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Full Length Research Paper

Genetic variation of selected quality protein maize inbred lines

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Maize (*Zea mays* L.) is a widely cultivated crop in South Africa and forms the main food crop of thousands of rural communities in the country. In order to improve food and nutrition security for marginalised communities, there is need to develop numerous elite quality protein maize (QPM) varieties. The success of a breeding programme is dependent on the existence of molecular variability among the germplasm. The diversity within 45 QPM inbred lines was evaluated using simple sequence repeat markers. Twenty seven simple sequence repeat primers amplified a total of 112 fragments among the inbred lines. The mean polymorphism information content was 0.48, with an average of 4.32 alleles per locus. Cluster analysis using Rogers (1972) genetic distance partitioned the inbred lines into two major clusters with four and nine sub-clusters each. The minimum genetic distances was 0.13 between CIM12 and CIM13, the average genetic distance was 0.32 and the maximum was 0.46. Cross combinations between QS1 and CIM19 and those between QS22 and CIM18 can potentially give substantial heterosis because of the moderate (0.46) genetic distances that were found between them. Hybrids between these parental lines need to be generated and evaluated in yield trials.

Key words: Quality protein maize, diversity, inbred lines.

INTRODUCTION

Maize is the most important grain crop in South Africa and plays a vital role especially in the diet of women, children, weaned babies and the sick in marginal rural areas of the Eastern Cape Province. It provides food security and a means of livelihood to the majority of people in the province who depend on the crop for daily calories and nutrients. Just like most crops, normal maize provides the recommended calorie amounts but it does not meet all the nutrient requirements of the human body. Quality protein maize (QPM) is a type of maize that contains nearly twice the amount of lysine and tryptophan found in normal endosperm maize.

The benefits of consuming QPM far exceed those of normal maize. QPM has a biological value of protein of 80% compared to that of milk which is 90%, while that of normal maize is 45%. The biological value is an

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indication of the amount of nitrogen that can be absorbed for synthesis of amino acids required for the various metabolic functions in the body (Prassana, 2001). QPM is a nutrition smart food crop providing an improved quality of protein especially for communities who cannot afford protein supplements. Additional benefits of QPM include higher protein retention in people, less sick days for infants, and a quick recovery time for malnourished children. When maize consumption rates of QPM were compared to those of normal maize, it was revealed that children need to consume 100g of QPM to achieve the daily recommended protein requirements compared to 500 g of normal maize (Nuss and Tanumihardjo, 2011). This means that, a harvest of QPM maize will go a longer way in providing nutrition than that of normal maize. Quality protein maize feeding trials reported a 60% increase in live weight for pigs fed on QPM compared to those fed on normal maize. The use of QPM for animal nutrition has resulted in farmers purchasing less soybean and fish meals as protein supplements and using the money for other inputs.

The development of QPM began in the 1970's in South Africa's KwaZulu Natal Province. However, its adoption and utilization has been remarkably low in South Africa and neighbouring countries (Van de Merwe, 1995). Due to the high dependency on maize by rural communities, it has become imperative to identify quality protein maize inbred lines that can be used in breeding programs to develop competitive hybrid and synthetic varieties. Only twelve white QPM hybrids and five QPM open pollinated varieties have been developed and registered in South Africa compared to more than one hundred normal maize varieties. Eighty eight percent of the QPM varieties available were developed by Quality Seed cc (QS) (DAFF, 2014). However, these varieties were bred mainly for high potential areas in the KwaZulu Natal Province (Dr Gevers, personal communication). On the other hand, the Eastern Cape's climate is characterized by a high evaporative demand, erratic rainfall and high summer temperatures (Van Averberke et al., 2011) not ideal for the existing QPM varieties.

With no more arable land available and the demand for agricultural produce continuously increasing, crop improvement can play a significant role in ensuring sustainable food security for marginal areas. However, the basis of crop improvement involves harnessing the variability among germplasm, which will facilitate selection of potential genotypes with potential for producing maximum heterosis when used in crosses. Detailed knowledge on the available QPM inbred lines is required for them to use them efficiently in breeding programmes.

At present no information is available on the genetic diversity between CIMMYT and QS QPM inbred lines. Therefore, determination of the genetic relationships of germplasm from these two sources would be of interest to maize breeders targeting the development of highly productive QPM cultivars not only for the Eastern Cape province of South Africa but for Southern Africa. Genetic diversity can be investigated using several techniques such as morphological or molecular markers.

Molecular markers can reveal genetic relationships among the inbred lines. Crosses between inbred lines that are genetically distant are expected to give a larger genetic variance among progenies than crosses between closely related lines (Hallauer and Miranda, 1988). Molecular markers have been extensively used in maize genetic studies for the analysis of genotype frequencies, identification of deviations at individual loci and for characterization of molecular variation within and between populations. Relative to other types of molecular markers, simple sequence repeats (SSRs) are technically simple to use, cost effective, co-dominant, robust and reliable, and they are transferrable between populations (Collard et al., 2008).

According to Shin et al. (2006), genetic distance measurement ensures a better understanding of the genetic structure and helps in genetic manipulation of genotypes for crop improvement. Genetic distances are estimated from assessment of genetic diversity between genotypes. Mondini et al. (2009) defined genetic diversity as the variety of alleles and genotypes present in a population, which is reflected in morphological and physiological differences between individuals of a population. Knowledge of the genetic distance of inbred lines enables those from different heterotic groups to be combined to form a heterotic pattern. Heterotic patterns can be used in selecting parents of crosses for line development. In addition the heterotic patterns can be used in selecting testers for evaluating the combining abilities of new inbred lines.

Maize breeders are also interested in selecting inbred lines that combine well and give high yields without necessarily making all possible crosses between them (Makumbi et al., 2011). In the present study, assessment of genetic diversity was of interest for broadening the current QPM genetic base. The highest genetic distance reported among CIMMYT-QPM inbred lines used in this study was 0.38 (Pfunde, 2013), which necessitated broadening the current QPM genetic base by including those from QS cc. The objective of this study was therefore to assess the genetic diversity among 45 QPM inbred lines using SSR markers.

MATERIALS AND METHODS

Forty-five white grained QPM inbred lines were sourced from CIMMYT –Zimbabwe (CIM) and Quality Seed cc (QS) in KwaZulu Natal Province, South Africa. The QPM inbred lines are described in Table 1.

Genetic diversity using SSR analysis

Quality protein maize inbred lines were planted in pots in a

Table 1. Names and heterotic groups of Quality Protein Maize inbred lines.

S/N	Inbred line	Heterotic group
1	IBL1	A
2	IBL2	B
3	IBL3	B
4	IBL4	-
5	IBL5	B
6	IBL6	A
7	IBL7	A
8	IBL8	B
9	IBL9	B
10	IBL10	B
11	IBL11	B
12	IBL12	B
13	IBL13	B
14	IBL14	B
15	IBL15	B
16	IBL16	B
17	IBL17	-
18	IBL18	-
19	IBL19	A
20	IBL20	A
21	IBL21	B
22	QSW1	F
23	QSW2	F
24	QSW3	F
25	QSW4	F
26	QSW5	M
27	QSW6	F
28	QSW7	F
29	QSW8	O
30	QSW9	T
31	QSW10	M
32	QSW11	F
33	QSW12	F
34	QSW13	F
35	QSW14	H
36	QSW15	B
37	QSW16	H
38	QSW17	H
39	QSW18	H
40	QSW19	H
41	QSW20	H
42	QSW21	H
43	QSW22	G
44	QSW23	G
45	QSW24	G

heterotic group unknown

glasshouse at the University of Fort Hare in February 2014. Maize genomic DNA was extracted from 2 week old leaves from each of the 45 QPM inbred lines. Extraction was carried out using a Wizard® genomic DNA purification kit (Promega) from 40 mg of maize leaf tissue that was freeze dried using liquid nitrogen.

In order to determine the quality of DNA, 2 µl of concentrated DNA sample was mixed with 10 µl of 6x loading dye. The mixture was loaded on a 0.8% agarose gel, and electrophoresis was carried out in a buffer with 0.5 Tris Borate Ethylenediaminetetraacetic acid (TBE) with a pH of 8.0, using a Gel XL Ultra horizontal gel system (Labnet International) at 100v for 90 min. A 1 kb ladder was used as the molecular weight marker. After electrophoresis, the DNA was stained with ethidium bromide and then visualised using a gel documentation system (Uvitec Cambridge, Alliance version 4.7). The quantity of DNA was determined by Ultraviolet absorbance using a spectrophotometer (Genova MK3 Life analyser, Jenway). For quantity assesement, 5 µl of the concentrated DNA sample, plus 995 µl of Tris EDTA (TE), was loaded into a cuvette which was then inserted into the spectrophotometer chamber for measurement.

The polymerase chain reaction conditions were in accordance with CIMMYT laboratory protocols (2005), with minor modifications. The final concentrations of the PCR reagents that were used for amplification were; 40 ng template DNA, 0.25 µM forward and reverse primers, 1 unit Takara Ex Taq DNA polymerase (Separations, 150 µM each of dNTPs, 1X Taq buffer (20 mM Tris-HCl, pH 8.0,100 mM KCL, 0.1 mm EDTA, 1 Mm DTT, 0.5% Tween 20, 0.5% NP-40, 50% glycerol).

A touchdown PCR programme was used as described by Senior et al. (1998), with a few modifications. The initial cycle had a denaturation temperature of 94ºC for one minute. The second cycle had ten cycles, starting with denaturation at 94°C for 1 min, followed by annealing. One cycle was performed for every 1ºC decrease in annealing temperature from 65 to 55°C. Ten cycles were therefore performed at 10 different temperature settings. Extension was done at 72°C for 1 min 30 s. Temperature settings for the next 30 cycles were as follows; denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min 30 s. The final extension was at 72°C for 5 min, and the holding temperature was 4ºC. Each of the 27 SSR primers amplified DNA of each of the 45 inbred lines.

After amplification, PCR products were electrophoresed on a vertical gel system with 12% acrylamide solution (non-denaturing gels). A mixture of 6 µl of the PCR sample and 2 µl of O'Gene 6x orange loading dye (Thermo Scientific) were loaded into a 1.0 mm wide gel well. Products were separated by electrophoresis in a Bio-Rad Mini Protean Tetra System. The gels were run for 90 min at 120 volts. After electrophoresis, the gel was stained with 5 µl of ethidium bromide in 70 ml of distilled water at room temperature for 35 min. The bands of DNA were then visualised using a gel documentation system (Uvitec Cambridge, Alliance version 4.7). Allele sizes of the SSR bands were determined by comparing them with the internal O'Gene 100 bp molecular weight marker (CIMMYT, 2005).

Statistical analysis

For molecular analysis, each SSR primer was considered as a locus, and each band as an allele. Deoxyribonucleic acid banding patterns from SSR gels were converted to binary form, one indicated the presence of a specific allele and zero indicated its absence. The polymorphism information content (PIC) for each SSR primer was determined as described by Smith et al. (1997), using the following formula:

$$1 - \sum_{i=I,n}^{n} f_i^2$$

Where, f_i is the frequency of the *i*th allele. Gene diversity was calculated to quantify the genetic variation among the maize inbred lines. Allele frequency was calculated for each locus across the set of inbred lines using the Power Marker software version 3.25. The resulting unrooted tree was visualized using Mega version 5. The genetic distances between genotypes were computed using Roger's (1972) genetic distances (RD). Cluster analysis was then carried out using the neighbour-joining tree (NJ) method.

RESULTS

Polymorphism of SSR markers

The 27 SSR primers amplified a total of 121 bands among the 45 inbred lines, to give an average allele richness of 4.32 alleles per locus. The highest number of alleles (7) was identified for primer Phi127 and Phi 109275. The polymorphism information content (PIC) was from 0.20 for Phi 213984 to 0.67 for Phi109275, with a mean of 0.48. The gene diversity ranged from 0.23 to 0.71 while the average gene diversity was 0.53. Primer Phi 109275 showed the highest gene diversity (0.71) as shown in Table 2.

Genetic distances among the CIMMYT and QS inbred lines

The highest genetic distance was found between inbred lines CIM18 x QS 22 and between CIM19 x QS1 (0.46). The next highest genetic distance was 0.45, and it was found between inbred lines CIM1 x QS10; CIM15 x QS4; CIM16 x QS4; CIM15 x QS1; CIM16 x QS1; CIM19 x QS17; CIM19 x QS22; CIM19 x QS5; CIM3 x QS11 and CIM4 x QS19. Conversely, the lowest genetic distance was found between CIMMYT lines CIM12 x CIM13 (0.13). The overall average genetic distance was 0.31.

The highest genetic distances among the QS lines was 0.45. Cross combinations that exhibited this distance were QS9 x QS12 and QS6 x QS10. Other QS cross combinations with a moderate genetic distance of 0.43 were QS11 x QS21 while crosses QS16 x QS21; QS17 x QS21; QS19 x QS21 and QS2 and QS4 had a genetic distance of 0.41. It was observed that cross combinations within the QS cluster showed higher genetic distances (0.45) than cross combinations within the CIMMYT cluster (0.38).

Cluster analysis

The unrooted tree clearly revealed two distinct groups, with 22 and 23 inbred lines for clusters- 1 and 2 respectively (Figure 1). The CIMMYT inbred lines were grouped separately from QS inbred lines, with the exception of QS9 which branched off from CIM10. The major clusters were further divided into sub-clusters. Cluster 1 was further divided into four sub- clusters while cluster 2 was further divided into nine sub-clusters. Inbred lines did not cluster clearly according to heterotic

Table 2. Allele frequency, allele number, gene diversity and polymorphism information content.

Marker	Bin No.	Allele frequency	Allele number	Gene diversity	PIC
PHI127	2.08	0.50	7.00	0.68	0.64
PHI053	3.05	0.54	6.00	0.65	0.62
PHI029	3.04	0.50	6.00	0.67	0.63
PHI072	4.01	0.50	6.00	0.67	0.63
NC130	5.00	0.51	6.00	0.68	0.64
PHI031	6.04	0.63	3.00	0.50	0.42
PHI034	7.02	0.59	6.00	0.61	0.58
PHI032	9.04	0.60	4.00	0.56	0.50
PHI050	10.03	0.67	4.00	0.52	0.48
NC133	2.05	0.74	3.00	0.40	0.35
UMC1061	10.06	0.66	3.00	0.47	0.39
PHI213984	4.01	0.87	2.00	0.23	0.20
PHI109275	1.00	0.46	7.00	0.71	0.67
UMC1109	4.10	0.58	4.00	0.58	0.52
PHI059	10.02	0.52	6.00	0.66	0.61
PHI046	3.08	0.87	3.00	0.23	0.21
PHI121	8.04	0.54	4.00	0.60	0.53
PHI101049	2.09	0.44	4.00	0.63	0.56
UMC1136	3.10	0.51	6.00	0.67	0.63
UMC1399	3.07	0.54	3.00	0.54	0.44
UMC1161	8.06	0.51	4.00	0.62	0.55
UMC1153	5.09	0.61	4.00	0.51	0.42
UMC1277	9.00	0.82	3.00	0.31	0.28
PHI112	7.01	0.50	4.00	0.64	0.58
UMC1122	1.06	0.78	3.00	0.37	0.33
PHI96100	2.00	0.78	3.00	0.37	0.34
PHI015	8.09	0.64	4.00	0.53	0.49
Mean	-	0.62	4.32	0.53	0.48

groups or pedigree information provided, except for a few inbred lines such as QS3 and QS4; QS18 and QS19 which belonged to heterotic group F and H respectively.

DISCUSSION

Polymorphism of SSR markers

The average number of alleles per primer obtained in this study is less than those reported in previous SSR studies. However, the average allele number is in accordance with previous studies (Khoza, 2012) who also recorded average alleles of 4.96 per locus while investigating the genetic diversity of 60 maize inbred lines. The moderate allele richness and gene diversity in this study indicated a moderate genetic base. The average PIC value obtained (0.48), was higher than that reported by Legesse et al. (2007) of 0.33. The lowest PIC (0.20) was reported for primer Phi213984 which identified two alleles.

Genetic distance between CIMMYT and QS lines

There was greater diversity between CIMMYT and QS inbred lines than among lines within the major. This was evident from the genetic distances observed between the two groups of inbred lines. The highest genetic distance was recorded for CIM19 and QS1 which belonged to different clusters and heterotic groups, A and F respectively. This cross combination has the potential to produce superior hybrids. Hallauer and Miranda (1988) reported that the more parental lines are genetically distant the more likely the manifestation of heterosis. The lowest genetic distance was recorded for CIM12 and CIM13, which belonged to the same heterotic group, 'B' and were also grouped into the same sub-cluster. This indicated that CIM12 and CIM13 are less likely to develop high performing hybrids because they have almost similar genetic backgrounds.

