedling upon stand establishment to maintain 44,444 plants ha^{-1} for maize and 250,000 plants ha^{-1} for common bean.

All treatments received fertilizer rates recommended: 110 kg N and 46 kg P_2O_5 ha^{-1} for maize and 46 kg P_2O_5 and 37 kg of N ha^{-1} for common bean. For maize, all the phosphorous and a third of N was applied as basal dose; while two-third N was side-dressed at 35 days after emergence. For common bean, all the fertilizer was applied at planting. Maize (cv BH-543 (154 days maturity)) and common bean (cv Hawassa Dume (102 days maturity)) varieties, were used in all years. In the maize-bean intercropping treatment, bean was planted at the same time as maize, between maize rows.

The treatments managed through conservation agriculture were sprayed with a broad-spectrum systemic herbicide (glyphosate) 10

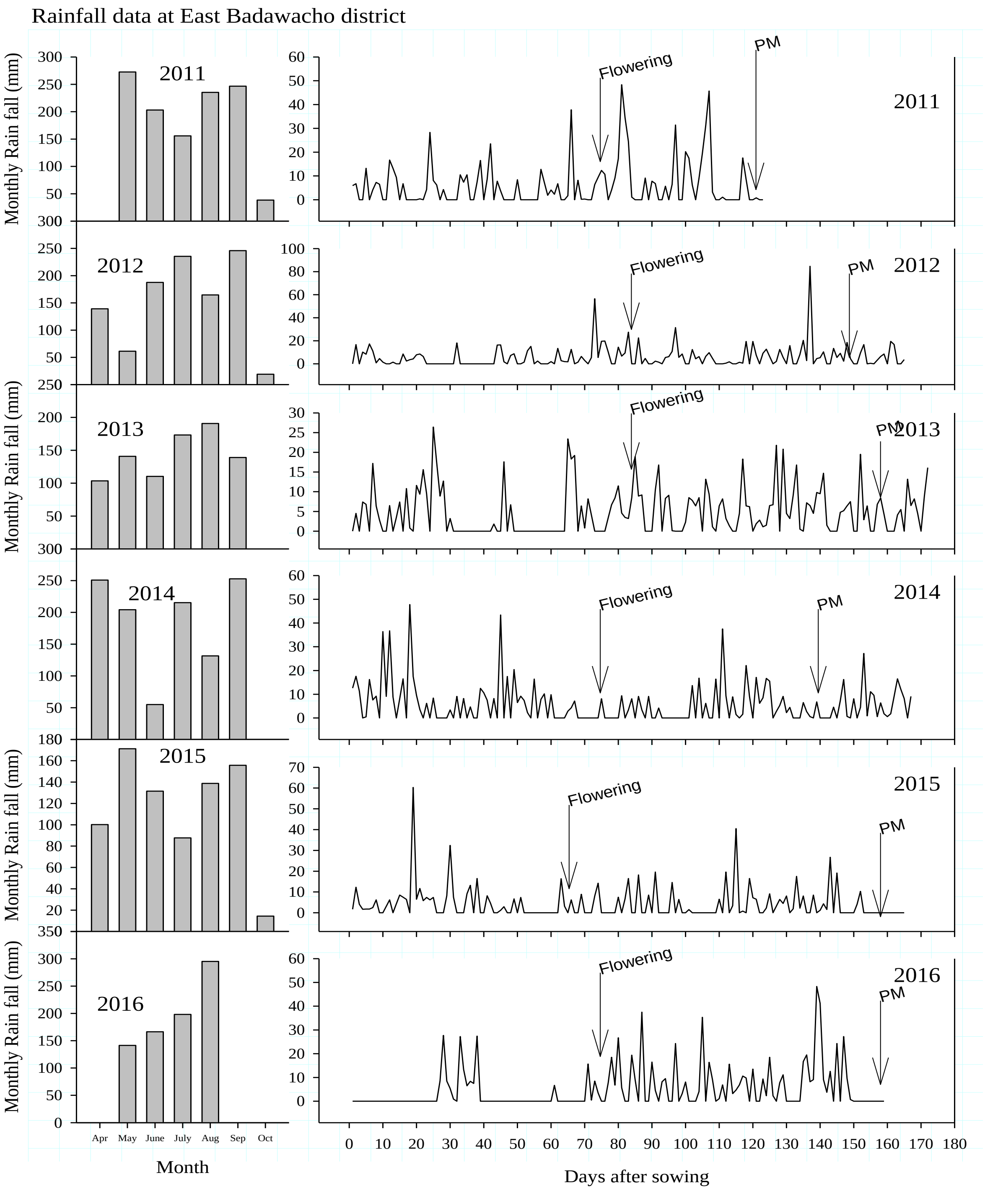

Figure 2. Cumulative monthly rainfall (bar graph) and daily rainfall distribution (line graph) during 2011 - 2016 cropping seasons at East-Badawacho. The arrows indicate flowering and physiological maturity (PM) stages of the crop.

days before planting at the rate of 3-L ha^{-1} to control weed and all plots were maintained weed free afterwards by hand weeding. The conventional farmer practice was hand weeded following the common practice done by farmers. Pest (stem borer) control method (chemical application) used was same for both CA and farmer's practice.

Measurements

Soil water measurement

Composite soil samples from three cores were taken at three depths, 0-15, 15-30 and 30-45 cm, at planting, at bean harvesting