Cross combinations with QS inbred lines showed moderate genetic diversity. This indicated that there was more diversity within the QS lines than there was among

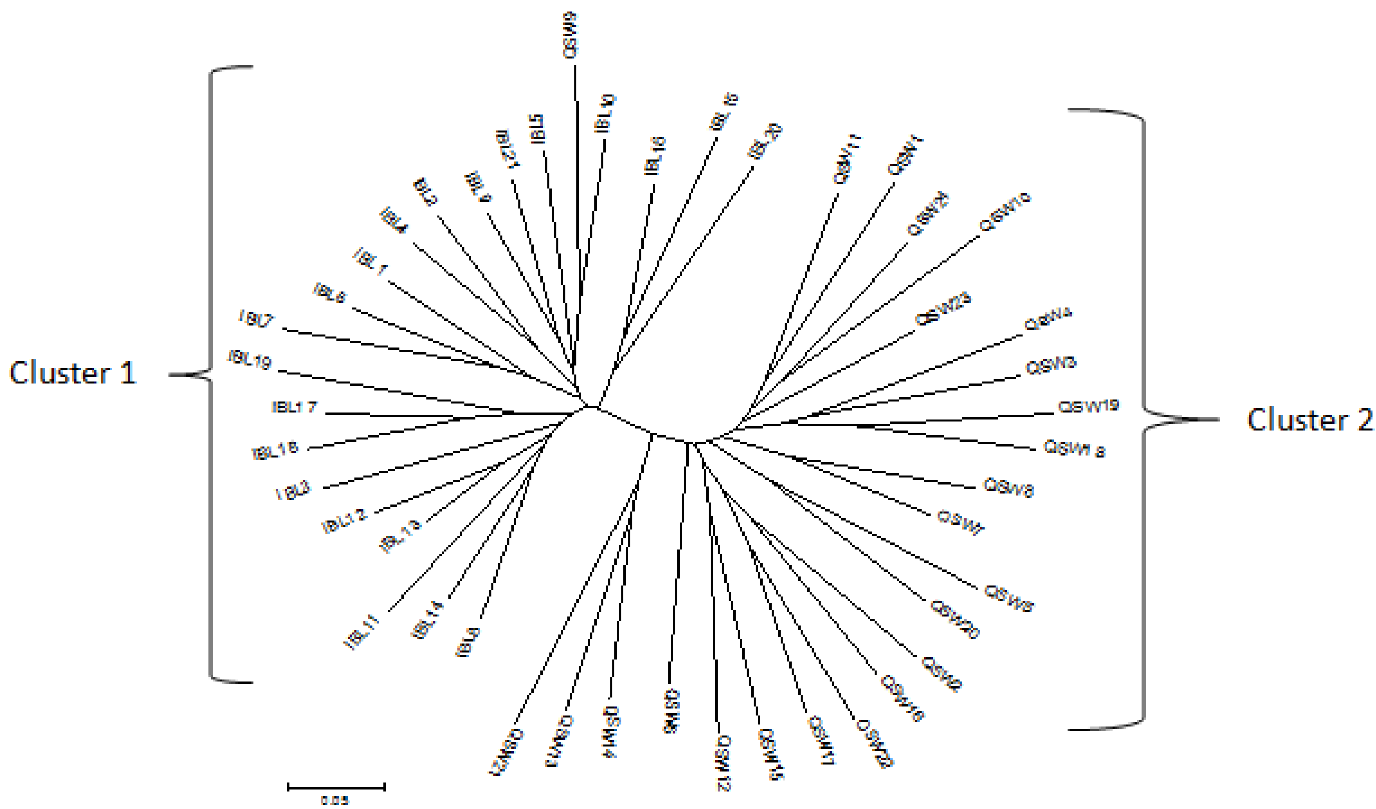

Figure 1. Unrooted tree for 45 QPM inbred lines based on Rogers (1972) genetic distance.

CIMMYT lines. The moderate genetic distance among QS lines may partly explain why it was possible to produce successful QPM hybrids that have been registered in South Africa so far. All inbred lines with a genetic distance ranging from 0.41 to 0.45 were taken from different sub-clusters within the QS cluster. The reason for low genetic distances for some maize inbred lines may be due to intensive breeding which aims to select germplasm suitable for similar agro-environments.

Cluster analysis

The QPM inbred lines were clustered into two major groups according to their source. CIMMYT-sourced inbred lines were distinctly separated from inbred lines sourced from Quality Seeds. The sub-clusters formed were expected because both CIMMYT and QS draw their inbred lines from different pools and populations (Warburton et al., 2005). QS9 was grouped with the CIMMYT inbred lines which have a tropical origin. According to the available information on QS9, its heterotic grouping is unknown but is assumed to have originated in the tropics (Gevers, Personal communication). Inbred lines CIM12 and CIM13 from CIMMYT were grouped together in the same sub-cluster because they share a common parent GQL5. The two lines probably inherited most of the genes from this common parent. Some of the lines were grouped according to heterotic groups while others were mixed.

According to Vivek et al. (2008), heterotic groups are subjective and are constantly evolving suggesting that heterotic groups such as ‘F’ F2834W may have been derived from the same population as H (Hickory King) which may explain the mix in groups in each cluster. The large number of sub-clusters found in the QS major cluster indicates a wider genetic diversity, as also shown by the several heterotic groups within that cluster. In comparison, the CIMMYT cluster showed only four sub-clusters and had two heterotic groups.

In conclusion, moderate genetic diversity was found in the selected QPM inbred lines based on 27 SSR markers. The clustering observed in this study was in agreement with some of the heterotic grouping. Quality protein maize inbred lines for hybridization can be selected based on the genetic distance information that was generated from this study.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Length of condyles and phalanges of Brazilian bovines (Nellore, Pantaneira, Curraleira) and water buffaloes (Murrah × Jafarabadi) measured by radiographic images: post-mortem study

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The diversity between the ruminants' digits makes it necessary to study the anatomical features in all these animals. For this purpose, the left forelimb and hind limb of Brazilian male bovine breeds (Curraleira, n=15; Pantaneira, n=15; Nellore, n=15) and water buffaloes (n=12) were used. Dorsopalmar/plantar radiographies were obtained. Then they were photographed, digitalized and analyzing by computer program ImageJ software. The lengths of the condyles (LC), first (P1), second (P2), third phalanges (P3), and the overall length (OL) of the digits, on the forefeet (FF) and hind feet (HF) were determined. The LC, P1, P2, P3 and OL of the digits were similar in the Curraleira and Pantaneira breeds in the FF and HF. In Nellore, the lengths of all bone measurements were greater than the bone lengths of the Curraleira and Pantaneira. No differences for LC and P1 lengths between Nellore and Buffaloes were observed. The buffaloes showed the lengths of the P2, P3 and OL greater than the bone measurements of all bovine breeds. No statistical differences between lateral and medial digits, of the FF and HF, were seen in bovines and buffaloes. Nevertheless, it was interesting to observe that the lateral digits appeared some millimeters greater, in Curraleira (FF=2.4 mm, HF=0.8 mm), Pantaneira (FF=0.7 mm, HF=1.1 mm), Nellore (FF=0.7 mm, HF=1.6 mm) and buffaloes (FF=0.2 mm, HF=0.9 MM). The lateral forefoot condyles were longer than medial forefoot condyles in all animals. A higher correlation between the length of P3 and the body weight in the hind foot of buffaloes was seen. Canonical analysis demonstrated similarity between the bovine digits and proved that buffalo digits were longer than in all bovines. The lengths of the digital bones are different among Curraleira, Pantaneira and Nellore breeds, and Buffaloes.

Key words: Cattle, digit, ruminants, radiography, bone, Bovidae.

INTRODUCTION

Bovine breeds raised in Brazil can be classified into two groups, as Creole and Exotic (Egito et al., 2007). Creole breeds (Curraleira, Pantaneiro, Caracu, Crioulo Lageano and National Polled), also referred to as native, local or naturalized breeds, including those derived from the first cattle populations introduced by the European conquerors around 1500s (Mariante and Egito, 2002). The most prominent exotic breeds in Brazil are the Indian zebu breeds such as Nelore, Gyr and their hybrids, which have been introduced over the last 100 years (Egito et al., 2007).

Buffaloes of the Murrah, Mediterrâneo, Jafarabadi and Carabao breeds were introduced in Brazil in the beginning of the 20th century. Nowadays, the population is of 2.8 million in Brazil and increasing at a faster rate than cattle (Malhado et al., 2007).

These bovine breeds and the buffaloes have mainly been studied with the aim of determining the genetic diversity to establish conservation programs (Serrano et al., 2004; Ramos et al., 2006; Egito et al., 2007; Malhado et al., 2007; Salles et al., 2013; Malhado et al., 2013). Moreover, few studies have been done to describe the normal aspects of digits in Nellore (Gonçalves et al., 2014) or to report diseases that affect the limbs in cattle and buffaloes (Barbosa et al., 2014).

Taking into account the importance of these Bovideos for Brazil, this study investigates the digits of these animals to obtain relevant data using measurements taken using dimensions of the digits, their interrelation as well as establishing the correlation between these measures and body weight.

The aims are to compare the various digital bones of bovines (Nellore, Pantaneira, Curraleira) and water buffaloes (Murrah × Jafarabadi) using the radiographic anatomical measurements, in order to check the differences in the lengths of the metacarpal/metatarsal condyle, the three phalanges and the total lengths of the lateral and medial digits, in the thoracic and pelvic limbs.

MATERIALS AND METHODS

The left digits (forefeet and hind feet) of males in the *Bovidae* family, 45 bovines (Curraleira, Pantaneira and Nellore breeds) and 12 water buffaloes (cross breed Murrah × Jafarabadi), were used in this study (Table 1). The Research Ethics Committee of Federal University of Goiás has approved this study (Nº 090/2011).

The Nellore and Curraleira breeds came from state farms in Goiás. The Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) of Mato Grosso do Sul donated the Pantaneira breed. Moreover, the buffaloes came from state farms in Pará.

The animals were slaughtered following all the sanitary protocols indicated by the Federal Inspection in Goiás. The thoracic and pelvic limbs were disjointed on the carpal-metacarpal and tarsal-metatarsal joints. Only the left limbs were used in this experiment, while another study was carried out using the contralateral limbs.

Before the radiographic procedures the limbs were washed, dried, and examined to confirm the absence of digital diseases.

Dorsopalmar and dorsoplantar radiographies were obtained in a stationary X-ray device by Tur in the T-350 model (Röntgentechnik GmbH, Potsdam, Germany) with the capability for 600 mA. Exposure indices were defined considering the thickness of the member, varying from 60 kV to 70 kV, 25 to 30 mA and 0.2 s. The focus-film distance was maintained at 90 cm and the central beam was positioned perpendicular to the cassette at the level of the proximal interphalangeal joint between the two feet. The radiographies were developed in a Vision Line LX-2 (Lotus, Curitiba, Paraná, Brazil) automatic processor.

The radiographies were done and evaluated following the Bargai et al. (1989) and the Geissbühler et al. (2010) instructions. After, by using a digital camera (DSC-w130, Sony Brasil Ltda., São Paulo, São Paulo) the radiographies were photographed, digitalized and compressed in a Joint Photographic Experts Group (JPEG) format. Using the computer program ImageJ software (Version 1.36b for Mac OS X) the lengths of the bones were determined.

Figure 1 outlines the length of the condyles (LC), length of proximal phalanx (P1), length of medial phalanx (P2), length of distal phalanx (P3) and the overall length (OL) for the medial digit (M) and lateral digit (L) in the forefeet (FF) and hind feet (HF). The measurements were done according to Schwarzmann et al. (2007) and Muggli et al. (2011).

For all the measurements, the means and standard deviations were calculated. The mean lengths assessed in each group were compared by ANOVA followed by the Tukey test. A correlation between the body weight with the obtained measurements were evaluated using Pearson's correlation. The Canonical analysis was used to express the similarities between the variables studied in each animal. All analyses were carried out by software R (R-Development Core Team, 2011) adopting $p<0.05$.

RESULTS

Examining the radiographies, the bovine bones silhouettes (condyles, first, second and third phalanges) appeared longer and thinner in Curraleira and Pantaneira than in Nellore. The bone silhouettes were bigger in Buffaloes (Figure 2). The lengths of the condyles, P1, P2, P3 and the overall length of the digits, in the forefoot and hind foot were similar ($p>0.05$) in the Curraleira and Pantaneira breeds (Table 2).

In Nellore, the length of all bones were greater than those of Curraleira and Pantaneira ($p<0.05$). No differences ($p>0.05$) were observed for LC and P1 lengths when the Nellore bone lengths were compared with bone buffaloes. The buffaloes showed greater lengths of the P2, P3 and OL than the bone measurements of all bovine breeds ($p<0.05$). Comparing the lateral and medial digits, of the FF and HF, no differences ($p>0.05$) were observed between all phalanges and OL for bovines and buffaloes. Nevertheless, it was interesting to observe that the OL of the lateral digits appeared some millimeters greater, but not significantly, in Curraleira (FF=2.4 mm, HF=0.8 mm),

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Table 1. Number of animals and digits, body weight and age of *Bovidae* family.

***Bovidae* family**	**Number of animals**	**Number of digits**	**Age (months)**	**Body weight (Kg)**
Curraleira	n=15	n=30	26.70±5.47	408±84.2
Pantaneira	n=15	n=30	26.35±8.97	456±83.7
Nellore	n=15	n=30	22.30±2.69	508±40.8
Buffaloes	n=12	n=24	34.50±1.17	525±45.5

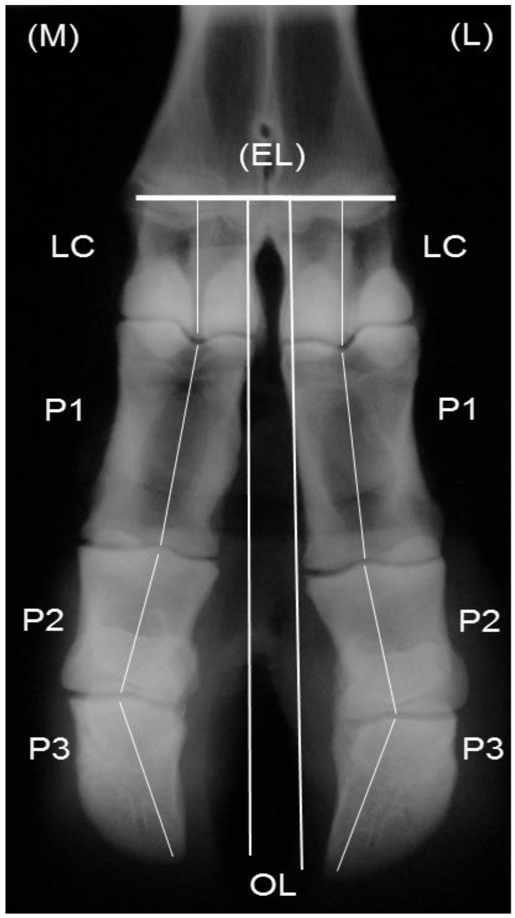

Figure 1. Schematic representation of the measurements in a plantarodorsal radiograph of the left hindfoot in male Nellore cattle. Medial digit (M), lateral digit (L), epiphyseal line (EL). LC, length of condyle; P1, length of proximal phalanx; P2, length of medial phalanx; P3, length of distal phalanx; OL, overall length.

Pantaneira (FF=0.7 mm, HF=1.1 mm), Nellore (FF=0.7 mm, HF=1.6 mm) and buffaloes (FF=0.2 mm, HF=0.9 MM).

In the forelimb, the lateral condyles were longer than the medial condyles (Table 2) in Curraleira (p=0.001), Pantaneira (p=0.009), Nellore (p=0.005) and buffalo (p=0.004). No differences were observed in the condyles of the hind limb (p>0.05). The results of the statistical correlation showed higher correlation (r=0.73, p=0.006) between the length of P3 and the body weight in the hind foot of buffaloes (Table 3). Canonical analysis demonstrated similarity between bovine digits and, proved that buffalo digits were bigger than in all bovines.

DISCUSSION

The differences between the bone silhouettes in the

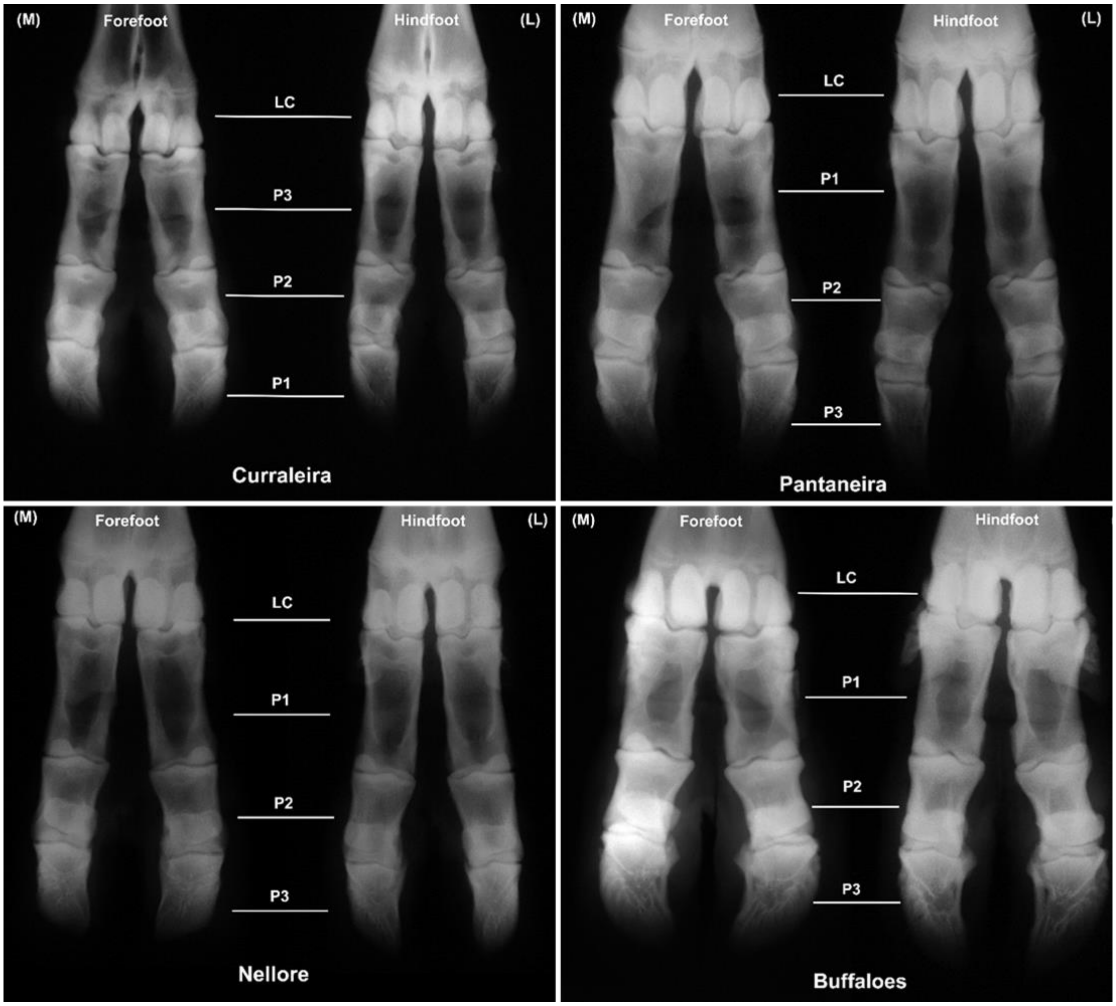

Figure 2. Plantarodorsal radiograph of the left forefoot and hind foot. Showing the differences between digital bones of the Curraleira, Pantaneira, Nellore and Buffalo males. Medial digit (M) and lateral digit (L). LC, condyle length; P1, proximal phalange; P2, medial phalange; P3, distal phalange.

radiographs of the bovines (Curraleira, Pantaneira, Nellore) and the buffaloes were confirmed by the length measurements of the condyles, P1, P2, P3 and OL in the forefoot and hind foot.

The bone lengths established in the present study were close to the lengths observed in different breeds of bovine, by direct measurements (Ocal et al., 2004; Nacambo et al., 2007; Radisic et al., 2012) and radiographic measurements (Schwarzmann et al., 2007; Muggli et al., 2011). As well as, in buffalo bones (Nourinezhad et al., 2012) and in wild animal radiographies (Keller et al., 2009).