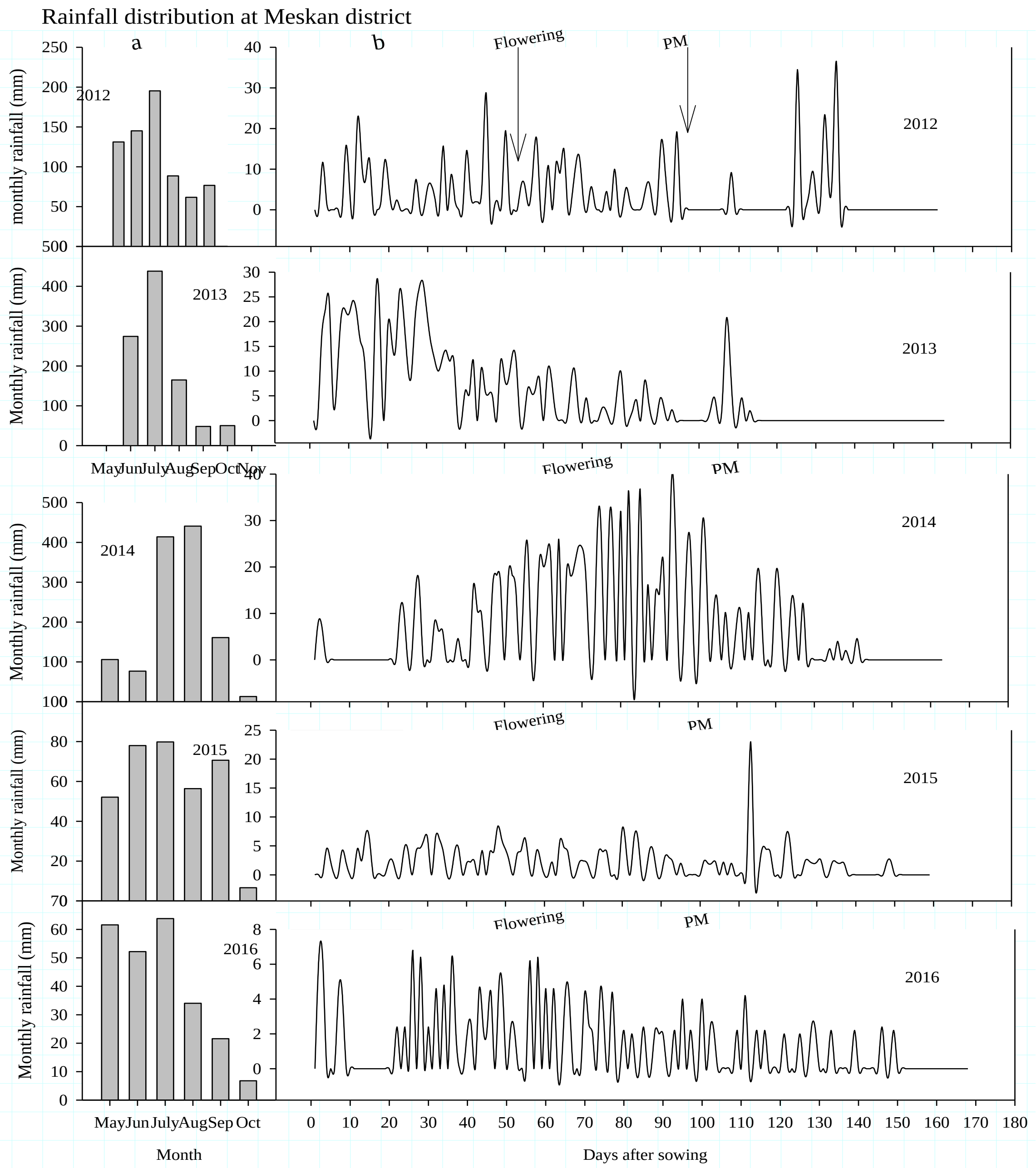

Figure 3. Cumulative monthly rainfall (bar graph) and daily rainfall distribution (line graph) during 2011 and 2016 cropping seasons at Meskan district. The arrows indicate flowering and physiological maturity (PM) stages of the crop.

and maize harvesting every year. The soil samples from each plot were weighed immediately after sampling and oven dried for 48 h at 105°C for final dry weight determination.

NDVI

Normalized Difference Vegetative Index (NDVI) was measured at vegetative and flowering stages at East Badawacho in 2016 using a Green Seeker^{TM} Handheld Optical Sensor Unit (NTech Industries, Inc., USA) (Govaerts et al., 2007; Verhulst et al., 2011).

Biomass yield

Above-ground biomass was measured at physiological maturity of maize from ten sample plant cut at ground level for fresh biomass measurement. From these ten sample plants, a 0.5 kg subsample was taken before oven drying for dry maize biomass weight measurement. For common bean, ten plants were cut at the ground level and dried for biomass. Biomass samples were dried in a fan-circulated oven set at 65°C until constant weight and expressed on dry weight basis (Karim et al., 2000). For common bean, the additional parameters of harvest index (HI), number of