The methods used for obtaining measurements on the radiographs had been established in previous studies (Schwarzmann et al., 2007; Nacambo et al., 2007; Keller et al., 2009; Muggly et al., 2011) and, in our study they were carried out thoughtfully to ensure accurate results. According to Nourinezhad et al. (2012) direct measurements have the advantage of direct visibility and better determination of measuring points, while in radiographic measurements, projection errors and poorer visibility of the surface of the bones may account for mistakes. The authors can assume that the measurements carried out in our study were accurate, since there was concordance with the results of the studies that used direct measurements.

The mean lengths of the P1, P2, P3 and the OL (plus and minus the condyles) in our study were compared with the mean values of bone lengths in other studies (Table 4) of bovines and buffaloes. The Curraleira and Pantaneira measurements were similar to the measurements in Holstein male cattle (Ocal et al., 2004). The lengths of the Nellore bones were similar as those described by Muggli et al. (2011) in heifers and steers of different breeds. For buffaloes, the mean values in our study were distinctively greater than the values of the

Table 2. Mean values (mm) ± standard deviation of the measurements in the radiographic images of the condyles and phalanges in the forefoot and hind foot of bovine males (Curraleira, Pantaneira, Nellore) and buffaloes male.

Measures	Curraleira	Pantaneira	Nellore	Buffaloes
Forefoot				
Lateral				
LC	28.90±0.60^{bA}	30.20±0.60^{bA}	36.00±1.00^{aA}	34.20±0.50^{aA}
P1	52.00±0.70^{b}	54.50±0.50^{b}	60.80±0.90^{a}	62.80±1.30^{a}
P2	34.10±0.90^{c}	35.90±0.90^{c}	40.00±1.90^{b}	44.30±1.80^{a}
P3	40.60±0.50^{c}	41.60±0.30^{c}	43.00±0.70^{b}	60.90±1.20^{a}
OL	161.10±2.40^{c}	166.80±1.90^{c}	181.50±2.00^{b}	205.80±4.60^{a}
Medial				
LC	27.40±0.40^{bB}	28.50±0.60^{bB}	32.50±0.40^{aB}	30.70±0.70^{aB}
P1	51.90±0.90^{b}	54.60±0.60^{b}	62.00±0.80^{a}	63.10±1.80^{a}
P2	33.90±0.90^{c}	35.80±0.70^{c}	39.50±0.70^{b}	44.10±1.90^{a}
P3	40.50±0.50^{c}	41.60±0.30^{c}	43.10±0.50^{b}	62.30±1.20^{a}
OL	158.70±2.50^{c}	166.10±2.00^{c}	180.80±1.90^{b}	204.10±4.10^{a}
		Hind foot		
Lateral				
LC	28.80±0.60^{b}	30.90±0.70^{b}	34.90±0.80^{a}	32.50±0.50^{a}
P1	50.76±0.80^{b}	58.00±10.0^{b}	65.10±0.90^{a}	64.80±1.50^{a}
P2	36.80±0.90^{c}	37.90±0.90^{c}	40.90±0.60^{b}	46.00±2.20^{a}
P3	39.00±0.60^{c}	39.80±0.60^{c}	42.60±0.40^{b}	62.70±1.20^{a}
OL	161.90±2.40^{c}	171.90±2.40^{c}	187.70±1.90^{b}	21.01±4.30^{a}
Medial				
LC	28.70±0.60^{b}	30.40±0.90^{b}	34.10±0.60^{a}	32.20±0.50^{a}
P1	55.50±0.70^{b}	57.40±0.80^{b}	64.90±0.60^{a}	63.80±1.70^{a}
P2	36.30±1.10^{c}	38.40±1.00^{c}	41.50±0.80^{b}	44.30±1.80^{a}
P3	39.80±0.60^{c}	40.40±0.50^{c}	41.80±0.50^{b}	62.40±1.60^{a}
OL	161.10±2.30^{c}	170.80±2.40^{c}	186.10±1.90^{b}	209.20±4.70^{a}

(LC) length of the metacarpal and metatarsal condyle, (P1) length of the proximal phalanx, (P2) length of the middle phalanx, (P3) length of the distal phalanx, (OL) lateral overall length. Means marked by different small superscripts (a and b) in a row indicate significant differences between animals regardless of lateral or medial ($p<0.05$). Means marked by different large superscripts (A and B) in a column differ significantly between the medial and the lateral bones in the same feet ($p<0.05$). Equals letters were omitted.

Khuzestan buffaloes (Nourinezhad et al., 2012).

There was no significant asymmetry between the lateral and medial bones, in the forefoot and hind foot, in both bovines and buffaloes. By direct measurements, Ocal et al. (2004) and Nourinezhad et al. (2012) reported no differences between the lengths of the phalanges and the total lengths of the three phalanges within the left and right thoracic and pelvic limbs. However, using radiographic measurements of Schwarzmann et al. (2007), Muggli et al. (2011) and Keller et al. (2009) described a lateral digit longer than medial.

Considering the differences between the bone lengths and the lateral and medial digits, it can be assumed that the digits bone length differences could be characteristics of artiodactyls (Keller et al., 2009), indicative of anatomical variations existent in individual cattle (Muggli et al., 2011) and/or, the differences among ruminants resulting from locomotion habits (Nourinezhad et al., 2012).

Even though the results have not pointed to a significant difference between the total lengths of the digits, the lateral digit were some millimeters longer than the medial in the forelimb (0.2 to 1.6 mm) and in the hind limb (0.8 to 2.4 mm). These results were similar with the findings observed by Muggli et al. (2011), the lateral digits were longer than the medial digits in the forelimb (1.8 mm) and in the hind limb (2.1 mm).

Nacambo et al. (2007), Keller et al. (2009) and Muggli et al. (2011) postulated that the length asymmetry between lateral and medial condyles were responsible for the difference in the length of the paired digits. Nourinezhad et al. (2012) hypothesized that such a length asymmetry of condyles bones exists in the water buffalo. In our study, the lateral metacarpal condyles

Table 3. Pearson's correlation (r) between the body weight and the measurements in the radiographic images of the condyles and phalanges in the forefoot and hind foot of the bovine males (Curraleira, Pantaneira, Nellore) and buffalo males.

Body weight (kg)	Variables (cm)	Forefoot				Hind foot			
		Lateral		Medial		Lateral		Medial	
		r	p*	r	p*	r	p*	r	p*
Curraleira (408±84.2)	LC	0.30	0.27	0.10	0.72	0.10	0.71	0.23	0.40
	P1	0.23	0.40	0.28	0.31	0.29	0.29	0.27	0.33
	P2	0.09	0.74	0.04	0.88	0.02	0.95	0.14	0.60
	P3	0.35	0.20	0.24	0.38	0.14	0.61	0.15	0.58
	OL	0.23	0.41	0.19	0.48	0.13	0.64	0.30	0.27
Pantaneira (456±83.7)	LC	0.42	0.12	0.09	0.75	0.42	0.12	0.01	0.96
	P1	0.11	0.68	0.19	0.50	0.12	0.66	0.11	0.70
	P2	0.01	0.96	0.26	0.35	0.15	0.59	0.22	0.43
	P3	0.00	0.99	0.22	0.42	0.11	0.68	0.01	0.96
	OL	0.07	0.80	0.05	0.85	0.18	0.52	0.11	0.70
Nellore (508±40.8)	LC	0.31	0.26	0.11	0.70	0.13	0.65	0.03	0.91
	P1	0.56	0.06	0.31	0.25	0.40	0.14	0.42	0.12
	P2	0.08	0.76	0.11	0.70	0.14	0.62	0.02	0.93
	P3	0.19	0.49	0.03	0.90	0.18	0.52	0.14	0.62
	OL	0.45	0.09	0.15	0.59	0.04	0.88	0.21	0.45
Bufalloes (525±45.5)	LC	0.47	0.12	0.40	0.19	0.45	0.14	0.14	0.67
	P1	0.18	0.56	0.21	0.51	0.32	0.31	0.31	0.31
	P2	0.53	0.07	0.41	0.18	0.36	0.24	0.30	0.34
	P3	0.22	0.50	0.22	0.49	0.36	0.25	0.73	0.006
	OL	0.18	0.58	0.33	0.29	0.47	0.12	0.34	0.28

(LC) length of the metacarpal and metatarsal condyle, (P1) length of the proximal phalanx, (P2) length of the middle phalanx, (P3) length of the distal phalanx, (OL) overall length. * Estimated probability of the correlation was statistically equal to zero by t-test.

were significantly longer than medial condyles in all animals of this study. Thus, our results support the assumptions reported in other studies.

A positive correlation between the body weight and the length of P3 was seen only in the buffalo hindfoot. Muggli et al. (2011) related no correlation between the body weight and measurements in the bone digits. Otherwise, Radišić et al. (2012) reported a higher degree of correlation between the body weight and hoof length in the front and hind limbs hooves of the Simmental bulls, ranged from 635 to 1370 kg.

It can be assumed that the greater P3 and, consequently, the wider capsule of the hooves in the buffaloes were the cause for the correlation that was observed in this study. According to Keller et al. (2009) the longer third phalanx correlated well with the longer dorsal wall length of the hooves in wild animals. Besides, Nacambo et al. (2007) hypothesized that the longer lateral condyles were responsible for the larger size of the lateral claws. In addition, Nuss et al. (2011) suggested that variations in shape and certain claw characteristics had been present in cattle individually, although the animal feet had been exposed to similar housing conditions.

The Canonical analysis confirmed the similarity between the Curraleira and Pantaneira bone lengths and that Nellore breeds showed bones with intermediate lengths compared to Pantaneira breeds and buffaloes. It also exposed that the digits of buffaloes were longer than digits of the bovine breeds.

The results showed that the measurements made in the radiographic image were consistent in identifying the anatomical differences among the Brazilian cattle and buffaloes. Therefore, data acquired in this present study might be useful as reference for researchers and for clinical practice, as well as to differentiate bone fragments in archaeological specimens.

Conclusion

The Curraleira and Pantaneira breeds have similar lengths of digital bones. The Nellore breed has intermediate lengths of the digital bones. Buffaloes have longer bones than the cattle bones.

Table 4. Comparison of the mean lengths of the condyles (LC), phalanges (P1, P2, P3) and total length (OL) in the forefoot and hind foot of cattle and buffaloes of the present study with other studies.

			Curraleira	Pantaneira	Nellore	Ocal et al. (2004)	Muggli et al. (2011)	Buffaloes	Nourinezhad et al. (2012)
Forefoot	Lateral	LC	28.90±0.60	30.20±0.60	36.00±1.00	NM	39.8±4.10	34.20±0.50	NM
		P1	52.00±0.70	54.50±0.50	60.80±0.90	52.21±0.81	63.20±0.73	62.80±1.30	51.20±3.50
		P2	34.10±0.90	35.90±0.90	40.00±1.90	36.68±0.74	42.95±0.65	44.30±1.80	36.20±2.00
		P3	40.60±0.50	41.60±0.30	43.00±0.70	47.29±0.71	59.25±0.77	60.20±1.20	41.00±4.10
		OL	161.10±2.40	166.80±1.90	181.50±2.00	136.18	165.40 ±1.60	20.58±0.46	128.40
	Medial	LC	27.40±0.40	28.50±0.60	32.50±0.40	NM	38.9±4.6	3.07±0.07	NM
		P1	51.90±0.90	54.60±0.60	62.00±0.80	52.04±0.81	62.35±0.73	6.31±0.18	53.10±3.70
		P2	33.90±0.90	35.80±0.70	39.50±0.70	36.42±0.52	43.35±0.61	4.41±0.19	35.40±2.20
		P3	40.50±0.50	41.60±0.30	43.10±0.50	48.63±0.45	60.60±1.06	6.23±0.12	42.30±3.90
		OL	156.70±2.50	166.10±2.00	180.80±1.90	137.90	166.3±1.61	20.41±0.41	130.80
Hind foot	Lateral	LC	28.80±0.60	30.90±0.70	34.90±0.80	NM	40.0±3.2	3.25±0.05	NM
		P1	50.76±0.80	58.00±10.0	65.10±0.90	53.79±0.78	64.65±0.81	6.48±0.15	53.60±3.40
		P2	36.80±0.90	37.90±0.90	40.90±0.60	38.10±0.52	46.30±0.44	4.60±0.22	38.30±2.20
		P3	39.00±0.60	39.10±0.60	42.60±0.40	46.11±0.31	59.40±1.01	6.27±0.12	40.10±3.80
		OL	161.90±2.40	171.90±2.40	187.70±1.90	138.00	170.35±1.80	21.01±0.43	132.00
	Medial	LC	28.70±0.60	30.40±0.90	34.10±0.60	NM	39.4±3.5	3.22±0.05	NM
		P1	55.50±0.70	57.40±0.80	64.90±0.60	53.18±0.78	64.00±0.73	6.38±0.17	52.10±3.40
		P2	36.30±1.10	38.40±1.00	41.50±0.80	38.15±0.52	45.50±0.44	4.43±0.18	37.70±2.30
		P3	39.80±0.60	40.40±0.50	41.80±0.50	47.19±0.40	61.30±0.90	6.24±0.16	40.70±3.80
		OL	161.10±2.30	170.80±2.40	186.10±1.90	138.52	170.8±1.66	20.92±0.47	130.50

NM- no mensure.

Conflict of Interests

The authors have not declared any conflict of interests.

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Proteins expression and germination of maize seeds submitted to saline stress

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Plants are frequently exposed to various biotic and abiotic stresses that impair their growth, development and limit the productivity. In the present work the objective was to evaluate the effect of saline stress on the germination and biochemical alterations by the expression of enzymatic systems in corn seeds treated with different concentrations of NaCl. Hybrid maize seeds (H1, H2, H3) were used during 2013 cropping season and solutions of sodium chloride (NaCl) to simulate the saline stress. The respective concentrations of NaCl (mol.m^{-3}) were zero (0.0 g/L); 25 (1.46 g/L); 50 (2.93 g/L); 75 (4.39 g/L) and 100 mol.m^{-3} (5.85 g/L), diluted into water. In order to evaluate the physiological potential of these seeds, we realized the germination test with four replications of 50 seeds, distributed between three germitest papers moistened with NaCl solutions, described above. The evaluation was performed on the seventh day, with the first count realized determining the percentage of normal seedlings on the fourth day after planting. The biochemical evaluation was performed by the expression of isoenzymes superoxide dismutase (SOD), esterase (EST), catalase (CAT), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH) and alpha amylase. Seeds of the three tested hybrids have lower germination when placed in conditions of greater concentration of salinity. The hybrid H3 that presents higher tolerance to salinity and hybrid H1 are most susceptible during the germination process. The hybrid with higher vigor, H3 had higher activity of alpha amylase and catalase enzyme groups. Esterase and alcoholic dehydrogenase were not effective in measuring the quality of maize grains under salt stress conditions.

Key words: *Zea mays*, physiological quality enzyme.

INTRODUCTION

Nowadays, maize is the cereal with higher volume of production in the world with approximately 990 million of tons. Brazil is the third higher world producer and the second in number of exportations, with a cultivated area around 17 million of hectares (CONAB, 2014).

Despite the expressive growth in area, production and productivity, the conditions for cultivation of this species, are not always appropriate to the agronomic performance as, the limiting nutrient availability and the occurrence of hydric deficit in the field.

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In situations where the integrated fertilization management and irrigation are not done correctly, increases occur in the concentration of salts in the soil making impossible the cultivation of most part of plants (Tôrres et al., 2004). The occurrence of saline soils in arid and semi-arid regions is due mainly to the low precipitation and high evapotranspiration. In these conditions, the salts are not leachate, accumulating in the soil or in the water, being harmful to the normal development of the plants. The high concentration of salt is a factor of stress for the plants, because it reduces the osmotic potential and provides the action of ions on the protoplasm. Besides this, it compromises the germination of seeds and leads to the ionic imbalance on cells and consequently, toxicity and stress (Deuner et al., 2011).

Salt stress affects the germination, as well as the vigor, metabolism and the seeds chemical constitution, being these effects dependent of other factors like; species, cultivar, phenological stages, intensity and the duration of the stress (Tester and Davénport, 2003). Most commonly used method for determining the adaptation and tolerance of different species to these stresses is the percentage of germination in salt substrate. The reduction in the percentage of germination in relation to the control is used as indicative of the level of tolerance of the species to the salinity (Góis et al., 2008).

Internal quality control programs beyond the germination and biochemical tests have been used (Catão et al., 2010). Besides this, researches have associated the electrophoreses technique to the physiological quality of maize seeds, being the molecular markers used like tools in the evaluation of this physiological quality. The selection of genotypes through the previous evaluation of physiological quality of seeds and their enzymatic system can provide parameters capable to help in the evaluation of new maize cultivars tolerant to different stresses (Abreu et al., 2014).

Therefore, the objective of the present work was to evaluate the effect of salt stress on germination and biochemical alterations by the expression of isoenzymatic systems in maize seeds submitted to different concentrations of NaCl.

MATERIALS AND METHODS

This study was conducted at Central Laboratory of Seeds, Department of Agriculture of Universidade Federal de Lavras (UFLA), in Lavras-MG. Maize hybrid seeds described as hybrids 1, 2 and 3 (H1, H2 and H3) were used during the 2013 cropping season. The materials were obtained from the same field under the same irrigation system and the three cited materials have the same male genitor. After the harvest, these seeds were stored in cold chamber at 10°C in multiwall paper bags until realization of the following tests and determinations.

Water content was determined by the oven method at 105°C for 24 h using five replicates of 50 seeds from each treatment (Brasil, 2009). The results were expressed in percentage.

In order to simulate the salt stress, sodium chloride solution (NaCl) was used. The concentrations of NaCl (mol.m^{-3}) were: zero (0.0 g/L); 25 (1.46 g/L); 50 (2.93/L); 75 (4.39 g/L) and 100 mol.m^{-3} (5.85 g/L) diluted in distilled water. At level zero, distilled water was used to moisten the substrate. The value of electric conductivity of these solutions was verified with aid of a conductivimeter; while Van't Hoff formula according to Salisbury and Ross (1991) was used to calculate the amount of NaCl to be added for the obtainment of each treatment.

In the germination test, for each treatment four replications of 50 seeds were used. Seeds were distributed between three germitest papers moistened with the NaCl solutions previously described. The amount of solution equivalent to 2.5 times the weight of dry paper was used. Following that, the rolls were kept in germinator type B.O.D regulated to the constant temperature of 25ºC. The evaluation was realized at seventh day after the installation of the test by determining the percentage of normal seedlings (Brasil, 2009). The first count was realized together with the germination test, by determining the percentage of normal seedlings at fourth day after the test installation.

The hypocotyl and root length were also determined. In this regard, seeds from each treatment, in five replications of 15, were sown equidistant from each other, according methodologies described to germination test. The measurement of hypocotyls and root length of seedlings was classified with an aid of a graduated ruler. The results were expressed in cm.seedling^{-1}.

In the biochemical evaluation, samples of 10 seeds of each treatment were collected and macerated in presence of PVP (polyvinylpyrrolidone) and liquid nitrogen in small container and afterwards stored at -86ºC temperature. The seeds were collected 48 h after the germination test installation.

For enzymes extraction, the extraction buffer (tris HCl 0.2 M pH 8 + 0.1% of β-mercaptoethanol) was added in the proportion of 250 μL for 100 mg of seeds powder. The material was homogenized in vortex and kept in refrigerator during 12 h followed by the centrifugation at 14000 rpm for 30 min at 4ºC and then applied in polyacrilamide gel. The electrophoretic run was realized in a discontinuous polyacrylamide gel system at 7.5% (separating gel) and 4.5% (concentrating gel) using tris-glycine pH 8.9 as standard buffer in the gel electrode system. In each gel channel, was applied 50 µL of the sample supernatant and the running was performed at 150 V for 5 h. At the end of running, the gels were revealed for the enzymes superoxide dismutase (SOD- EC.1.15.1.1.), catalase (CAT- EC.1.11.1.6.), esterase (EST- EC 3.1.1.1.), malate dehydrogenase (MDH- EC 1.1.1.37.) and alpha amylase (α-AMI-EC 3.2.1.1.), according to the protocols established by Alfenas (2006). Evaluation of the gels was realized on transilluminator, being considered the variation of intensity of bands.

Factorial experiment in randomized complete design of (3x5) was used, including three maize hybrids (H1, H2, H3) and five different salinity levels (0, 25, 50, 75, 100 mols.m^{-3}). The data, previously submitted to the normality tests and homocedasticity of variances, were submitted to analysis of variances and the averages were compared by the Scott-Knott test at 5% of probability. The statistical analyzes were realized with aid of SISVAR® statistical program (Ferreira, 2011). Evaluation of the enzymatic patterns was made according to the intensity of the bands.

RESULTS AND DISCUSSION

The medium water content of seeds in the moment of the test was of 12.3% with maximum variation of 1%. This uniformity between the tested materials is important once accentuated variations accelerates the deterioration process and the formation of products which entail immediate damages, like the free radicals, masking the final result (Marcos Filho, 2005).

Table 1. Percentage of normal seedlings in the first count of germination test (PCG) of three maize hybrids, submitted to 5 levels of salt concentrations.

Concentration ($mol.m^{-3}$)	Hybrids		
	H1	H2	H3
0	65^{Aa}	70^{Aa}	71^{Aa}
25	47^{Bb}	57^{Ba}	55^{Ba}
50	28^{Cb}	44^{Ba}	39^{Ca}
75	9^{Dc}	31^{Ca}	22^{Db}
100	6^{Db}	19^{Da}	6^{Eb}
CV(%)		16.4	

Means followed by the same lowercase letter in line, and capital in the column do not differ at 5% probability by Scott Knott test.

Table 2. Percentage of germination of three maize hybrids, submitted to 5 levels of salt concentrations.

Concentration ($mol.m^{-3}$)	Hybrids		
	H1	H2	H3
0	98^{Aa}	100^{Aa}	96^{Aa}
25	94^{Aa}	96^{Aa}	90^{Aa}
50	80^{Bb}	84^{Ba}	86^{Aa}
75	52^{Cc}	80^{Ba}	76^{Bb}
100	42^{Dc}	64^{Cb}	72^{Ba}
CV (%)		9.6	

Means followed by the same lowercase letter in line, and capital in the column do not differ at 5% probability by Scott Knott test.

The decrease in water availability caused by the reduction of osmotic potential of saline solutions reduced gradually the percentage of normal seedlings at fourth day of evaluation for all the hybrid seeds accordingly verifying the first count of germination (PGG) (Table 1). For the H1, the vigor reduction is more pronounced in the concentration of 75 $mol.m^{-3}$, not differing statistically from the 100 $mol.m^{3}$ concentration. For the H2, the concentrations of 25 and 50 $mol.m^{-3}$ did not present significant differences. The hybrid seeds H3 had a continous and proportional decline with increasing salt concentration, statistically differing from each concentration at which they were submitted.

In analyzing each salt concentration in different maize hybrids function, it was observed that when the concentration of 0 $mol.m^{-3}$ was used, the three hybrids did not present significant diferences between them (Table 1). Already in the concentrations of 25 and 50 $mol.m^{-3}$, the hybrids H2 and H3 had presented higher percentage of normal seedlings in the first count in comparison to the H1. In the concentraion of 75 $mol.m^{-3}$ all maize hybrids were significantly different between them, with the better performance of the H2, followed by the H3 and H1. In the higher concentration of 100 $mol.m^{-3}$, the H2 had higher percentage of normal seedlings, while the hybrids H1 and N3 did not differ between them.

The reduction in the osmotic potential induced by treatment with NaCl, caused alterations in the germinative parameters of maize seeds in different intensities. The percentage of germination decreased proportionally to the increase in the osmotic potential with NaCl for all the hybrid seeds (Table 2). In the absence of salt (0 $mol.m^{-3}$), the percentage of seeds germination achieve levels superior than 95%. Seeds subjected in the 25 $mol.m^{-3}$ solution recorded percentage of germination equal or superior than 90% and, however, did not differ statistically in the control and between them. The reduction in the percentage of H1 germination, comparing the difference between the control treatment and the higher osmotic potential, was of 56% points, more drastic than the reduction of H2 (36% points) and of H3 (28% points). In the concentration of 50 $mol.m^{-3}$, H3 presented 86% of germination value above the considered minimum pattern for the commercialization of maize seeds, according to the Ministry of Agriculture, Cattle and Supply (MAPA). This minimum value for the remaining concentrations of H2 and H1 was not verified.

The decrease in the percentage of germination with increasing of the salt stress may be due to the osmotic effect, as well as the effect known as physiological drought; like the toxic effect resulting from the acumulation of ions in the protoplasm and the nutritional imbalance occasioned by the inhibition of absorption and transport of nutrients (Tôrres et al., 2004). Similar results were found by Moraes and Menezes (2003) on soybean seeds, confirming the decrease in germination with the reduction of osmotic potential of solutions in the substrate. In rice, Lima et al. (2005) verified decrease in the germination of all the cultivars, in function of the increase in salt concentration, and suggested that the salinity affects the development of normal seedlings and decreases the viability and vigor of seeds.

The root length was less compromised by the water restriction occasioned by the presence of salts, than the development of the shoot part (Tables 3 and 4). These results can be explained by the fact that the seedlings submitted to higher levels of water stress, in general tend to invest higher biomass and to develop higher radicular system as strategy of survival. The distribution of the root system in depth/length on grounds of the water failure is considered as indicative parameter of drought tolerance and can confer adaptation in some species.

There was no variation between the three hybrids analyzed in the treatment of 0 $mol.m^{-3}$. In the concentrations of 25, 50, and 75 $mol.m^{-3}$, the hybrids H1 and H2 presented results statistically superior. In the treatment of 100 $mol.m^{-3}$, the hybrid H1 had higher length. Beside this, comparing the variations inside each hybrid, it is noted that in all of them occurs reduction of the root length with increase in water stress (Table 3).

In relation to the data about shoot length (Table 4), it

Table 3. Length (cm) of seedlings root system of three maize hybrids submitted to five levels of osmotic potential.

Concentration ($mol.m^{-3}$)	Hybrids		
	H1	H2	H3
0	23.16^{aA}	23.57^{aA}	22.98^{aA}
25	20.54^{aB}	20.47^{aB}	17.32^{bB}
50	18.43^{aC}	18.58^{aC}	14.43^{bC}
75	12.98^{aD}	16.79^{aD}	12.08^{bC}
100	12.42^{aE}	11.95^{bE}	8.27^{cD}
CV (%)		11.43	

Means followed by the same lowercase letter in line, and capital in the column do not differ at 5% probability by Scott Knott test.

Table 4. Length (cm) of seedlings shoot of three maize hybrids submitted to five levels of osmotic potential.

Concentration ($mol.m^{-3}$)	Hybrids		
	H1	H2	H3
0	10.43^{aA}	9.54^{bA}	9.47^{bA}
25	8.38^{aB}	7.43^{bB}	7.49^{bB}
50	6.72^{aC}	6.41^{aC}	5.63^{bC}
75	5.05^{aC}	4.75^{aD}	4.27^{bC}
100	1.98^{cD2}	2.65^{aE}	0.37^{bD}
CV (%)		12.48	

Means followed by the same lowercase letter in line, and capital in the column do not differ at 5% probability by Scott Knott test.

was observed that in the concentration of 25 $mol.m^{-3}$, the hybrid H1 presented the higher length of shoot in relation to the other hybrids. However, in the concentration of 50 and 75 $mol.m^{-3}$, H3 had lower shoot length. In the concentration of 100 $mol.m^{-3}$, the higher length was observed with H2 hybrid. All maize hybrids recorded a reduction in the shoot length according the osmotic potential decreased, mainly due to the reduction of the metabolism occasioned by the lack of water, which harms the plant development.

Generally, the water stress caused damage in the radicular and shoot growth of maize seedlings. Similar results were found in other studies reported by Kaya et al. (2006), Ávila et al. (2007) working with canola, and Conus (2009) on maize, where they observed that salt stress decreased shoot and roots lengths.

Figure 1 illustrates the expression pattern of enzymes alpha amylase, superoxide dismutase, catalase, esterase and malato desidrogenase in seeds of three maize hybrids submitted to imbibition. The activity of the enzyme alpha amylase can be evidenced by achromatic bands in blue background due to the reaction of the iodine with amylase. In the locals where the enzymes were present, the starch was hydrolyzed. This enzyme has high activity in the beginning of the germinative process and, over time, the activity decreases. This behavior is due to the reduction of starch content while the germination is occurring. Being an enzyme responsible for starch hydrolyses, genotypes which present high levels of alpha amylase expression have higher capacity in providing starch for the embryos, justifying the better performance in the final germination (Franco et al., 2002). However, Abreu et al. (2014) working on maize seeds, claims that water restriction to the seeds (lower availability of water) results in delay of imbibition process. Once the enzymes responsible for starch degradation in maize seeds are “synthesized again” at the moment of imbibition, there will be higher acumulation of alpha amylase enzyme as soon as there is an increase of this water restriction.

In general, it is observed from this study, that H1 presented lower activity of alpha amylase in all saline concentrations, except in the higher concentration of 100 $mol.m^{-3}$, when compared to the hybrids H2 and H3 (Figure 1A). The same result was observed in relation to the first count of germination and germination (Tables 1 and 2). However, comparing the electrophoretic profile of hybrids H2 and H3, it is observed that the H3 presents lower activity of this enzyme, differently from what was presented in Tables 1 and 2. Besides this, it was observed that in the concentration of 100 $mol.m^{-3}$, the activity of enzymes is higher for the three hybrids.

The enzyme SOD is directly involved in the celular defense against the free radicals being responsible by the convertion of superoxide radical into hydrogen peroxide. However, it was observed from Figure 1B that there was no increase in the expression of this enzyme with increasing osmotic potential, suggesting that the stress occasioned by the water restriction for the three maize hybrids was not enough for increasing activity of this enzyme.

Another enzyme involved in the removal of hydrogen peroxide, formed from the activity of SOD is the enzyme CAT. The compound hydrogen peroxide is toxic for the seeds but not for the cells, the catalases enzymes act on hydrogen peroxide neutralizing it. The higher activity of CAT for the three hybrids in higher concentrations of stresses (50, 75 and 100 $mol.m^{-3}$) can be atributed to the activity of this enzyme for removing the hydrogen peroxide accumulated in occasion of saline stress. As verified in Table 2 for H1, the reduction in germination decreased significantly from the potential of 75 $mol.m^{-3}$ and suggests higher deterioration level of these seeds.

In relation to the enzyme EST, it was observed that in the control treatment (0 $mol.m^{-3}$) for hybrids H1 and H2, the activity of this enzyme was higher when compared to H3 (Figure 1C). In other concentrations, high activity was observed only for H2. The high activity of this enzyme is related to the higher efficiency of the membrane system, and according to the Tables 1 and 2, the H2 can be observed to present higher quality when compared to H1.

The enzyme MDH is an important molecular marker of

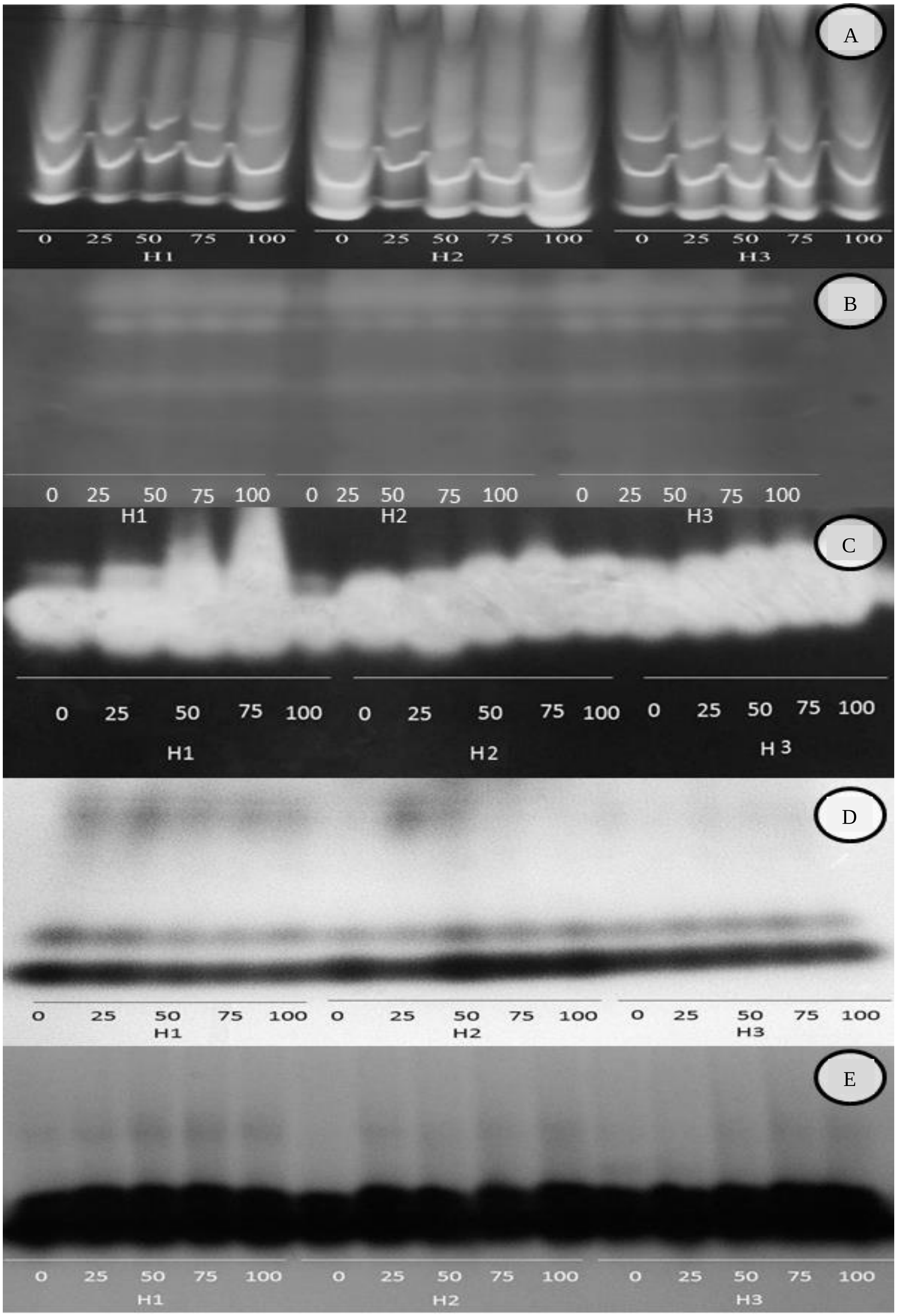

Figure 1. (A) Expression pattern of enzymes alpha amylase, (B) superoxide dismutase, (C) catalase, (D) esterase and (E) malato dehydrogenase of three maize hybrids (H1, H2, H3), submitted to five levels of osmotic potential (0, 25, 50, 75, 100 mols.m^{-3} of NaCl).

seeds respiration. The accumulation of salt in solution and consequently water restriction, take seeds to a condition of lack of oxygen. In Figure 1E, higher expression of this enzyme with increasing osmotic potential was observed; there was also decrease in seeds respiration and increase in the deterioration process.

However, this enzyme is codified for five loci and it is found in great abundance in different cellular organelles, in mitochondria's and cytoplasm's. Due to this alteration in your expression, it is only observed when it occurs in seeds, a very large deteriorating process, which makes this enzyme an inefficient marker of physiological quality.

Conclusions

Seeds of the three maize hybrid tested presented low percentage of germination when placed in conditions of higher salt concentration.

The hybrid with higher vigor, H3, had lower activity of the enzymatic groups alpha amylase and esterase. Superoxide dismutase and malato desidrogenase were not efficient in measuring the quality of maize seeds under salt stress.

The hybrid H3 is the one which presents higher tolerance to the salinity and the hybrid H1 is the most susceptible during the germinative process.

Conflict of Interests

The authors have not declared any conflict of interests.

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Beneficiaries' perception of selected rural women empowerment projects in Ogun State, Nigeria

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Problems militating against women development in rural areas revolve round their inability to develop themselves in their chosen economic activities. This study examined the beneficiaries' perception of selected agricultural empowerment projects components targeted at women in rural communities of Ogun State, Nigeria. The projects were Cassava: Adding Values Africa (C:AVA), Justice, Development and Peace Movement (JDPM) Micro finance (MICRO) and National Programme for Food Security (NPFS), implemented by Justice Development and Peace Movement (JDPM) and Ogun State Agricultural Development Programme (OGADEP). Interview guides were used to elicit information from 139 randomly selected members of 16 purposively selected groups beneficiaries of selected women empowerment in the study area. The study revealed that the rural women predominantly had have high perception about the effects of the projects on their livelihood as 58.27, 72.66, 82.01, 90.65, 61.15 and 56.83% of the women perceived that the projects had improved their product packaging, access to credit facilities, knowledge and skills, business expansion, balanced emotion and increased income respectively. The data was subjected to Chi -Square analysis and the result showed that there is a significant relationship between the nature of occupation the women engaged in and the effect of the projects (x^2 =15.38, p<0.05), while other socio-economic characteristics were not significantly related with the projects' effect. It was inferred from this study that participants of the OGADEP and JDPM projects had high perception of the effects of the projects on their livelihood. This study recommended that governmental and non-governmental organizations of rural orientation should focus more on empowering rural women and other rural household members in order to transform rural communities.