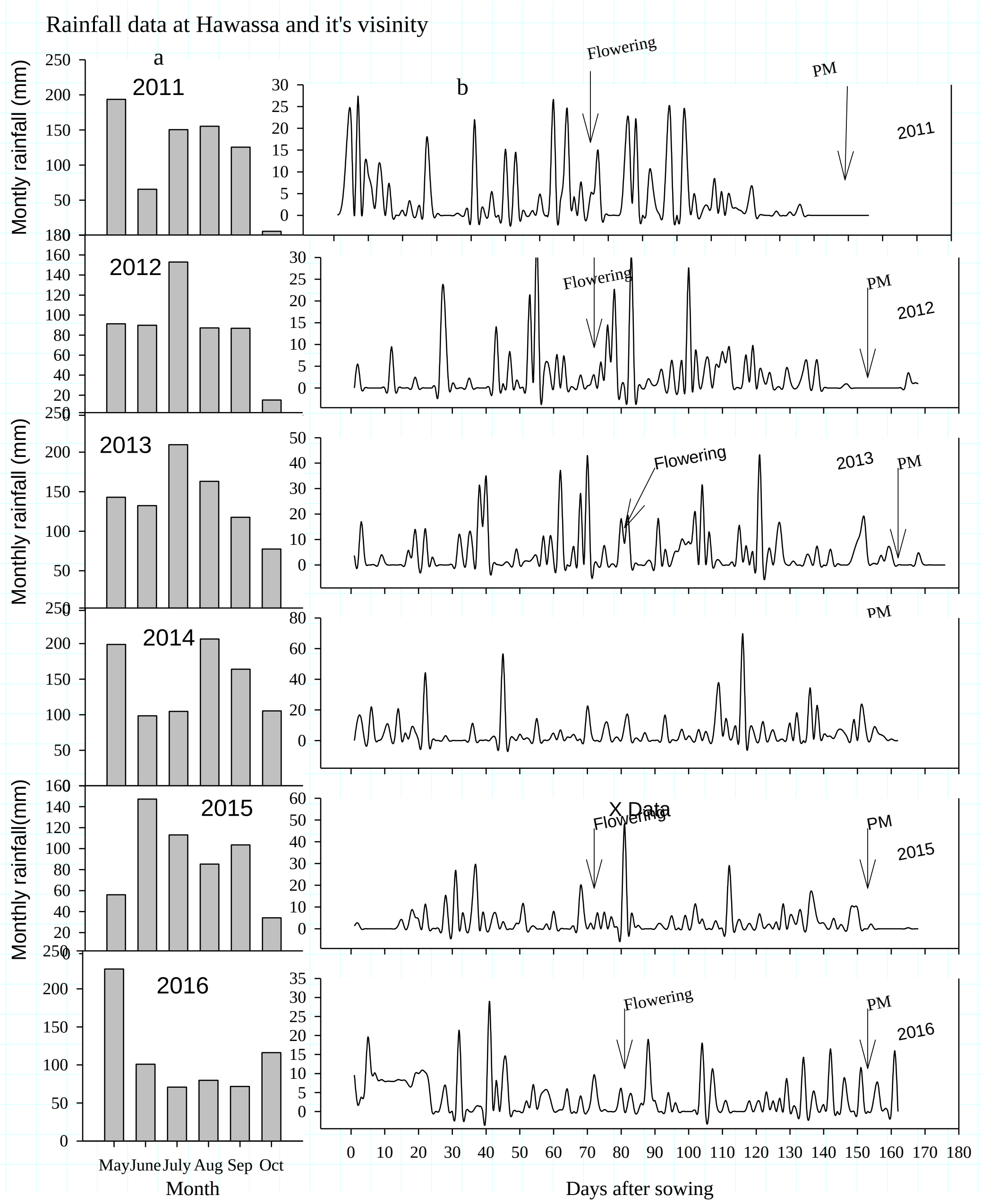

Figure 4. Cumulative monthly rainfall (bar graph) and daily rainfall distribution (line graph) during 2011 to 2016 cropping seasons at Hawassa-Zuriya district. The arrows indicate flowering and physiological maturity (PM) stages of the crop.

pods per plant (PPP), number of seeds per pod (SPP), thousand seed weight (TSW) and plant height (PH) stand count at harvesting time were collected in addition to biomass and grain yield.

Grain yield and yield components for the component crops

Grain yield, pods per plant and number of seeds per pod were assessed for common bean. Plants in the middle 11 rows, from an area of 82.5 m^2 were hand harvested at physiological maturity. Ears were shelled, grain weight and grain moisture content measured, and yield was adjusted for 12.5% grain moisture content. For common bean, total number of pods per plant (PPP) and seeds per pod (SPP) were counted from ten plants and ten pods, respectively. The yield data was then adjusted to 10% moisture content for common.

Statistical analysis

Normality of data was checked prior to analysis of variance (ANOVA) using Shapiro-Wilk normality test. ANOVA for each year

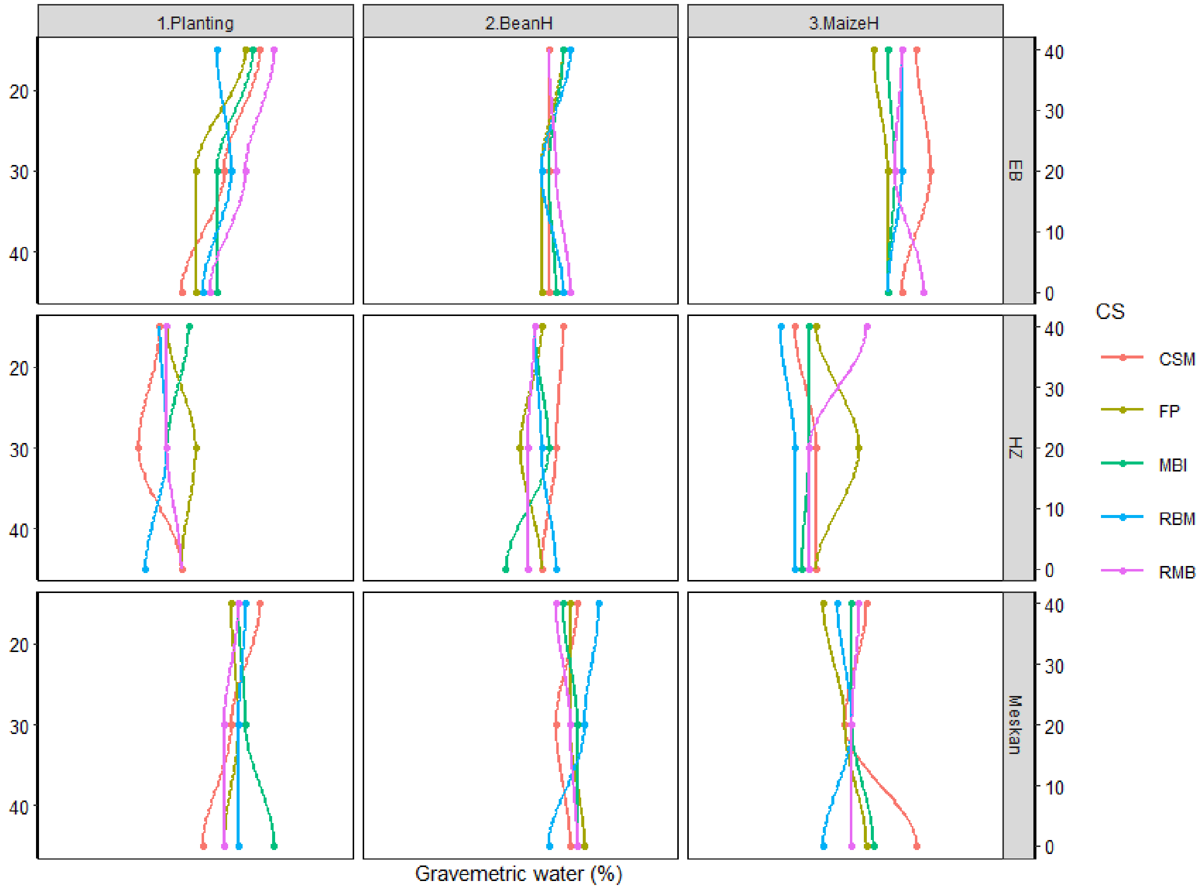

Figure 5. Mean gravimetric soil moisture content (%) for different cropping systems (CS) grown under conservation (CA) and conventional (CN) tillage practices at East-Badawacho (EB), Meskan and Hawassa-Zuriya (HZ) in 2013, 2015 and 2016 cropping seasons. CSM = Continuous sole maize (CA); FP = farmers' practice continuous maize (CN); MBI = maize bean intercropping (CA); RBM = rotation bean maize (CA); RMB = rotation maize bean (CA).

was done for yield and other traits using SAS version 9.0. Analysis was done for each year independently and for all combined years. Means were separated using LSD test. Graphs were developed using sigma plot 10.0 (Systat Software, San Jose, CA).

RESULTS AND DISCUSSION

Soil moisture content

At East-Badawacho, there was significant difference in soil moisture at planting between treatments at 15-30 cm soil depth (Figure 5). The highest soil moisture content was obtained in bean maize rotation treatment. At soil depth of >30 cm the difference in soil moisture was significant at planting time. At maize harvesting, the difference in soil moisture was significant at 0-15 cm soil depth and the highest soil moisture was obtained from CA sole maize (Table 1). At Meskan, a significant difference in soil moisture was detected at bean harvesting at 0-15 cm soil depth. The highest soil moisture was observed in the CA sole maize. At soil depth >30 cm, the difference was significant between treatments at planting, bean harvesting and maize harvesting time. At planting time, at soil depth of >30 cm the highest soil moisture value was obtained from bean maize rotation. At bean harvesting time, the highest soil moisture value was recorded in FP sole maize; whereas at maize harvesting, the highest value obtained from CA sole maize at similar soil depth (Table 1). At Hawassa Zuriya, the difference was significant between treatments >30 cm soil depth, with the highest value obtained from bean maize rotation at planting. At bean harvesting, there was significant soil moisture difference between treatments at soil depth of 0-15 and >30 cm. The highest value was obtained from bean-maize rotation at 0-15 cm soil depth; but at soil depth >30 cm the highest soil moisture was obtained from FP-sole maize.

The result from this study highlighted that the existence of difference for soil moisture holding capacity between tillage practice across cropping systems at different soil depth. Mostly the highest soil moisture at soil depth of above 30 cm under CA highlights that CA practice

Table 1. Average gravimetric soil moisture content (%) at planting, bean harvesting (Bean_H) and maize harvesting (Maize_H) at East-Badawacho, Meskan and Hawassa-Zuriya districts at the three soil depths (0-15, 15-30 and 30-45cm) for different cropping systems in 2013, 2015 and 2016 cropping seasons.

Depth (cm)	Cropping system	East-Badawacho			Meskan			Hawassa-Zuriya		
		Planting	Bean_H	Maize_H	Planting	Bean_H	Maize_H	Planting	Bean_H	Maize_H
0-15	FP	27^{a}	26^{a}	24^{b}	25^{a}	27^{ab}	17^{a}	16^{a}	23^{a}	16^{a}
	RBM	23^{a}	27^{a}	28^{ab}	27^{a}	31^{a}	19^{a}	15^{a}	22^{a}	11^{a}
	MBI	28^{a}	26^{a}	26^{ab}	26^{a}	26^{b}	21^{a}	19^{a}	22^{a}	15^{a}
	RMB	31^{a}	24^{a}	28^{ab}	26^{a}	25^{b}	22^{a}	16^{a}	22^{a}	23^{a}
	CSM	29^{a}	24^{a}	30^{a}	29^{a}	28^{ab}	23^{a}	15^{a}	26^{a}	13^{a}
15-30	FP	20^{b}	23^{a}	26^{a}	26^{a}	27^{a}	20^{a}	20^{a}	20^{b}	22^{a}
	RBM	25^{ab}	23^{a}	28^{a}	26^{a}	29^{a}	21^{a}	16^{ab}	23^{ab}	13^{a}
	MBI	23^{ab}	24^{a}	27^{a}	27^{a}	28^{a}	21^{a}	16^{ab}	24^{ab}	15^{a}
	RMB	27^{a}	25^{a}	27^{a}	24^{a}	27^{a}	21^{a}	16^{ab}	21^{ab}	15^{a}
	CSM	24^{b}	24^{a}	32^{a}	25^{a}	25^{a}	20^{a}	12^{b}	25^{a}	16^{a}
30-45	FP	20^{ab}	23^{a}	26^{a}	24^{ab}	29^{a}	23^{ab}	18^{a}	23^{ab}	16^{a}
	RBM	21^{ab}	26^{a}	26^{a}	26^{ab}	24^{b}	17^{b}	13^{a}	25^{a}	13^{a}
	MBI	23^{a}	25^{a}	26^{a}	31^{a}	28^{ab}	24^{ab}	18^{a}	18^{b}	14^{a}
	RMB	22^{a}	27^{a}	31^{a}	24^{ab}	28^{ab}	21^{ab}	18^{a}	21^{ab}	15^{a}
	CSM	18^{b}	24^{a}	28^{a}	21^{b}	27^{ab}	30^{a}	18^{a}	23^{ab}	16^{a}

Columns with the same letter are not significantly different at $P < 0.05$. FB = Farmers' practice continuous maize (CN); RBM = rotation bean maize (CA); MBI = maize bean intercropping (CA); RMB = rotation maize bean (CA); CSM = continuous sole maize (CA).

contributed more for soil moisture infiltration compared with FP. This more efficient soil water conservation ability of CA than FP provided the chance to harvest higher yield especially under seasons with random drought stress. In line with findings from this study, different investigators reported higher soil moisture under CA compared to FP (Zerihun et al., 2014), higher water infiltration rate more by 15% at low moisture area under CA. But, at potential area (Bako) the infiltration rate of water was less by 16% compared with FP (Liben et al., 2018). Furthermore, in a previous study, higher infiltration rate has been reported from no till practice with four different crop residue conditions (no till with: no input (control), inorganic fertilizer, residues, residue + inorganic fertilizer) compared with conventional practice with four residue conditions mentioned for no till (Kabirigi, 2015). At maize harvesting time, the difference was significant between treatment at soil depth of >30 cm (Table 1). Conservation agriculture is also one way of improving soil moisture management through combining the four principle of conservation agriculture (reducing soil disturbance, maintain permanent soil cover, controlling in field traffic and crop rotation) (Benites and Navarrete, 2003).

NDVI

There was significant difference in NDVI among treatments with the highest observed for rotation and sole maize under CA compared with farmers practice (Table 2). Higher NDVI values for CA than CN at vegetative and flowering reflected higher growth for CA treatments than CN (Table 3) (Verhulst et al., 2011). This was because drought stress conditions enhanced earlier

Table 2. Mean square and mean of NDVI measured at East-Badawacho district for different cropping systems grown under conservation (CA) and conventional (CN) practices in the 2016 cropping season.

Source of variation	DF	Mean Square	Cropping system	NDVI
Farmer	4	0.004	Farmer practice (CN)	0.58^{b}
Cropping system	2	0.04**	Sole maize (CA)	0.74^{a}
error	8	0.004	Maize rotation (CA)	0.73^{a}
CV	-	8.93		
Mean	-	0.68		
LSD	-	-	-	0.09

Table 3. Mean yield (t/ha) and above-ground biomass (t/ha) of maize for different cropping systems (CS) (continuous sole maize (CSM), maize bean intercropping (MBI), rotation maize bean (RMB) and farmers' practice (FP)) grown under conservation (CA) and conventional (CN) tillage practices and % mean performance deviation of each cropping systems against farmers' practice at East-Badawacho, Hawassa-Zuriya and Meskan during 2011 and 2016 cropping seasons.