Key words: Beneficiaries, perception, empowerment, rural women

INTRODUCTION

Gender issues cannot be excluded from agricultural and rural development in Nigeria, Africa and the entire world. Rural women in Nigeria represent a high percentage of the Nigerian 140 million populations (NPC, 2006). Although both men and women in rural areas carry out their economic activities in agricultural related work, women across the globe have always played major roles in agriculture. They contribute substantially to food production and food security.

Food and Agriculture Organization (FAO, 2007)

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reported that the majority of the world's poor live in rural areas, and 70 percent of the rural poor are women, majority of whose principal resource is agriculture. A study by the International Food Policy Research Institute (IFPRI), pointed out that if women farmers were given equal access to resources, developing countries would see significant increases in agricultural productivity. Women produce almost half the world's food, but they often work in difficult conditions, with low pay and inadequate access to land, capital, information and they are mainly into subsistence agriculture. Approximately 50% of these set of people are also subjected to a very low scale form of production, with little or no access to information, resources and social amenities that could improve their productivity (Iheduru, 2002).

Due to issues affecting the world in relation to women development, a lot of awareness project were directed at women empowerment by the African Governments and international organizations committed to implementing actions in the area of poverty reduction and the economic empowerment of women. Over the last 25 years, the role of women in agriculture has become a familiar and well-developed subject. This led to the establishment of gender equality around the world. United Nations Development Fund for Women (UNIFEM) was established as a separate fund within the United Nations Development Program (UNDP) in 1984 with the aim of empowering women, bearing in mind the outcome of Beijing Conference. Hence, more than 100 countries announced new initiatives to improve the status of women. Furthermore, various countries showed their commitments by outlining modalities for implementation of these plans, thus an overwhelming majority of African Governments placed poverty reduction and empowerment of women among their top priorities for action (Perpetua, 1999).

Due to the recognition of the roles played by rural women in agricultural production, both governmental and non-governmental organization embarked on various projects, which further popularize and improved the living condition of the women. The current issue in rural women development is now tending towards, recognizing the possible ways of sustaining all developmental projects directed at women. According to Adisa and Okunade (2005), many rural development projects and programmes which are gender specific have been introduced to take care of women's needs in the rural areas of the country. These programmes and projects include Better Life Programme for Rural Women (BLPRW), Women-In Agriculture (WIA), the Family Support Programme (FSP), Fand those introduced by international agencies UNDP, UNICEF. All these aimed at achieving women empowerment.

In general the problem militating against women development in rural areas, revolve round their inability to develop themselves in the area of their chosen economic activities, because they are regarded as not the original owners or possession of land which they can use as collateral in securing funds to improve on their activities. Another challenge is the societal culture that regards women as minor in the society while they are deprived of access to information that could better the lives.

This study focuses to analyze the perception of beneficiaries of three selected rural women empowerment projects [Cassava: Adding Values for Africa (C: AVA), NATIONAL PROGRAMME FOR FOOD SECURITY (NPFS) and Microfinance in Ogun State, Nigeria with the following objectives to:

i) Describe the socio-economic characteristics of rural women involved in the selected projects.
ii) Assess the perception of rural women about the need for selected women empowerment project in Ogun State.
iii) Determine the perception of the beneficiaries on the effect of the projects on their livelihoods.

Hypothesis of the study

It was hypothesized that there are no significant relationships between the socio-economic characteristics of the respondents and the perceived effect of the selected project on the livelihood of beneficiaries.

METHODOLOGY

The study was conducted in Ogun State, which is located in the southwestern part of Nigeria. It is situated within the tropics covering16, 409.29 square kilometers with a population of about 4,054,272 (National population commission 2006).

Purposive selection of two senatorial districts out of the three existing senatorial districts, Ogun west and Ogun central Senatorial was chosen due to the existence of the selected projects.

From Ogun central senatorial district, two local governments area selected were Ifo and Ewekoro local governments and from Ogun west senatorial district Egbado south and Ado Odoota Local government area was chosen.

The selected organizations worked with women group of an average seventeen members therefore fifty percent (50%) of each beneficiary women group selected. Interview guide and with three key trained researchers assistant that are trained for a period of two days were employed in obtaining useful data from the respondents.

The consent of the group members was sought verbally and based on their agreement and ethical approval of the group members, the interview guide was administered during the meeting days of the selected women group.

The data were collected by the researcher and the research assistant who translated the interview guide to the illiterate respondents in their local language, while the literate respondents administered the interview guide on their own. The data obtained was statistically analyzed using both descriptive and inferential statistics appropriately. The hypothesis of the study was tested using chi-square analysis. A total of 143 respondents were selected for this study as shown in Table 1.

RESULTS AND DISCUSSION

Socio-economic characteristics of the respondents

Table 2 revealed that the average age of sampled

Table 1. Ogun State Senatorial Districts (showing sampling procedures and sample size).

Implementing organisation	Selected senatorial districts	Selected local government area	Name of group	Number in group	Selected member 50%
Ogun State Agricultural Development Programme (OGADEP)	Ogun Central	Ifo	CAVA		
			Ifelodun	14	7
			Soboje	16	8
			NPFS		
			Oredegbe	14	7
			Igbesiaye Rorun	22	11
		Ewekoro	CAVA		
			Obalagbe	14	7
			Agbelere	14	7
			NPFS		
			Ajegunle Fufu	14	7
			Ajegunle Gari	14	7
Sub Total				**122**	**61**
Justice Development and Peace Movement (JDPM)	Ogun West	Yewa	CAVA		
			Owolowo	22	11
			Ifelodun	22	11
			Microcredit		
			Tepamose	18	9
			Ore Ofe Oluwa	30	15
		Adoodoota	CAVA		
			Groups 1	24	12
			Group 2	18	9
			Microcredit		
			Itesiwaju	14	7
			Halleluyah	16	8
Sub total				**164**	**82**
Total				**2 86**	**143**

Total number of respondents= (61+82), 143.

Table 2. Distribution of respondents by their socio-economic characteristics (n=139).

Variable	Frequency	Percentage	Mean/Mode
Age			
≤ 30 years	20	14.39	
31 - 40 yeas	42	30.22	
41 - 50 years	45	32.37	43.62 years
51 - 60 years	27	19.42	
Over 60 years	5	3.6	
Total	**139**	**100**	
Educational Level			
Adult Education	6	4.32	
No formal education	38	27.34	
Primary	62	44.60	Primary
Secondary	22	15.83	
Tertiary	11	7.91	
Total	**139**	**100**	

Table 2. Contd.

Marital status			
Married	95	68.35	
Separated	9	6.48	
Single	13	9.35	Married
Widowed	22	15.83	
Total	**139**	**100**	
Economic activity			
Artisanship	4	2.88	
Farming	28	20.14	
Food processing	60	43.17	Food Processing
Street Banking	47	33.81	
Total	**139**	**100**	
Experience			
Less than 10 years	77	55.40	
10-15 years	39	28.06	
16-20years	16	11.51	
Total	**139**	**100**	
Household size			
1-5	66	47.48	
6-10	56	40.29	7 People
11-15	11	7.91	
Not indicated	6	4.32	
Total	**139**	**100**	
Religion			
Islam	48	34.53	
Traditional religion	2	1.44	Christianity
Christianity	89	64.03	
Total	**139**	**100**	
Membership of social organisation			
Yes	118	84.89	
No	21	15.11	
Total	139	100	
Social status			
Community chief	8	6.78	
Executive member	46	38.98	
Group leader	19	16.10	
No position	45	38.14	
Total	139	100	
Nativity			
Native	98	70.5	
Non native	41	29.5	
Total	139	100	

Source: Field survey 2012.

women was 43.62 years with more than half (about 63%) of the women between the ages of 31 and 50 years, 22.02% above 50 years and 14% less than 30 years. It was revealed that very few (less than 25%) of the respondents had more than primary education, close to half (44.60%) had primary education while one out of every four women (27.34%) had no formal education at all, About two out of every three (68.35%) of the women were married, 15.83% were widow while the remaining (15.83%) are neither married nor separated.

Majority (43.17%) of the women engage in food processing, 33.81% engage in trading while 20.14% and 2.88% of respondents are farmers and artisans respectively. More than half (55.40%) of the respondents have less than ten years of experience in their respective economic activities while 39.57% have above ten years of experience. Furthermore, a high percentage of (64.03%) were Christians while 34.53% were Muslims, only 5.76% of the respondents were Community Chiefs. Also, about half of the women (46.76%) are either executive members of their association or group leaders while 15.11% of the women respondents are non-members of social groups In general, 70.50% of respondents were natives of their various localities while 29.5 % were non-natives.

The result from Table 2 implies that respondents are within the economically active age group; and it is in support of Fakoya and Daramola (2005) who observed that respondents within this age bracket are more innovative, motivated and adaptable individuals who can cope with wisdom of challenges. This was also the opinion of FAO (1997), Yinusa (1999) and Ayinde et al. (2002). For educational status the women had a low level of formal education, this is in line with the findings of Longe (1988) which reiterated that women are generally not literate in the rural areas. Furthermore Sokoya (2003) observed that women are poorer than their male counterpart because they lack adequate access to educational opportunities.

With the majority of the women engagement in food processing is an indication that the food Processing is a popular enterprise among rural women. This finding is justified by Ogunlela and Adekanye(2009) who opined that women are known to be more active in agricultural activities in sub-saharan African countries. Afolabi (2004) also pointed out that women have virtually taken over the production and processing of staple food.

On religion basis, Christianity is a popular religion among the respondents. The result further showed the dichotomy in the religion spread in Nigeria that northern part is dominated by Muslims while the southern part has relatively more Christians as also reiterated by WHO (2001).

This result also showed that the women are active members of their various social groups. Group membership helps members to become better informed about the world and about new technologies, and groups may serve as a stepping stone to some higher gains or status and serious involvement in the group activities made them contribute meaningfully to their community development. This assertion is in agreement with Awotunde (1990) that regular participation of all members is a suitable indicator of success.

The fact that majority of the group members were natives would have a positive bearing on the sustainability of the project. This observation may be attributed to the geographical and occupational distribution as well as infrastructural provision of the respondent's household which favours farming (Fapojuwo, 2007).

Description of the perception of beneficiaries about the need for the projects

Respondents level of perception was measured by using a combination of adopted 22 indicatorson a five point likert scale. Results of analysis on the perception of beneficiaries about the need for the projects shows a low perception of less than mean score of 66, and above as having high perception about the need for the projects**.** The result showed that more than half of the respondents (53.24%) adjugded high perception while (46.76%) had low perception about the need for the projects (Table 3).

The distribution of the sampled beneficiaries while considering the projects individually indicated that across all the projects considered the respondents had high perception on the need for the projects except in JDPM C: AVA with low perception. However, the general breakdown of the perception statement result further indicated that individual responses was based on their expectations of what empowerment means to them, their local orientation, belief and knowledge about the goals of the organisation and how the projects affect their livelihood activities.

Perception of respondents on the effect of the projects on their livelihood

Table 4 showed that across all the projects, respondents had high perception for the types of effect the empowerment has on their livelihood. The result x-rayed that respondents had strongly agreed that the projects provided them with opportunities to develop in the area of product packaging (56.12%) and that their products' quality had increased (44.60%), 44.60 and 35.25% strongly disagreed that they had been able to reduce wastages from processing and had improved knowledge on modern tools usage respectively. About 40.29% of the rural women had witnessed increased customer patronage within and outside their communities. Higher proportions also strongly disagreed that their profit margin had not increased (40.29%) and that they would like to continue as the time spent on project meetings was commendable to achievements (37.41%).

The perception of respondents on the effect of the project on their livelihood implies that rural women had mixed feelings towards the projects as they had positive perception of the product packaging, business expansion

Table 3. Description of the perception beneficiaries about the need for the projects.

	Statements	SA	%	A	%	U	%	D	%	SD	%
1	The projects is concerned with the well-being of rural women	124	(89.21)	15	(10.79)	00	(0.00)	00	(0.00)	00	(0.00)
2	The projects should be scrapped	15	(10.79)	9	(6.47)	00	(0.00)	20	(14.39)	95	(68.35)
3	The projects concentrates on the felt needs of rural women	15	(10.79)	0	(0.00)	00	(0.00)	5	(25.18)	9	(64.03)
4	The project is a waste of time	26	(18.71)	5	(3.60)	15	(10.79)	42	(30.22)	51	(36.69)
5	There is increased in the sales of my product	9	(6.47)	0	(0.00)	9	(6.47)	39	(28.06)	82	(58.99)
6	Even though, I have increase in my sales but project is cumbersome	19	(13.67)	60	(43.17)	21	(15.11)	10	(7.19)	29	(20.86)
7	The officers of the projects are always friendly	3	(2.16)	2	(1.44)	15	(10.79)	55	(39.57)	64	(46.04)
8	The project brought increased unemployment	33	(23.74)	55	(39.57)	33	(23.74)	14	(10.07)	4	(2.88)
9	There is reduction in my waste generation	19	(13.67)	20	(14.39)	15	(10.79)	50	(35.97)	35	(25.18)
10	There is no improvement in the quality of my product	34	(24.46)	16	(11.51)	6	(4.32)	28	(20.14)	55	(39.57)
11	There is increase in the number of customers outside my community	5	(3.60)	19	(13.67)	0	(0.00)	40	(28.78)	51	(53.96)
12	The project has not really added value to my product	24	(17.27)	30	(21.58)	15	(10.79)	32	(23.02)	38	(27.34)
13	The project made me realise the role of belonging to the groups	29	(20.86)	7	(5.04)	12	(8.63)	52	(37.41)	39	(28.06)
14	I feel I can do better without the project	20	(14.39)	13	(11.35	15	(10.79)	44	(31.65)	47	(33.81)
15	The project has reduced my spending on labour	24	(17.27)	13	(11.51)	1	(0.72)	16	(11.51)	59	(42.45)
16	The project disturbed my relationship with my family due time demand	27	(19.42)	27	(19.42)	8	(5.76)	31	(22.3)	46	(33.09)
17	The project made me have information on the latest innovation	32	(23.02)	5	(3.6)	7	(5.04)	43	(30.94)	52	(37.41)
18	The project is just a top bottom approach	57	(41.01)	16	(11.51)	11	(7.91)	5	(3.60)	50	(35.97)
19	I think I have an improved income	8	(5.76)	14	(10.07)	8	(5.76)	47	(33.81)	62	(44.60)
20	The project has reduced my level of access to information	8	(5.76)	12	(8.63)	22	(15.83)	53	(38.18)	44	(31.65)
21	The project is an eye opener to increased food availability	55	(39.57)	38	(27.34)	10	(7.19)	29	(20.86)	7	(5.04)
22	The technology training is too advance	27	(19.42)	11	(7.91)	25	(17.99)	34	(24.46)	42	(30.22)

Note: 1=strongly agree, 2=agree, 3=undecided, 4= disagree, 5= strongly disagree for positive statements

Note: 5=strongly disagree, 4=disagree, 3=undecided, 4= agree, 5= strongly agree for negative statements

Source: Field Survey (2012).

expansion, improved knowledge and skills, increased income and balanced emotion while they had negative perception of the credit facilities. This assertion supports Sen (1993) that extension and projects directed at women change the quality of life of women through the vehicle of technology transfer; hence, it is necessary to improve their production potentials by treating them as economic factors, not as dependent members of the family.

Description of perception of project effect on livelihood

Table 5 presented the result for the respondent's perception from each perceived effect benefits derived from the projects.

It revealed that by individual projects, JDPM-C: AVA had high effect on access to credit facilities, improved knowledge and skills, business expansion and increased income; OGADEP-C: AVA had high effect on product packaging, access to credit facilities, business expansion, balanced

Table 4. Respondents perception on the of the project on their livelihood

Statement	Very High	High	Average	Low	Very Low
Production packaging					
The project provided me with opportunity to develop in the area of products packaging.	78(56.12)	18(12.95)	10(7.19)	12(8.63)	21(15.11)
The quality of my product had not increased.	2(1.44)	10(7.19)	18(12.95)	47(33.81)	62(44.60)
Credit facilities					
I have more and better access to finance	33(23.74)	23(16.55)	17(12.23)	31(22.30)	35(25.02)
The time for loan processing has not reduced	45(32.37)	12(8.63)	17(12.23)	19(13.67)	46(33.09)
Improved knowledge and skills					
I have reduced wastage from my processing	2(1.44)	3(2.16)	25(17.99)	47(33.81)	62(44.60)
I have no improved knowledge on modern tools usage	7(5.04)	32(23.03)	26(18.71)	5(17.99)	49(35.25)
Business expansion					
I experienced improvement in my sales level	19(13.67)	18(12.95)	24(17.27)	18(12.95)	57(41.01)
I did not witnessed an increase customer patronage within and outside my community	28(20.14)	6(4.32)	4(2.88)	45(32.37)	56(40.29)
Increased income					
My expenditure on labour has been reduced	6(5.04)	7(5.04)	35(25.18)	49(35.25)	62(44.60)
My profit margin has not increased	28(20.14)	6(4.32)	4(2.88)	45(32.37)	56(40.29)
Balance emotion					
The time I spend on project meeting is commendable to my achievement, I would like to continue	31(22.30)	9(6.47)	5(3.60)	42(30.22)	52(37.41)
The officers provides timely solutions to problems	47(33.81)	16(11.51)	18(12.95)	12(8.63)	46(33.09)

Source: Field survey (2012).

balanced emotion and increased income; JDPM-MICRO had high effects on improved knowledge and skills, business expansion, balanced emotion and increased income; while OGADEP-NPFS had high effects on all except improved knowledge and skills and increase income. Further analysis indicated that OGADEP-C: AVA had more effects than other projects.

Result of tested hypothesis

There is no significant relationship between the socio-economic characteristics of the respondents and the effect of the selected project on the livelihood of beneficiaries.