Parameter		East-Badawacho		Hawassa-Zuriya		Meskan	
		Yield	TDM	Yield	TDM	Yield	TDM
Season	2011	4.4^{a}	9.4^{b}	6.1^{ab}	14.0^{a}	3.6^{b}	10.8^{ab}
	2012	4.2^{a}	14.0^{a}	3.9^{c}	11.4^{b}	1.8^{c}	10.2^{ab}
	2013	4.5^{a}	9.8^{a}	6.8^{a}	9.8^{bc}	1.3^{c}	6.2^{c}
	2014	4.4^{a}	16.9^{a}	5.1^{b}	9.2^{bc}	4.4^{ab}	12.4^{a}
	2015	2.6^{b}	9.5^{a}	3.2^{c}	8.3^{c}	4.6^{a}	11.3^{ab}
	2016	3.6^{ab}	10.1^{a}	3.5^{c}	5.7^{d}	4.7^{a}	8.3^{bc}
CS	CSM	4.0^{a}	12.4^{ab}	4.7^{b}	10.0^{ab}	3.6^{a}	9.6
	FP	3.8^{a}	10.1^{b}	5.6^{a}	10.9^{a}	3.2^{a}	10.9
	MBI	3.6^{a}	11.5^{ab}	4.3^{b}	8.8^{b}	3.4^{a}	10.0
	RMB	4.3^{a}	12.9^{a}	4.7^{ab}	10.0^{ab}	3.5^{a}	9.9
		Percent mean deviation of cropping systems against farmer practice					
CS	CSM	5.3	22.8	-16.1	-8.3	12.5	-11.9
	FP	-	-	-	-	-	-
	MBI	-5.3	13.9	-23.2	-19.3	6.2	-8.3
	RMB	13.2	27.7	-16.1	-8.3	9.4	-9.2
	CA/FP (%)	4.4	21.5	-18.5	-11.9	9.4	-9.8

Columns with different letters are significantly different at P<0.05.

reduction of the NDVI values (Verhulst et al., 2011). NDVI was significantly affected by tillage conditions, increasing their values from conventional practice to CA on maize in sub-Saharan Africa as also reported previously (Gracia-Romero et al., 2018). The NDVI adequately described the effect of residue mulch on the growth of both rice and wheat crops (Jat et al., 2019), which is also associated with higher grain yield in Western India.

Mean performance of cropping systems for grain yield

At East-Badawacho the data combined across seasons (six years) and cropping systems showed that using a CA practice had higher yield performance than FP by 4% (Table 3). While considering six-year average by each cropping system, RMB and CSM had a higher grain yield advantage over FP by 13 and 5%, respectively. However, maize-bean MBI had inferior yield performance by 5.3% compared with FP considering maize yield only; but inter cropping has bonus yield from common bean, which is an advantage of inter cropping. This confirmed that additional yield of common bean obtained from MBI makes the system more productive compared with the farmer practice and other cropping systems (Table 3). In line with this study's finding, a higher yield advantage was also reported (Yilmaz et al., 2008) from 67% maize mixed with 50% bean or cowpea in both 1 maize:1 bean and 2 maize:2 bean or in one row and two row planting patterns compared to solitary cropping of the same species (Yilmaz et al., 2008).

Under each season, MBI had a 4% advantage compared to FP on maize grain yield during the worst season (2012). The reason may be due to the space between maize rows covered by common bean which helped to protect soil moisture from evaporation and make it available for maize and common bean crops. During the remaining five years (relatively good season compared with 2012 rain fall), the MBI cropping system had inferior performance for maize grain yield compared to FP; without considering the grain yield advantage obtained from common bean. Similarly, there were significantly enhanced yields (7%) under rain fed agriculture from no till in dry climates when the other two CA principles were implemented; but a reverse result was reported, that is a yield reduction by 12% when no till is applied alone (Cameron et al., 2014). RMB had higher grain yield advantage than FP by 25, 15, 5, 26 and 20% in 2012, 2013, 2014, 2015 and 2016, respectively. Only in the first season (2011), RMB under CA had a lower grain yield advantage than FP by 1%. CSM also had higher grain yield advantage than FP by 15, 7, 11 and 16% in 2012, 2013, 2014 and 2015, respectively; but during the starting year (2011) and last year (2016) of the experiment, the performance of CSM under CA had lower performance than FP.

At Hawassa-Zuriya, RMB out yielded FP in 2011 and 2013 by 19 and 2%, respectively. Similarly, higher benefits of crop rotation over continuous sole maize and inter cropping also has been reported (Thierfelder et al., 2012). Result from the six-year and cropping systems combined showed that CA had lower performance compared with farmer practice by 19% (Table 3) which in line with the report of an overall reduction of 6% from no-till (Cameron et al., 2014). When no-till is combined with the other two conservation agriculture principles of residue retention and crop rotation, its negative impacts are minimized and significantly increases rain fed crop productivity in dry climates (Cameron et al., 2014). This suggests that the combination of the three CA components may become an important climate-change adaptation strategy for drier regions of the world.

At Meskan, six-year and cropping systems combined data analysis showed higher performance (9%) was obtained from CA (Table 3). The variation in the performance of cropping systems was due to the seasonal rainfall variability. The combined data analysis at East-Badawacho and Meskan also showed that CA had higher grain yield advantage (7%) than FP. Across seasons, combined data analysis of each cropping systems: CSM, RMB and MBI had higher grain yield compared with FP by 13, 6 and 9%, respectively (Table 3). Considering individual seasons and cropping systems, MBI had higher grain yield advantage than FP in 2011, 2012, 2013 and 2014 by 0.2, 86, 37 and 8%, respectively. RMB also had higher grain yield (109, 68 and 4%) than FP during 2012, 2013 and 2016, respectively. CSM had also superior grain yield (0.2, 71, 59, and 2%) than FP in 2011, 2012, 2013 and 2016, respectively. The higher grain and biomass yield obtained from CA indicated that, under CA maize might have better water use efficiency compared with FP. High water use efficiency has been reported in permanent raised beds with 30% standing crop residue retention compared to treatments ploughed once at sowing with 30% standing crop residue retention and conventional tillage (Araya et al., 2012). Survey results on determinant factors for adoption of crop rotation in Arsi-Negele, Ethiopia, indicated regular education, farming experience (number of years the farmer spent in the agriculture) and frequency of contacts with extension workers in a year had significant contribution for adoption of the practice (Musa, 2014).