The socio-economic characteristics considered were age, religion, marital status, educational level, social status and native status. To test for the relationship between the variables, Chi-square was used. The significance of the relationship was determined at 0.05 level of significance.

The result of the Chi -square analysis on Table 6 shows that, there is a significant relationship between the nature of occupation and effect of the

Table 5. Respondent's perception of effects of the projects on their livelihood activities.

Product packaging	**JDPM CAVA**	**OGADEP CAVA**	**JDPM MICRO**	**OGADEP NPFS**	**TOTAL**
Positive	14(10.07)	24(17.27)	17(12.33)	26(18.71)	81(58.27)
Negative	28(20.14)	4(2.88)	21(15.11)	5(3.60)	58(41.73)
Total	42	28	38	31	139(100)
Credit facilities					
Positive	35(25.18)	28(20.14)	35(25.18)	25(17.99)	104(74.82)
Negative	7(5.04)	0(0.00)	25(17.99)	6(4.32)	35(25.18)
Total	42	28	38	31	139(100)
Improved knowledge and skills					
Positive	31(22.30)	14(10.07)	24(17.27)	15(10.79)	114(82.01)
Negative	11(7.91)	14(10.07)	14(10.07)	16(11.51)	25(17.99)
Total	42	28	38	31	139(100)
Business expansion					
Positive	41(29.50)	28(20.14)	3726.62)	20(14.39)	126(90.65)
Negative	1(0.72)	0(0.00)	1(0.72)	11(7.91)	13(9.35)
Total	42	28	38	31	139(100)
Balanced Emotion					
Positive	15(10.79)	16(11.51)	29(20.86)	25(17.99)	65(46.76)
Negative	27(19.42)	12(8.63)	9(6.47)	6(4.32)	74(53.24)
Total	42	28	38	31	139(100)
Increased income					
Positive	25(17.99)	22(15.83)	31(22.30)	1(0.79)	79(56.83**)**
Negative	17(12.23)	6(4.32)	7(5.04)	30(21.58)	60(43.17)
Total	42	28	38	31	139(100)

Source: Field survey 2012

Table 6. Chi square analysis of respondents' socio-economic characteristics and the effect on the selected projects.

Variable	**Coefficient**				
	x^2	**df**	**Contingency**	**p-value**	**Decision**
Age	11.68	4	0.15	0.5	NS
Educational status	5.65	5	0.2	0.34	NS
Marital status	3.14	4	0.15	0.53	NS
Nature of occupation	15.38	3	0.32	0	S
Years of experience	3.15	3	0.15	0.37	NS
Household size	0.26	3	0.04	0.97	NS
Religion	0.6	2	0.07	0.74	NS
Membership	0.72	1	0.07	0.39	NS
social status	3.84	3	0.18	0.28	NS
Nativity	1.01	1	0.85	0.6	NS

df = Degree of freedom. Source: Field Survey (2012).

Projects (x^2 =15.38, $p<0.05$), therefore, the null hypothesis is rejected, while accepting the alternate. This means that the nature of occupation determines the effect of the selected projects on the livelihood of the beneficiaries. Therefore a respondent engaged in cassava flakes garri production might derive more benefit as a result of the nature of the project more than respondents that are involved in cassava flour or fufu production. This could be as a result of the sophisticated technological dimension introduced to garri production. This view supports Aworh (2008) that technology has improved livelihood through generation of employment in the rural areas, reduce post-harvest cassava losses and provide a good source of income to farmers and processors.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

Supplemental irrigation levels in bell pepper under shade mesh and in open-field: Crop coefficient, yield, fruit quality and water productivity

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This study aims to evaluate the effects of supplemental irrigation in bell pepper crop under shade mesh and in open-field to improve management of water resources. The experimental design was a randomized complete block with four replications and ten treatments in factorial arrangement (five irrigation levels combined with two shade levels). Irrigation treatments were 0.25, 0.50, 0.75 and 1.0 rate of crop evapotranspiration and the control (no supplemental irrigation). Shading treatments were 0 and 50% reduction of photosynthetically active radiation, compared to open field conditions. Crop coefficient was influenced by rainfall, especially during initial growth stage period when it was high and Kc values were 0.71, 1.17, and 0.92. Treatments under shade and open-field had no significant interaction effect, alike between the years of study. The yield in open-field and under shade mesh showed better performance in 0.75 and 0.50 of ETc, respectively. Maximum water productivity and irrigation water productivity was obtained in open-field and deficit irrigation plots. Under shade, highest fruit quality was obtained; heavier fruits, less dry matter, no sunscald and increasing value added to production. Comparing water consumption in open-field and shading, it can be obtained up to 14 to 25% water saving, significantly improving yield and fruit quality.

Key words: *Capsicum annuum*, dual crop coefficient, drip irrigation, strategies for efficient irrigation.

INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is a member of the Solanaceous family, native to Mexico, Central America and northern South America (Echer et al., 2002; Filgueira, 2003). It is an important crop in many parts of the world, given their economic importance, ranking second in world production. Major producing countries

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Table 1. Monthly climatic data of the experimental area during the 2013-2014 and 2014-2015 seasons.

Month	Relative humidity mean (%)		Insolation (hour)		Evaporation (mm)	
	2013-2014	2014-2015	2013-2014	2014-2015	2013-2014	2014-2015
Nov	71.60	71.0	229.2	173.1	144.5	131.8
Dec	69.45	76.1	286.2	211.0	175.2	142.2
Jan	73.15	78.3	219.2	212.4	158.3	142.0
Feb	73.79	79.7	211.2	218.0	137.9	123.9
Mar	76.87	77.6	212.6	208.4	112.8	114.1
Sum	-	-	1376.8	1189.6	850.8	804.7

are China, Mexico, Turkey, Indonesia, Spain and United States (FAOSTAT, 2013). It is considered one of the ten species of greatest economic importance in the Brazilian vegetable market. The area cultivated annually is about 13,000 ha, with fruit production close to 290,000 t, generally grown in open field (Marouelli and Silva, 2012).

The widespread lack of water for agricultural production has led to frequent need for strategies aimed at optimizing the efficiency of its use (Padrón et al., 2014). The strategy to ensure food supply to the world population for the next 25 years inevitably includes a parallel increase in agricultural production. A large part of this effort is relayed on irrigation; therefore the big challenge is to improve the efficiency and performance of agriculture, water and energy from existing irrigated fields (Mukherji and Facon, 2009; Melgarejo and López, 2012). The scarcity of water resources necessitates appropriate management and use of the available water, given that the agricultural sector is one of the activities that demand more water (Albuquerque et al., 2012). Therefore, techniques to minimize consumption of irrigation water and increase yield with water use efficiency are necessary.

Temperature affects the vital functions of plants such as germination, transpiration, respiration, photosynthesis, growth and flowering (Goto and Tivelli, 1998). Therefore, planting in a protected environment may reduce the effects of temperature to the plant (Santos et al., 2009). In agriculture, the use of shade mesh has increased to attenuate the flux of solar radiation received by the crop (Pezzopane et al., 2004). Excess radiation, usually accompanied by high temperatures can cause damage as: flower abortion; reduced yield and incidence of fruit physiological disorders such as: blossom end, rot and sunscald causing significant loss (Espinoza, 1991; Olle and Bender, 2009). Also, shade mesh reduces water requirements and increases the efficiency of irrigation water use (Möller and Assouline, 2007).

Effective management of water resources is the key to sustainability and profitability of the crop, thus encouraging the development of new techniques for the analysis and efficient water management. This study aims to evaluate the effects of supplemental irrigation levels in bell pepper crop yield and water use efficiency under shade mesh and in open-field.

MATERIALS AND METHODS

This field study was conducted at the experimental area of the Polytechnic School of the Federal University of Santa Maria (altitude of 110 m, and 29°41'25"S, and 53°48'42"W), during the Spring-Summer seasons of 2013-2014 and 2014-2015. The soil is classified as typical dystrophic yellow argissolo, with a loam texture (Streck et al., 2008). The climate of the region, according to the Köppen classification is subtropical humid (Cfa). According to the National Institute of Meteorology (INMET), mean annual evaporation, temperature and rainfall range from 800 to 1200 mm, 18 to 20°C and 1450 to 1650 mm, respectively. Table 1 shows the summary of the mean monthly climate data during the experiment. The insolation and evaporation in season 2013 to 2014 were higher than those in 2014 to 2015, except mean relative humidity which was reduced in 2013 to 2014. Solar radiation, evaporation, rainfall and daily temperature during the experimental period are shown in Figure 1. The monthly mean solar radiations, temperature and rainfall were higher in 2013 to 2014 than in 2014 to 2015. The daily mean temperatures in 2013 to 2014 were higher than those in 2014 to 2015 except December and March. The monthly maximum temperatures were higher in December, January and February. The rainfall cumulative was higher in 2013 to 2014 (892.8 mm) and 2014 to 2015 (834 mm); the maximum monthly rainfalls were in November and December, respectively. The monthly radiation in 2013 to 2014 was higher in December and January.

The experimental design was a randomized complete block with four replications and ten treatments in factorial arrangement (four irrigation levels combined with shade mesh). Irrigation treatments were: 25% ($I_{0.25}$), 50% ($I_{0.50}$), 75% ($I_{0.75}$), and 100% ($I_{1.0}$) rate of crop evapotranspiration (ETc) and the control [no irrigation (I_0)]. Shading treatments were 50% reduction of the photosynthetically active radiation (according to the manufacturer) and open field conditions (control, 0% shading). There were 40 experimental plots, each of 5.0 m long and 2 m wide (10 m^2), for a total area of 400 m^2, not including border plants. The variety of bell pepper was Arcade, widely used in the region. Two-month old plants were transplanted in the field, with 1.0 m separation between rows and 0.4 m between plants (density of 2.5 plants m^{-2}) on 16 November 2013 and 23 November 2014. Shade mesh (polyethylene black shade mesh) was supported with metallic cable and forming rectangular structure with the highest point at 2 m. The shade mesh was set two weeks before transplanting. The level of shading was verified by using digital radiometer (Model MS-100). Leaf temperature was measured in each plot with an infrared thermometer gun (Model: AR 320).

One drip irrigation tape was placed next to each row; emitters were spaced 0.2 m apart and had a flow rate of 0.8 L h^{-1} per

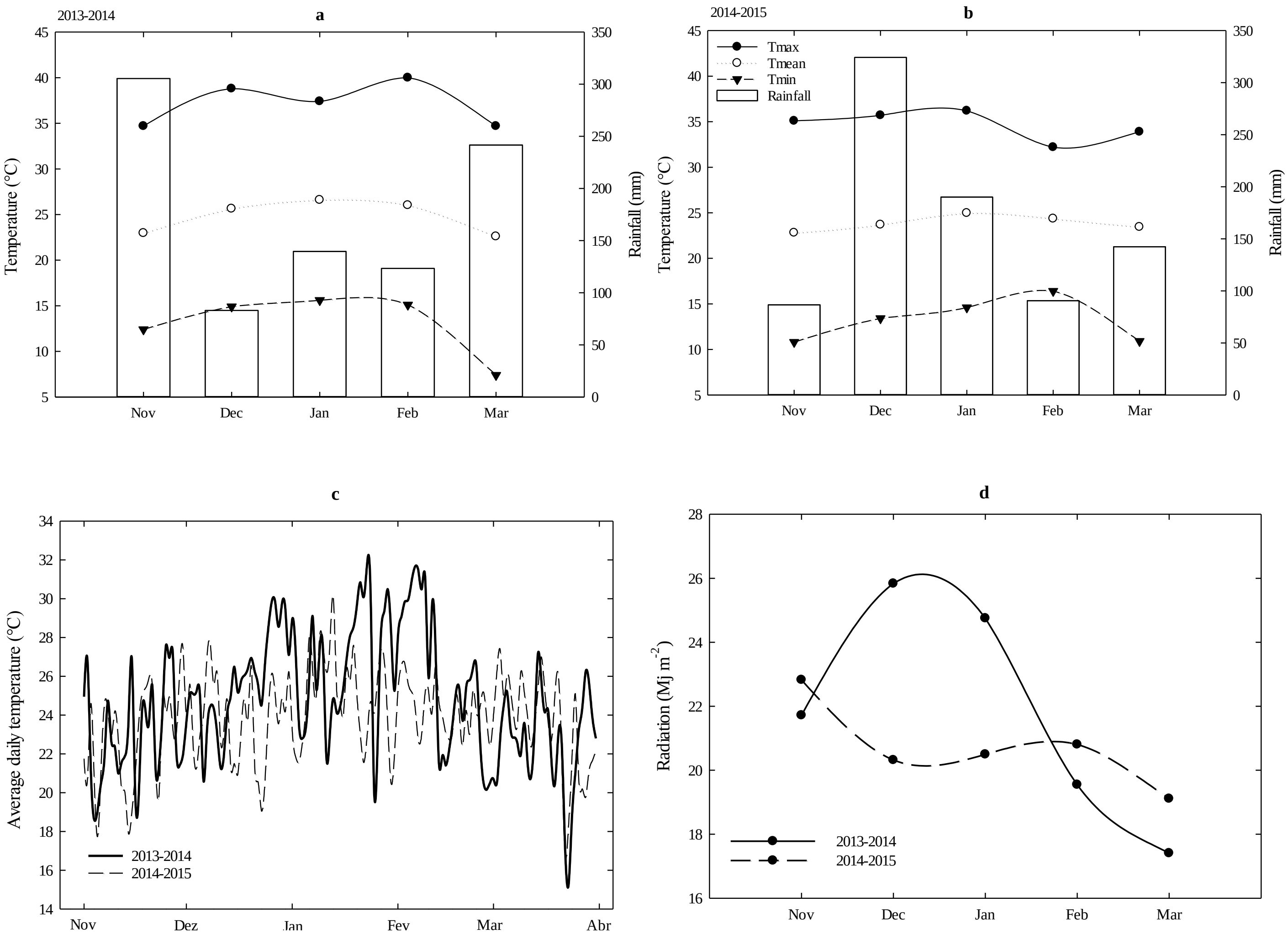

Figure 1. (a, b) Climograph of the experimental area, (c) average daily temperature and (d) radiation during the seasons 2013-2014 and 2014-2015.

emitter. In each experimental plot there was a ball valve for regulating irrigation time, pressure, and uniformity.

Irrigation strategy

During the first 20 days after transplanting 100% of crop evapotranspiration (ETc) was applied to all treatments to ensure plants establishment. Levels of supplementary irrigation were applied from 20 to 119 days after transplant and the frequency of daily watering was established. After effective rainfall exceeded reference evapotranspiration, irrigation was applied two days after the event.

Crop reference evapotranspiration (ETo) and crop evapotranspiration (ETc) were calculated using Equations 1 and 2. The use of reference evapotranspiration leads to increasing uncertainty comparing actual evapotranspiration. There are other models that can estimate evapotranspiration reference than have had successful results. Also, they are useful for selecting the best model when researchers must apply temperature-based models on the basis of available data (Valipour and Eslamian, 2014; Valipour, 2014a, b, c; Valipour, 2015a, b). Weather data were collected from an automatic weather station located 1 km from the experimental area. Crop reference evaporation was calculated based on the method of FAO Penman-Monteith (Allen et al., 2006), (Equation 1) as follows:

$$\mathrm{ETo} = \frac{0.408\,\Delta\,(Rn - G) + \gamma\,\frac{900}{\mathrm{T}+273}\,U_2.(e_s - e_a)}{\Delta + \gamma\,(1 + 0.34\,U_2)} \quad (1)$$

where ETo is the reference evapotranspiration (mm day^{-1}), Rn, G and T are net radiation value at crop surface (MJ m^{-2} day^{-1}), soil heat flux density (MJ m^{-2} day^{-1}) and mean daily air temperature at 2 m height (°C), respectively. Also, u_2, e_s e_a, (e_s - e_a), Δ and γ represent wind speed at 2 m height (m s^{-1}), saturation vapor pressure (kPa), actual vapor pressure (kPa), saturation vapor pressure deficit (kPa), slope of the saturation vapor pressure curve (kPa/°C) and psychrometric constant (kPa/°C), respectively.

Crop evapotranspiration (ETc) was calculated with the method of dual crop coefficients for each crop phoenological stage (Allen et al., 2006), (Equation 2) as follows:

$$\mathrm{ETc} = (\mathrm{K_{cb}} + \mathrm{K_e}) \times \mathrm{ETo} \quad (2)$$

where ETc is crop evapotranspiration (mm), ETo is reference crop evapotranspiration (mm) and splitting K_c into two separate

Table 2. Average soil attributes of the experimental area.

Soil layers (m)	Bulk density (g cm^{-3})	Field capacity (m^3 m^{-3})	Wilting point (m^3 m^{-3})	Water content (m^3 m^{-3})	Infiltration (mm h^{-1})	Texture
0-0.2	1.42	0.31	0.14	0.18		Loam
0.2-0.4	1.38	0.34	0.17	0.17	15.0	Clay-loam
0.4-0.6	1.36	0.37	0.23	0.13		Clay

Table 3. Crop evapotranspiration, effective rainfall and irrigation applications during two seasons in bell pepper.

Treatment	2013-214				2014-2015			
	Etc (mm)	Rainfall[Z] (mm)	Irrigation[Y] (mm)	Days irrigation	ETc (mm)	Rainfall[Z] (mm)	Irrigation[Y] (mm)	Days irrigation
I_0	-		-	-	-	543.8	-	-
$I_{0.25}$	140.1		100.4		125.2		70.4	
$I_{0.50}$	280.1	345.1	200.9	74	250.4		140.8	57
$I_{0.75}$	420.2		301.3		375.5		211.2	
$I_{1.0}$	560.2		401.8		500.7		281.5	

[Z]Effective rainfall; [Y]Effective Irrigation.

coefficients, basal crop coefficient (K_{cb}) and soil water evaporation coefficient (K_e).

Before the plants were transplanted, sampling points in the experimental area were randomly selected to determine basic soil attributes, including soil texture, bulk density, field capacity, and permanent wilting point (Table 2). Also, an infiltration test of wet bulb was performed to design the irrigation system.

Soil water content over the season was measured before and after irrigation every two days (four readings per experimental plot), with a portable time domain reflectometry (TDR-100). The two metallic sensor (0.2 m rods of the TDR) were inserted vertically within the row between plants. Also soil water monitoring was performed with neutron probe (CPN Model 503, DR), with calibration previous to execution of the experiment (Padrón et al., 2015). PVC tubes (50 mm) were installed between row (1 m distance) and plant of each experimental plot at a depth of 0.7 m. Readings was performed once a week at 0.125, 0.30 and 0.50 m of soil depth.