Generally, any expansion of CA should be done with caution in drier areas, as implementation of the other two principles (residue retention and crop rotation) is often challenging in resource-poor and vulnerable smallholder farming systems, thereby increasing the likelihood of yield losses rather than gains. A yield benefit with no-till in combination with the other two CA principles in dry climates is probably because of improved water infiltration and greater soil moisture conservation (Serraj and Siddique, 2012). This finding suggests that if no-till applied in combination with the other two conservation agriculture principles, CA can become an increasingly important strategy to deal with soil moisture stress due to climate change. It is precisely resource-poor and vulnerable smallholder farming systems that will have the greatest challenges adopting the other two principles, most notably the retention of crop residues due to strong competition for residues by livestock and other uses (Erenstein et al., 2012; Giller et al., 2009). The comparative productivity analysis between continuous maize, maize intercropped with cowpea or pigeonpea and maize in rotation with cowpea or sunnhemp, showed marked benefits of rotation especially in CA systems (Thierfelder et al., 2012). Higher maize grain yield under CA practices has been reported compared with the maize grain yield from conventional practice (Kabirigi, 2015).

In combined data analysis across framers' fields for each year, the highest grain yield was at East Badawacho (4.5 t ha^{-1}) and Hawassa-Zuriya (6.8 t ha^{-1}) districts in 2013 cropping season. At Meskan, the highest yield was recorded in 2016. For data combined across season at each district, the highest grain yield obtained from RMB, FP and CSM at East Badawacho, Hawassa-Zuriya and Meskan, respectively, compared with the other cropping systems. CSM was the second-highest yielder cropping system at the three districts. RMB was also high yielder at Hawassa-Zuriya. At East-Badawacho, RMB and CSM had higher grain yield over FP with values of 13.2 and 5.3%, respectively. At Meskan, CSM, RMB

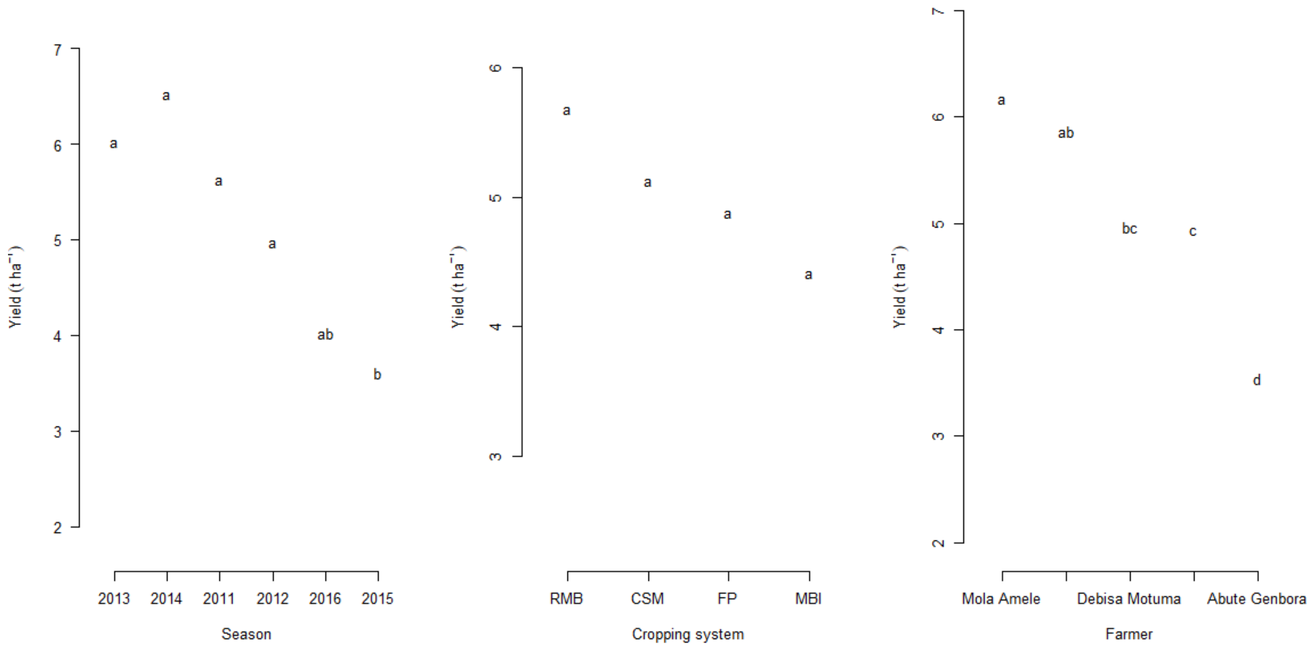

Figure 6. Maize yield variation among seasons, cropping systems and farmers used for the study during 2011 to 2016 cropping seasons at East-Badawacho district in Ethiopia. FP, RMB, CSM and MBI are farmers' practice, Rotation maize bean, continuous maize and maize bean intercropping, respectively. 2011 to 2016 are seasons. The bars indicate interquartile yield range for the seasons, cropping systems and farmers used for the study and bars with the same letter are not significantly different at $P < 0.05$.

and MBI under CA had higher grain yield than FP. Under combined data analysis across location and season the highest grain yield was obtained from RMB, FP and CSM in East-Badawacho, Hawassa-Zuriya and Meskan districts (Figures 6 to 8). For combined data across seasons and cropping systems, CA had higher mean grain yield performance than FP at East-Badawacho and Meskan with the magnitude of 4.4 and 9.4%, respectively. The GGE-biplot graphical analysis showed that BAMR3 and SM3 cropping practice under CA were more suitable for East-Badawacho but for Meskan and Hawassa-Zuriya, the three practices (BAMR1, SM1 and FP1) were good performing practices but the other seven combinations were not represented for three testing locations (Figure 9).

Mean performance of cropping systems for biomass yield

In the across season and cropping systems analysis for biomass yield, the mean performance of cropping systems under CA was 22% compared with FP at East-Badawacho (Table 3). In across season combined data analysis, MBI, CSM and RMB exhibited higher biomass yield than FP by 14, 28, and 23%, respectively. During each season, MBI had higher performance than FP in 2012, 2014, 2015 and 2016 with magnitude of 4, 30, 24, and 52%, respectively. RMB had higher biomass yield than FP; with the value of 14, 17, 31, 77 and 42% in 2012, 2013, 2014, 2015 and 2016, respectively, except in 2011 (first experimental season). CSM had higher biomass yield (4, 3, 29, 30, 64, 17%) than FP in 2011, 2012, 2013, 2014, 2015 and 2016, respectively. Generally, the higher maize grain and biomass yield in 2016 evidence is supported by availability of high chlorophyll content in maize leaf at vegetative and flowering stage of the crop compared with FP (Table 2).

At Hawassa-Zuriya, MBI had higher biomass yield than FP in 2011 and 2016 by 11 and 2%, respectively. RMB exhibited higher biomass yield in 2011, 2013 and 2016 with the magnitude of 42, 2 and 7%, respectively. CSM also had higher biomass yield with the value of 53% in 2011 cropping season, this treatment had also inferior performance compared with FP during the other cropping seasons. Previously, significantly higher stover yield from CA practices compared with the conventional practices (Kabirigi, 2015).

At Meskan, the combined data across seasons and cropping systems showed that CA had inferior performance by 10% compared with FP. While considering each cropping systems at each season, MBI had higher biomass yield than FP in 2011, 2013 and 2016 with the magnitude of 20, 53, and 56%, respectively. RMB also had higher biomass yield than FP in 2011, 2013 and 2016 with value of 21, 41, and 26%,

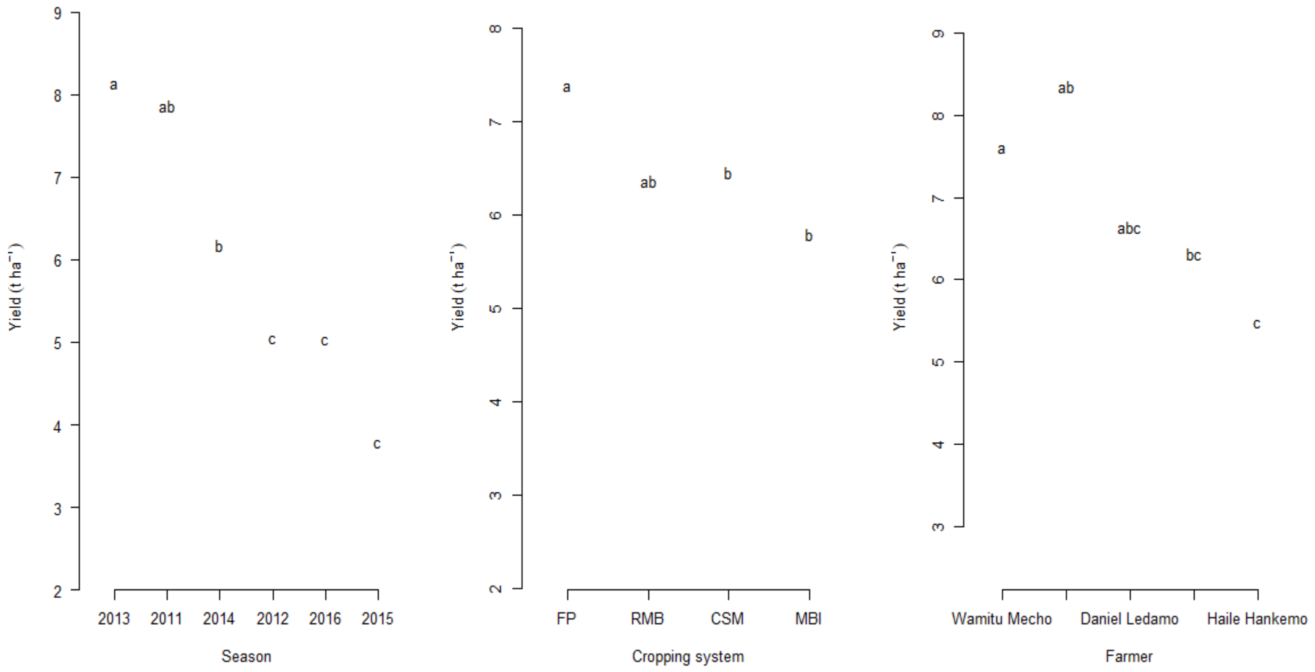

Figure 7. Maize yield variation among seasons, cropping systems and farmers used for the study during 2011 to 2016 cropping seasons at Hawassa-Zuriya district in Ethiopia. FP, RMB, CSM and MBI are farmers' practice, Rotation maize bean, continuous maize and maize bean intercropping, respectively. 2011 to 2016 are seasons. The bars indicate interquartile yield range for the seasons, cropping systems and farmers used for the study and bars with the same letter are not significantly different at $P < 0.05$.

respectively. Similarly, CSM had higher biomass yield than FP in 2011, 2013 and 2016 with magnitude of 11, 55, and 83%, respectively.

In across season and location combined data analysis for TDM, RMB and CSM had higher biomass advantage over FP by 7 and 2%, respectively; but the performance of MBI was lower by 22%. For each cropping system in each season combined across locations, MBI showed TBM yield in 2011 and 2016 with magnitude of 6 and 36% compared to FP, respectively. However, during the remaining seasons, this treatment had inferior performance than FP. RMB also had relatively higher biomass advantage than FP in 2011, 2013, 2015 and 2016; with magnitude of 22, 14, 14, and 25% respectively. CSM had better performance over FP in 2011, 2013, 2015 and 2016 with magnitude of 28, 20, 14, and 22%, respectively. The overall TDM performance of CA was higher by 7% compared with FP based on the average data from across six-year locations analysis.

For the data combined across cropping systems under each location, the highest TDM value was obtained in 2014, 2011 and 2015 at East-Badawacho, Hawassa-Zuria, and Meskan, respectively with values of 16.9, 14.0 and 11.3 t ha^{-1}, respectively. All cropping systems under CA had higher TDM at East-Badawacho and Meskan over FP; whereas at Hawassa-Zuria, FP had higher performance for grain yield and TDM compared with the other cropping system under CA (Table 3). At East-Badawacho, CA showed higher performance (21.5%) compared with FP for TDM. However, at Hawassa-Zuriya and Meskan districts, the overall performance of CA was lower than FP for TDM (Table 3). Similar to the higher TDM under CA than FP found at East-Badawacho in this study, higher biomass production from maize rotation compared to continuous sole maize has been reported for research conducted for long term CA trials in Zimbabwe under CA (Thierfelder et al., 2012). In this study, the increase in grain and biomass yield under no tillage is in contrast with the inferior performance of CA with zero tillage and wheat straw mulch compared with conventional practice (Mehmood et al., 2014).