Fruit were picked weekly during two months (60-120 day after transplanting) for yield, in both years. Fruit yield per plot was determined by harvesting 20 plants from center rows. To evaluate incidence of sunscald, number of affected fruit was determined. Fruit diameter, length and dry weight were determined at each harvest, using fruit from five plants per plot. Fruit samples were dried at 65°C until constant weight was obtained. Water productivity (WP) and irrigation water productivity (IWP) were calculated with the fresh total yield (kg ha^{-1}) divided by crop evapotranspiration (ETc) Equation (3) and total irrigation water applied Equation (4), respectively (Heydari, 2014; Molden et al., 2010), as follows:

$$\mathrm{WP} = \frac{\text{Total yield (kg ha}^{-1})}{\text{evapotranspiration (mm)}} \quad (3)$$

$$\mathrm{IWP} = \frac{\text{Total yield (kg ha}^{-1})}{\text{Irrigation water applied (mm)}} \quad (4)$$

Herbicides, fungicides and insecticides were applied as necessary. Fertigation was according to the nutritional needs of the crop and chemical analysis of the soil. Fertigation was performed with irrigation (daily) calculated to produce 40 t ha^{-1}. All plants received 368 kg ha^{-1} of a complete fertilizer ($13N$-$14P_2O_5$-$13K_2O$), 290 kg h^{-1} of ammonium nitrate (36% N) and 396 kg ha^{-1} of potassium nitrate (35% K_2O). Statistical analysis was performed using the SPSS software package (SPSS V17.0). Significant differences between means for different treatments were compared using Tukey test at $P<0.05$. Data from all years were pooled when no treatment interactions were found.

RESULTS AND DISCUSSION

Crop evapotranspiration, effective rainfall and effective irrigation are shown in Table 3. In 2013-2014, crop was irrigated 74 times and total irrigation applied was 401.8 mm. In 2014 to 2015, crop was irrigated 57 times and total irrigation was 281.5 mm. Thus, in 2014 to 2015 number of irrigations and irrigation volume were reduced. ETc in 2014-2015 was 59.5 mm lower than in 2013 to 2014. Effective rainfall was also higher in 2014 to 2015 than in 2013 to 2014.

Soil moisture and daily effective rainfall is shown in Figure 2. The 0-0.25 m soil profile presented sharp moisture decrease between irrigations. Soil moisture below 0.250 m depth remained almost constant in all treatments. Soil moisture under shade remained near field capacity and presented an average decrease of 0.02 m^3 m^{-3} when compared to treatments on open field. In general, under shade mesh soil water content was higher compared to treatments in open field. Shading reduces demand for crop evapotranspiration, causing reduction of transpiration, resulting in decreased soil water uptake in bell pepper (Díaz-Pérez, 2013; Möller et al., 2004; Kittas et al., 2009).

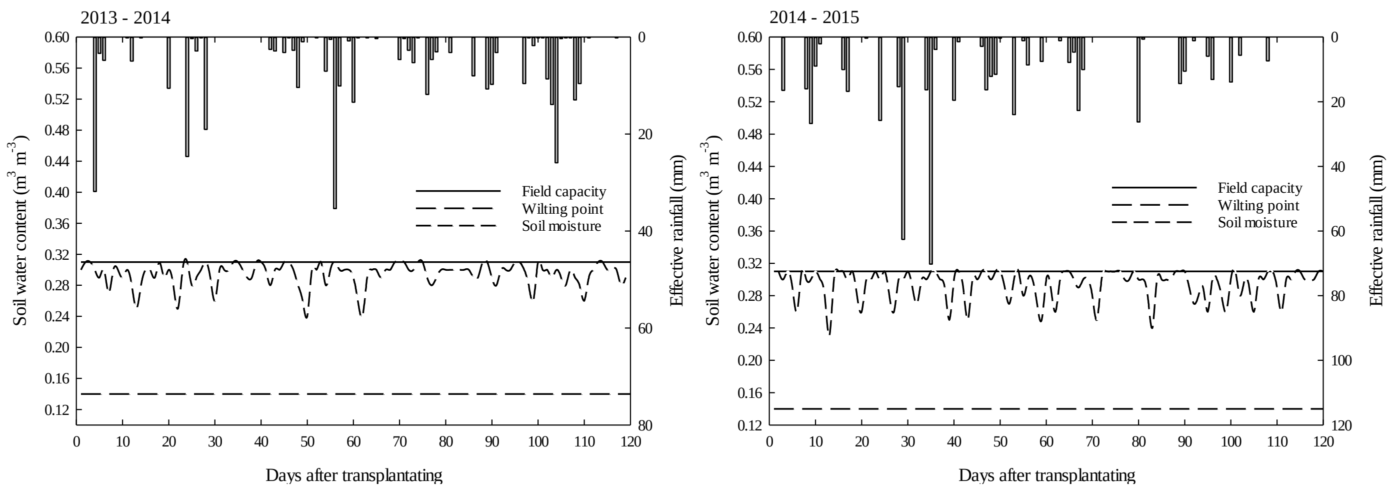

Figure 2. Soil moisture and effective rainfall. Soil moisture in the 0-0.30 cm depth layer and effective rainfall.

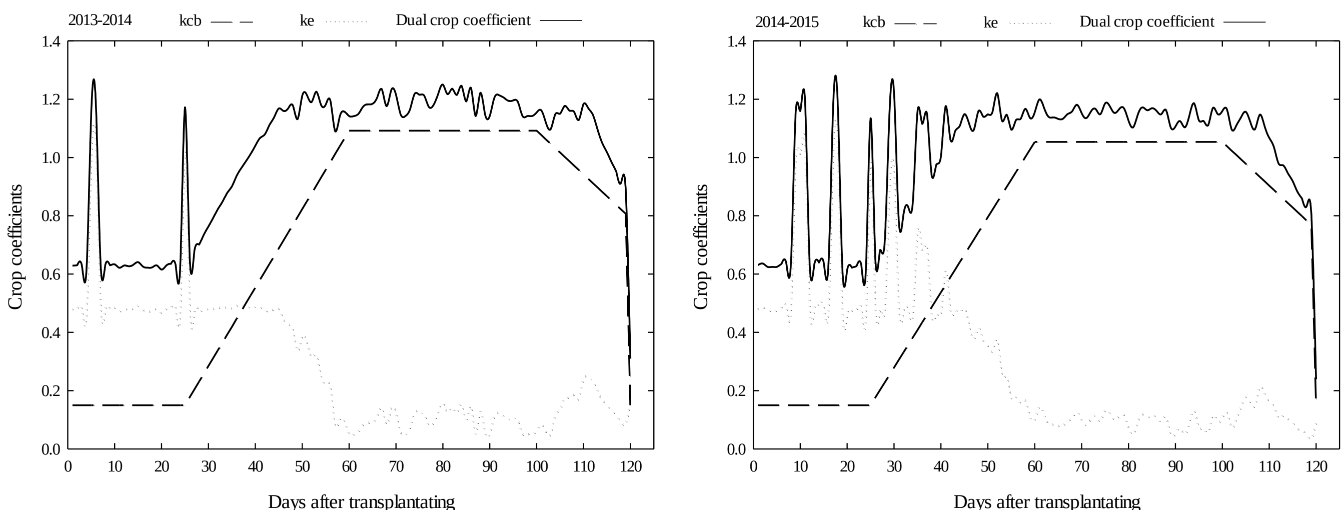

Figure 3. Dual crop coefficient for bell pepper in a sub-tropical region.

The dual crop coefficient during the study is shown in Figure 3. In both seasons, crop coefficient K_{cb} gradually increased reaching the highest values between 60 and 100 days after transplanting. Coefficient K_e was highest during the first 50 days after transplanting and then decreased. Both factors were most affected by the frequent rainfall in the 2014 to 2015 season. The biggest difference between the values of K_c and K_{cb} occurred in the initial crop growth stage where evapotranspiration was mainly composed of soil evaporation, while crop transpiration, was relatively small.

Dual crop coefficient values at different stages of bell pepper crop growth are shown in Table 4. At different stages Kc values were similar or higher than those recommended by FAO-56 values. Shukla et al. (2013) reported that early in the season Kc values were higher than those of the classic Kc curve reported in the literature (e.g., FAO-56) and increased as the crop developed until it reached maximum values at crop maturity. Due to increased water table at the time of transplanting, soil evaporation losses were higher than those of other regions leading to increased ETc and as a result increased Kc; Kc for bell pepper reached values of : Kc_{ini}=0.86, Kc_{med}=1.21, Kc_{fin}=1.28. Also, rainfall during the initial stage further increased the surface soil water content, which resulted in increased soil evaporation. Results show that Kc for pepper grown using sub-irrigation is highly dependent on the soil water content of the bare soil area. In semiarid climate, Kong et al. (2012), reported increased Kc values (Kc_{ini}=0.66-0.69; Kc_{med}=1.19-1.30; Kc_{fin}=0.89-0.93) (FAO 56).

There was no significant interaction effect for treatments

Table 4. Average of dual crop coefficients (Kc) for bell pepper irrigation in a sub-tropical region.

Crop growth stage	2013-2014	2014-2015	Average	FAO-56[z]
Initial growth	0.67	0.74	0.71	0.6
Crop development	1.03	1.05	1.04	
Mid-season	1.19	1.15	1.17	1.15
Late season	0.97	0.87	0.92	0.80

[z]Kc recommended by FAO-56.

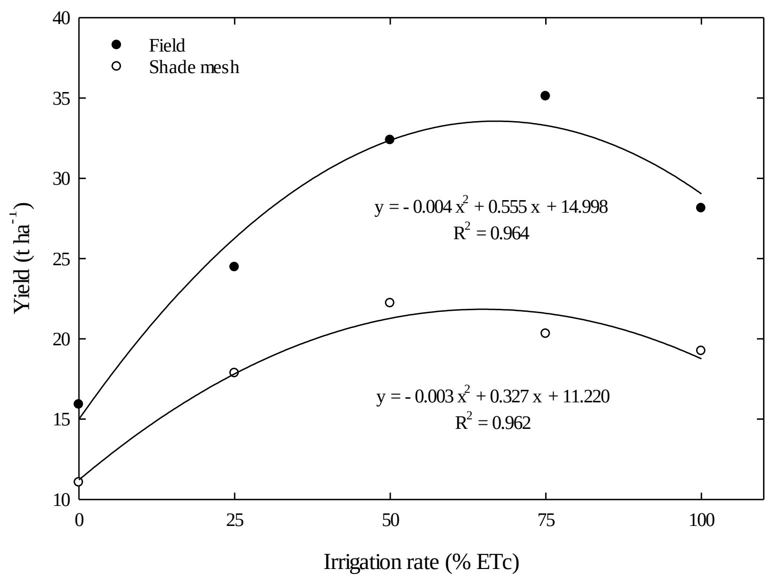

Figure 4. Relationship between fruit yield and irrigation rate applied as fraction of crop evapotranspiration in bell pepper grown under shade mesh or in open field.

under shade mesh and open-field between the years of study for yield data. Ilahy et al. (2013) reported that there were no significant differences between shaded and non-shaded conditions in commercial yield of sweet pepper 'Herminio'. However, statistical difference between the levels of irrigation on yield, fruit weight, fruit dry matter and number fruit per plant was found. Fruit weight showed significant interaction between treatments under shade netting and open field.

The relationship between fruit yield and irrigation rate under shade mesh and open-field is shown in Figure 4. Fruit yield in open-field was increased with $I_{0.50}$ and $I_{0.75}$. In the 2013 to 2014 season, values ranged from 13.8 t ha^{-1} in I_0 to 37.1 t ha^{-1} in $I_{0.75}$. Treatment I_0 showed a reduction in yield of 54.7% and that of $I_{1.0}$ was reduced by 19.9% relative to maximum yield ($I_{0.75}$). Kara and Yıldırım (2015), reported similar results in *Capsicum annuum* L. cv. Carliston with different irrigation levels (0.2, 0.5, 0.8, 1.0 and 1.2 ETc); yields were 18.78, 20.60, 21.57, 18.90 and 15.16 Mg ha^{-1}, respectively, with maximum yield with 0.8 ETc. Padrón et al. (2014), evaluated irrigation frequency and irrigation rates finding that daily irrigation resulted in better crop performance compared to irrigation every other day; crop yields were similar with daily irrigations at 60, 80 or 100% ETc. Moreover, Sezen et al. (2015), reported the highest yield values with full irrigation (44.2-47.8 t ha^{-1}) and deficit irrigation of 50 and 75% (34.9-36.0 t ha^{-1} and 40.8 to 47.2 t ha^{-1}, respectively).

Our results showed that cultivation of bell pepper was affected by deficit irrigation as well as excess water caused by high rainfall. Bell pepper crop irrigated more frequently tends to be more efficient in water use, without affecting yield, compared to a crop irrigated less frequently. Also, Yildirim et al. (2012), studying the effect of different irrigation treatments (0.0, 0.2, 0.5, 0.8, 1.0, 1.2 of ETc) in bell pepper to determine stress with a fixed interval of 7 days throughout the whole drought season, reported yields of 3.25, 8.64, 16.93, 20.08, 27.67 and 24.61 t ha^{-1}, respectively. They mentioned that the most important factor that affect growth and yield in pepper crops is the amount of irrigation water applied throughout the development period.

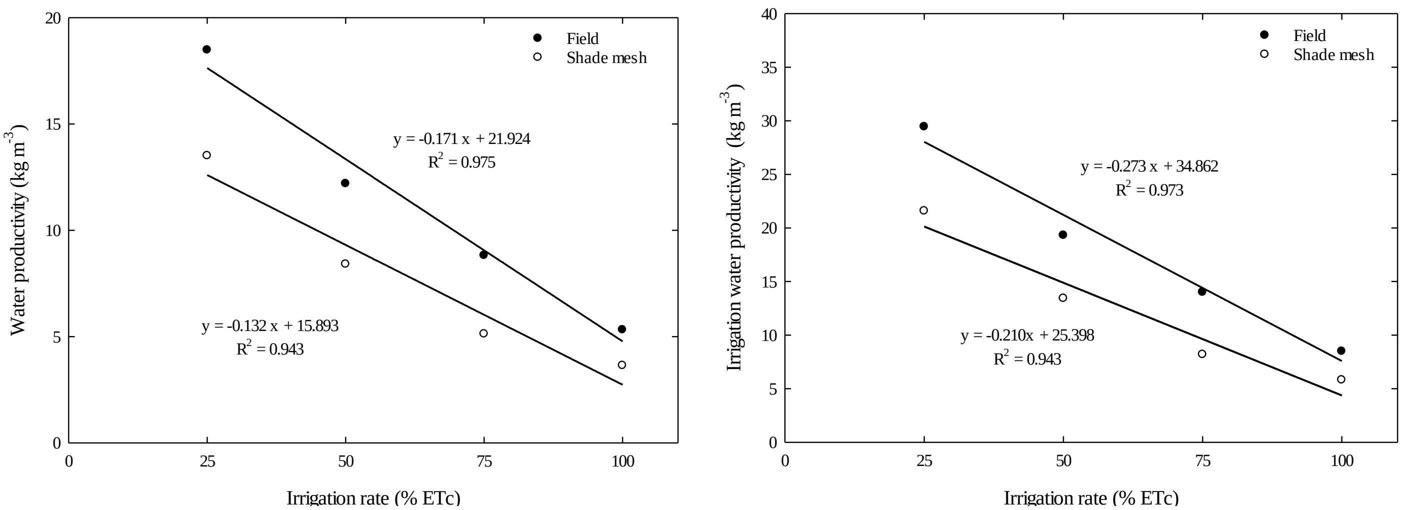

Figure 5. Water productivity and irrigation water productivity in bell pepper under shade mesh and in open-field.

The maximum yield under shade mesh was obtained at $I_{0.50}$. Yield ranged from 11.0 t ha^{-1} in I_0 to 22.2 t ha^{-1} in $I_{0.50}$. Treatment I_0 presented 50.3% reduction in yield and 13.4% reduction in $I_{1.0}$. Díaz-Pérez (2014), studying the effect of shade levels of 0, 30, 47%, 63 and 80% in *Capsicum annuum* cv. Heritage, reported that yield increased with increasing shade level up to 35% shade and then decreased with increasing shade levels; Möller and Assouline (2007), reported yield in Sweet pepper *C. annuum* cv. Selika between 5.93 and 9.26 kg m^{-2} in 30% shade. Ilahy et al. (2013), reported yield in (*C. annuum* cv 'Beldi'), grown at 0, 50 and 100% shade, varied between 0.9 kg/plant and 1.15 kg/plant, respectively. Also, López-Marin et al. (2012) reported that commercial yield of sweet pepper cv 'Herminio' ranged from 2.55 kg/plant under unshaded conditions compared to 2.53 kg/plant in shaded conditions.

The open-field treatments showed highest cumulative yields and the trend was observed throughout the harvest period, which is probably due to increased radiation and greater photosynthetic activity. López-Marin et al. (2012), reported highest yields with 40% shade. This was attributed to reduction of the incident radiation. Also, it was observed that the crop was negatively affected by high temperatures, with shading decreasing average daily temperature by 2.5°C at noon and 0.6°C in the morning, creating a micro climate favorable to crop development. Díaz-Pérez (2013), reported that moderate levels of shade (30 and 47%) were the most favorable for plant growth and function in bell pepper. Möller and Assouline (2007), reported that 30% mesh black shade reduces solar radiation, wind speed, water needs and increases the efficiency of water use in bell pepper.

In open-field, fruit sunscald was observed with greater intensity 55 to 85 days after transplanting, when the crop had reduced leaf area, with yield losses of 1.4, 2.2, 2.3%, 3.3 and 1.7% in irrigation treatments I_0, I_{25}, I_{50}, I_{75} and $I_{1.0}$, respectively. Under shade mesh fruit showed no sunscald. Ilahy et al. (2013), reported yield losses due to sunscald ranging from 0.69 kg/plant in open-field and 0.18 kg/plant in shady conditions.