Common bean performance

Regarding the common bean performance, for bean rotation the mean was 2978 kg ha^{-1} for grain yield and for inter cropping the mean value was 935 kg ha^{-1}across seasons and locations. The grain yield and biomass production from inter cropping is the additional gain in produce on maize yield for farmer. The combined mean data across location and season also showed that, the biomass yield of bean from bean rotation and inter cropping were 5045 and 1658 kg ha^{-1}, respectively (Table

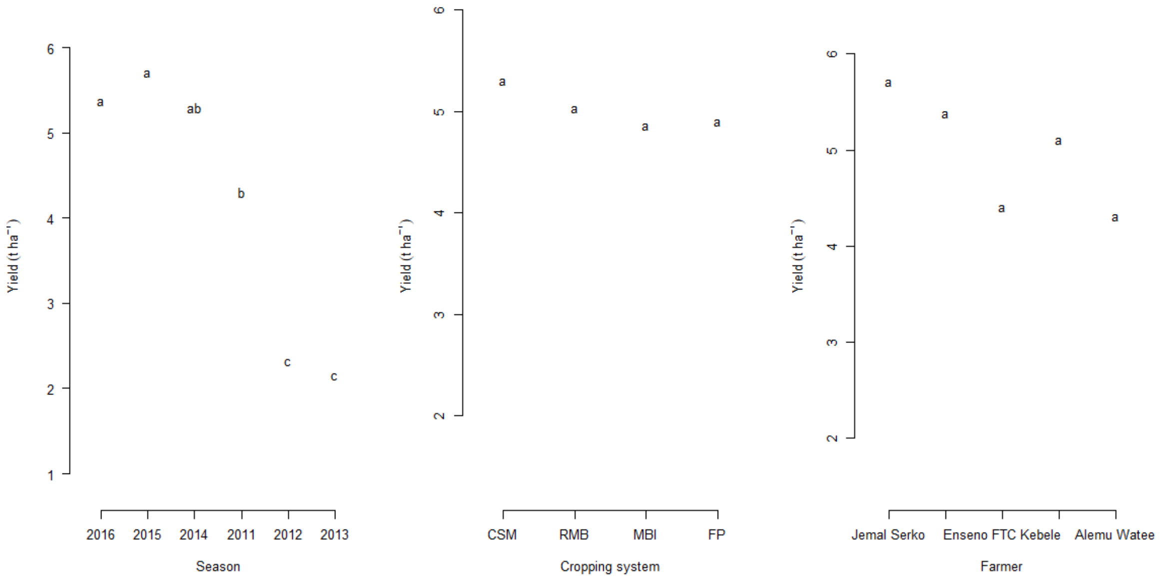

Figure 8. Maize yield variation among seasons, cropping systems and farmers used for the study during 2011 to 2016 cropping seasons at Meskan district in Ethiopia. FP, RMB, CSM and MBI are farmers' practice, Rotation maize bean, continuous maize and maize bean intercropping, respectively. 2011 to 2016 are seasons. The bars indicate interquartile yield range for the seasons, cropping systems and farmers used for the study and bars with the same letter are not significantly different at P < 0.05.

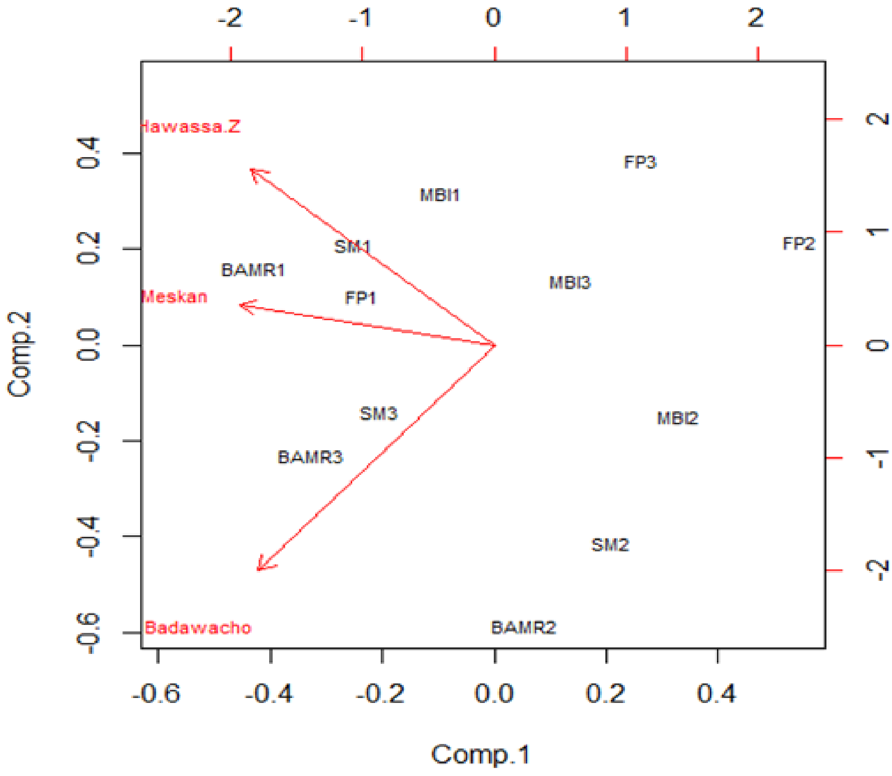

Figure 9. The "discrimination and representativeness" view of GGE biplot for maize yield from four cropping systems (sole maize (SM), maize after bean rotation (BAMR), maize bean intercropping (MBI) and farmers' practice (FP)) grown under conservation (CA) and conventional practices (CN) at East-Badawacho, Hawassa-Zuriya and Meskan during 2011 - 2016 cropping seasons.

Table 4. Mean performance of common bean combined data across (season and location) 2011-2016 under CA.

Treatment	TDM (t/ha)	GY (t/ha)	HI (%)	PPP (#)	SPP (#)	TSW (gm)	SHAV #/ha	NP/m^2 (#)	PH (cm)
Bean rotation (CA)	5.1^{a}	3.0^{a}	59.0^{a}	19.0^{a}	6.0^{a}	257.0^{a}	1648.0^{a}	17.0^{a}	50.0a
Inter cropping (CA)	2.0^{b}	1.0^{b}	56^{a}	13.0^{b}	5.0^{b}	254.0^{a}	788.0^{b}	8.0^{b}	44.6b
CV (%)	34.2	32.2	30.5	35.4	12.7	17.5	21.4	21.4	20.3
F-test	***	***	ns	***	***	^{ns}	***	***	***

TDM= Total dry matter, GY= grain yield, HI= harvest index in %, PPP= pod per plant (#), SPP= seed per pod (#), SHAV= stand count at harvest (#), NP/m^2= number of plants per meter square (#), PH= plant height (cm).

4). Bean rotation had higher performance than inter cropping under CA practice for HI, PPP, TSW and PH (Table 4).

Conclusion

The overall assessment of cropping systems under CA and FP indicated that, cropping systems under CA performed better than the farmer practice both under normal and poor-quality seasonal rainfall conditions. Soil moisture content from CA practices was higher than that of famer practices. Under rainfall shortage conditions, the crop yields from cropping systems under CA were higher compared with the farmer practice for grain yield and biomass due to CA practices conserving soil moisture. During the presence of rainfall shortage, maize-bean inter cropping had relatively higher potential compared with the other cropping systems under CA and farmer practice. Considering production from maize crop only, maze rotation had relatively higher maize grain yield and biomass potential compared with others. Considering the merit in reduction rainfall risks and having addition yield from common bean, maize-bean inter cropping is better.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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