Water productivity and irrigation water productivity both decreased with increasing irrigation rate (Figure 5). Both WP and IWP were higher in open field than under shade mesh. Plots without irrigation showed reduced WP (3.0 kg m^{-3} in the open-field and 2.1 kg m^{-3} under shade mesh). Values of WP in open-field oscillated between 18.5 kg m^{-3} in $I_{0.25}$ and 5.3 kg m^{-3} in $I_{1.0}$ and under shade mesh between 13.5 kg m^{-3} in $I_{0.25}$ and 3.6 kg m^{-3} in $I_{1.0}$. IWP values in open-field varied between 29.4 kg m^{-3} in $I_{0.25}$ and 8.5 kg m^{-3} in $I_{1.0}$ and under shade mesh between 21.6 kg m^{-3} in $I_{0.25}$ and 5.8 kg m^{-3} in $I_{1.0}$. The WP and IWP values of this study were similar to those previously reported. Kong et al. (2012), through drip irrigation determined WP values between 7.76 kg m^{-3} and 10.71 kg m^{-3} in bell pepper. Sezen et al. (2015), reports of WP of 6.9 kg m^{-3} and IWP of 5.7 kg m^{-3} by applying irrigation water of 570.4 mm for the whole growing season at intervals of 3 to 6 days. Guang-Cheng et al. (2010), determined the WP and IWP hot pepper in greenhouses values oscillate between 6.7 to 10.4 kg m^{-3} and 6.3 to 10.6 kg m-3, respectively. Demirel et al. (2012) determined the values WP and IWP in pepper grown in the Thrace region of Turkey varying from 2.4 to 7.0 kg m^{-3} and 0.3 to 9.1 kg m^{-3}, respectively. Kara and Yıldırım (2015), reported WUE in *C. annuum* L. cv. Carliston with irrigation levels of 0.2, 0.5, 0.8, 1.0 and 1.2% of ETc, between 6.0, 4.1, 3.6, 2.7, and 2.1 kg m^{-3}, respectively. Moreover, Yildirim et al. (2012), reported results of WUE and IWUE in bell pepper with irrigation treatments (0.0, 0.2, 0.5, 0.8, 1.0, 1.2 of ETc) of 1.6, 3.3, 5.3, 5.5, 6.9 and 5.7 kg m^{-3} and 2.0, 3.8, 6.0, 6.1, 7.5, and 6.2, respectively.

Table 5. Average of fruit quality in bell pepper under shade mesh and in open-field.

	Treatments	Fruit per plant (number)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Dry matter (%)
Field	I_0	6^{b}	13.4	5.6^{b}	126.50^{bC}	5.3
	$I_{0.25}$	7^{ab}	14.8	6.6^{a}	134.00^{bBC}	5.3
	$I_{0.50}$	9^{a}	14.3	6.1^{ab}	142.10^{bAB}	5.3
	$I_{0.75}$	9^{a}	14.9	5.9^{ab}	153.20^{bA}	5.2
	$I_{1.0}$	8^{ab}	15.0	5.5^{b}	144.70^{bAB}	5.0
	Sig.	*	ns	*	*	ns
Shade	I_0	5^{b}	16.9	6.0	154.20^{aC}	5.1^{a}
	$I_{0.25}$	7^{ab}	16.5	6.5	176.60^{aB}	5.0^{ab}
	$I_{0.50}$	8^{a}	18.0	6.7	179.50^{aB}	5.2^{a}
	$I_{0.75}$	8^{a}	16.4	6.2	188.6^{aAB}	4.9^{ab}
	$I_{1.0}$	7^{ab}	17.8	6.3	198.90^{aA}	4.6^{b}
	Sig.	*	ns	ns	**	*

Letters indicate significant differences at *P<0.05 and **P<0.01. Sig., significance.

The peaks were obtained from 1.0 ETc treatment.

Results on average of fruit quality in bell pepper under shade mesh and in open-field are presented in Table 5. Plots in open-field presented difference on fruit quality, number of fruits per plant, fruit diameter, fruit weight and under shade mesh, the number of fruit per plant, weight and fruit dry matter. The variable that showed interaction effect between under shade mesh and in open-field was the fruit weight. The quality of fruit in the open-field and under shade mesh were affected significantly, showing the smallest fruit diameter, length, and weight and lowest number of fruits per plant. Irrigation levels of 50 and 75% ETc obtained the best fruit quality. However, the maximum fruit weight and lower fruit dry matter content was presented by treatment $I_{1.0}$, under shade mesh. In summary, highest quality fruit (increased weight and reduced dry matter and sunscald incidence) was obtained under shade. Sezen et al. (2015), reported increased fruit yield to augmented fruit number In addition, a uniform supply of water in the soil throughout the growing season is needed to prevent poor fruit size and shape and to improve yield.

Rylski and Spigelman (1986), reported changes in plant development due to the shading affected. According to the authors, shading affected fruit set, number of fruits per plant, fruit location on the plant, fruit development and yield. Also, the lowest number of fruits per plant was obtained under 47% shading at 5 plants m^{-2} density, under 47 and 26% shading at 6.7 plants m^{-2} density. Under shading, individual fruits were larger and had a thicker pericarp. Shading reduced sun-scald damage of the fruits from 36% in full sunlight to 3 and 4% under 26 and 47% shading. The highest yield of high-quality fruits was obtained with 12 to 26% shade. On the other hand, Milenković et al. (2012), refer shading of pepper plants affected both fruit yield and quality. Total and marketable yield increased with 40% shading level and then decreased (with 50% shade). Shading of pepper (40%) may be an option to reduce heat stress conditions and extend the spring-summer season toward September and concludes the photoselective, light-dispersive shade nets provide a new, tool for crop protection. Changing the light intensity and radiation spectrum has a large impact on the total production system.

Maximum production efficiency in open-field was 34.2 t ha^{-1} with 69.4% of ETc; under shade mesh it was 20.1 t ha^{-1} with 54.5% of ETc. The difference of maximum production efficiency was of 14 t ha^{-1} and reduction of 14% of ETc. Bell pepper under 50% shade could save 14 to 25% of irrigation water. Moreover, the sunscald in fruit is improved by increasing the number of plant per square meter.

Conclusions

Yield of bell pepper in open-field was significantly higher with 75% of ETc; it was highest with 50% ETc under shade. The point of maximum production efficiency was 69.4% of ETc in open-field and 54.5% of ETc under shade. Thus, under 50% shades, there may be up to 25% water savings. Crop coefficients values found in this study will be useful for irrigation scheduling, dual crop coefficients in bell pepper, developed for a region with a humid subtropical climate. Results indicate that the WP and IWP values decreased with increasing irrigation level in both open field and shaded conditions. Irrigation with increased frequency tends to increase irrigation water use efficiency. Drip irrigations at 50% ETc and 75% of ETc may be recommended.

Conflict of Interest

The authors have not declared any conflict of interest.

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Fertilization with poultry litter in a corn crop for silage

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The increase in broiler production in Brazil has increased the production of poultry litter. Thus, the management and adequate disposal of this waste is recommended. Agricultural use is a promising solution. This study evaluated the productivity of corn for silage with different fertilizations of poultry litter. The production of Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Mineral Matter (MM) and Organic Matter (OM), and microbial activity of the soil such as Microbial Biomass Carbon (MBC), Metabolic Quotient (qCO_2) and Microbial Quotient (*q*MIC), were evaluated. There were treatments composed of a control that received mineral fertilization (33 Kg N, 80 Kg P_2O_5 and 80 Kg K_2O) and treatments with poultry litter Rice Straw (RS) and Wood Shavings (WSH) (doses of 2.5; 5.0; 7.5 and 10.0 Mg ha^{-1}). The treatments with poultry litter received, at sowing, 1/3 of the mineral fertilization. The evaluation of the production of corn for silage was carried out in the R4-R5 phase, and soils collected in the 0 to 10 cm layer in the post-poultry litter (A) and post-herbicide (B) periods. The microbial communities were influenced by anthropic activity in every treatment, with fall in MBC and the significant increase in the qCO_2 in the control and the *q*MIC in the treatments that received poultry litter, demonstrating greater efficiency in mineralization of nutrients. As regards DM productivity, there was no difference in the treatments; however, treatments 7.5 RS and 10 WSH presented higher levels of protein and ether extract similar to the control treatment. This study concluded that the treatments with poultry litter favored the use of soil OM, which had less impact on microbial activity, and proposed that the substitution of 2/3 of the fertilization would not cause losses or decrease in the production or characteristics of the corn silage.

Key words: *Zea mays*, organic fertilization, organic matter and poultry waste.

INTRODUCTION

Exponential population growth demands more food, and to satisfy this necessity, larger quantities of wastes are produced that, if not managed correctly, pollute the air, water and soil. Thus, the inclusion of the concept of

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sustainability and environmental management become important factors in current agricultural and cattle-raising activities (Costa et al., 2014).

The search for sustainable development represents one of the greatest challenges facing humanity. Brazil, the third largest producer of chicken in the world in 2013, produced 12,308 million tons, with the state of Paraná responsible for 29.4% of the national carcass slaughter (UBABEF, 2014). According to Marín (2011), the mean final waste production of poultry litter is 2.2 kg per broiler unit. Studies show that the litter can be reused 1 to 6 times without significant differences as regards mortality, weight gain, feed intake, feed efficiency and quality of the carcasses, demonstrating some beneficial properties for flocks with acquired immunity (Fukayama, 2008).

Current poultry exploitation is characterized by the production of precocial chickens. This development is anchored in the advances of genetics, nutrition, health and management. The increasing production of chickens leads to larger quantities of poultry litter (UBABEF, 2014). According to Costa et al. (2009), poultry litter is a material rich in nutrients and, thanks to its low-cost availability, is included in the fertilization of commercial cultures.

Due to these characteristics, poultry litter is being employed in the fertilization of large cultures like corn (*Zea mays* L.), which is one of the main cereals cultivated and consumed worldwide (Bobato, 2006). Known for its versatility of use, social aspect and consequence of human and animal production, the need to increase the productivity of the culture per planted area has produced an increase in the consumption of this product. In the 2012/2013 harvest, Brazil produced 77.887 million tons of corn, occupying an area of 15,726.3 thousand hectares (CONAB, 2014). This ingredient is also traditionally the material that is used the most for silage, due to its high yield potential in the production of mass and its bromatological quality, confirming the production of good silage (Ferreira, 2001).

Corn for silage requires special care as regards fertilization, due to the total harvest of the plant, which does not leave straw residue and thus extracts large quantities of nutrients from the soil. This can lead to decrease in the productivity and the quality of the silage. In order to maintain the desired characteristics of good silage, it is necessary to efficiently replace (through chemical fertilization: green/organic) the nutrients absorbed by the culture and exported in the harvest (França and Coelho, 2001).

The effect of organic matter on productivity can be direct, by means of nutrient supply or by bettering the physical properties of the soil, which improves the environment and stimulates plant development. Poultry litter, in addition to being rich in nutrients, improves crop productivity at a reduced cost. However, it should be emphasized that the use of organic fertilizers promotes the slow and gradual release of nutrients with the advantage of increasing the soil organic matter (SOM) content. The SOM content is considered one of the main indicators of sustainability and environmental quality in agroecosystems (Sá et al., 2001).

Alterations in the soil environment due to the addition of organic waste can affect soil microorganisms that are considered indicators of sustainability and environmental quality in agrosystems. Soils that have high microbial biomass (SMB) content are capable of not only storing, but also cycling more nutrients in the system (Berthrong et al., 2013). Soil respiration is defined as the biological oxidation of organic matter to CO_2 through the metabolism of aerobic microorganisms and occupies a key role in the carbon cycle of terrestrial ecosystems.

The evaluation of soil respiration is the most frequent technique to quantify microbial activity. It is positively related to organic matter content and microbial biomass (Alef and Nannipieri, 1995). The relationship between SMB and total organic carbon, called the microbial quotient (*q*MIC), is used as an indicator that reflects the efficiency of the transformation and conversion of organic matter C into microbial C (Baretta et al., 2005).

There is a verifiable necessity for wider studies related to the use of poultry litter as organic fertilizer in the productivity of cultures, as well as its effect on soil microorganisms. This study evaluated the productivity of corn for silage and the behavior of microbiological attributes of the soil with the use of poultry litter in the fertilization.

MATERIALS AND METHODS

The study was developed on the experimental farm of the Universidade Estadual do Norte do Paraná – *Campus* Luiz Meneghel (UENP/CLM), in Bandeirantes city (state of Paraná) (23°06'36"S; 50°27'28"W) (mean altitude of 420 m in typical eutrophic RED LATOSOL (LVef3) with no-tillage corn over black oats. The chemical characteristics are described in Table 1.

Random block design was considered, with subdivided plots. The blocks had four rows, the main plot nine treatments. The plots consisted of a control treatment with mineral fertilization (MF) (33 Kg N, 80 Kg P_2O_5 and 80 Kg K_2O per hectare). The treatments with poultry litter received, at sowing, 1/3 of the mineral fertilization (initial dose of the plant), while the plots of the MF treatment received the rest in the sowing furrows (Table 2).

A hybrid cultivar of corn (*Zea mays* L.) 2b810PW, of medium to high productivity, with a density of 55,000 plants ha^{-1} and spacing between rows of 0.90 m, was used for the test. Each experimental plot was 6.3 m long and 5.0 m wide, with a total area of 31.5 m^2. The cover fertilization was carried out when the plants presented 5 to 6 totally unrolled leaves in the vegetative stages V5 and V6 (Magalhães and Durães, 2008) and received 200 kg ha^{-1} of urea (Raij et al., 1996).

The litter was collected from poultry that had the same producer (2nd flock of broilers). The chemical characteristics of the litters are found in Table 3. The nutrient levels of the poultry litter are found in Table 4.

A collection of soil for chemical analysis was carried out at the beginning of the experiment and used to determine the need for lime. A dose of 1.2 Mg ha^{-1} was distributed in the total area of the experiment (Caires et al., 2006).

The soil collections for the microbiological analyses were carried

Table 1. Chemical properties of the soil at 0 to 20 cm deep.

Organic Mat. ($g.Kg^{-1}$)	pH ($CaCl_2$)	P ($mg.dm^{-3}$)	K ($cmol_c.dm^{-3}$)	Ca ($cmol_c.dm^{-3}$)	Mg ($cmol_c.dm^{-3}$)	Al ($cmol_c.dm^{-3}$)	CEC
22.77	4.44	5.19	0.16	5.89	1.93	0.35	12.91

P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Al = Aluminum; CEC = Cation Exchange Capacity.

Table 2. Description of the treatments, formed by mineral fertilization (MF), rice straw (RS) and wood shavings (WSH).

Treatment	Description
MF	Mineral Fertilization – MF
2.5 RS	1/3 MF + 2.5 Mg ha^{-1} of Rice Straw (RS)
5.0 RS	1/3 MF + 5.0 Mg ha^{-1} of Rice Straw (RS)
7.5 RS	1/3 MF + 7.5 Mg ha^{-1} of Rice Straw (RS)
10.0 RS	1/3 MF + 10.0 Mg ha^{-1} of Rice Straw (RS)
2.5 WSH	1/3 MF + 2.5 Mg ha^{-1} of Wood Shavings (WSH)
5.0 WSH	1/3 MF + 5.0 Mg ha^{-1} of Wood Shavings (WSH)
7.5 WSH	1/3 MF + 7.5 Mg ha^{-1} of Wood Shavings (WSH)
10 WSH	1/3 MF + 10.0 Mg ha^{-1} of Wood Shavings (WSH)

Table 3. Chemical analysis of the poultry litter (wood shavings [WSH] and rice straw [RS]).

Poultry litter	P	K	Ca	Mg	S	C	N	C/N	B	Cu	Fe	Mg	Zn
	g kg^{-1}					%			mg kg^{-1}				
WSH	6.2	17.8	16.4	5.5	3.1	30.6	1.5	19.3	37.2	243.8	2114.8	348.3	203
RS	3.9	13.7	11.2	4.0	2.9	28.7	1.2	23.3	28.4	159.8	4853.4	444.3	130.5

P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; S = Sulfur; C = Carbon; N = Nitrogen; C/N = Carbon/Nitrogen ratio; B = Boron; Cu = Copper; Fe = Iron; Mn = Manganese; Zn = Zinc; WSH = wood shavings and RS = rice straw.

Table 4. Nutrient levels of the poultry litter in each experimental plot.

Trat.	Parameter (1)										
	N	K	Ca	Mg	S	P	B	Cu	Fe	Mg	Zn
	kg ha^{-1}						g ha^{-1}				
2.5 RS	30.8	34.3	28.1	10.2	7.2	9.9	71.2	399.6	12133.6	1110.8	326.2
5.0 RS	61.6	68.6	56.2	20.4	14.4	19.8	142.4	799.2	24267.2	2221.6	652.4
7.5 RS	92.4	102.9	84.3	30.6	21.6	29.7	213.6	1198.8	36400.8	3332.4	978.6
10 RS	123.2	137.2	112.4	40.8	28.8	39.6	284.8	1598.4	48534.4	4443.2	1304.8
2.5 WSH	39.6	44.6	41.2	13.8	7.7	15.6	93.0	609.6	5286.9	870.9	509.1
5.0 WSH	79.2	89.2	82.4	27.6	15.4	31.2	186.0	1219.2	10573.8	1741.8	1018.2
7.5 WSH	118.8	133.8	123.6	41.4	23.1	46.8	279.0	1828.8	15860.7	2612.7	1527.3
10 WSH	158.4	178.4	164.8	55.2	30.8	62.4	372.0	2438.4	21147.6	3483.6	2036.4

(1) P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; S = Sulfur; C = Carbon; N = Nitrogen; C/N = Carbon/Nitrogen ratio; B = Boron; Cu = Copper; Fe = Iron; Mn = Manganese; Zn = Zinc; WSH = wood shavings and RS = rice straw.

out at 0 to 10 cm deep in the inter-row of the culture at two different times: post-poultry litter (A) and post-herbicide application (B). Each sample was composed of 7 simple subsamples, homogenized and sieved in a 2 mm mesh at a humidity adjusted to 60% of field capacity. Microbial biomass carbon (MBC) was determined by the fumigation-extraction method (Vance et al., 1987). Walkley and

Black (1934) determined the method used to analyze total organic carbon (TOC). The MBC/TOC ratio was calculated from the MBC values and the TOC contents (Sparling, 1992). Respiratory activity was determined by the quantification of C-CO_2, according to Silva et al. (2007) and the metabolic quotient (qCO_2) is obtained by the ratio between respiration and MBC.

In order to determine the mean productivity of dry mass per plant and per hectare, plants were collected from plots composed of three rows, two linear meters long (6 plants/row, totaling 18 plants per plot. All of the plants were then weighed and shredded into particles with a mean size of two centimeters using a forage harvester (JF92-Z10 series 2). Duplicate samples were removed from each plot. The samples were weighed and placed in a laboratory oven at 65°C with artificial ventilation for 72 h to determine the percentage of dry matter. After the determination of the dry matter (DM), the levels of mineral matter (MM), ether extract (EE) and crude protein (CP) were determined (Detmann et al., 2012).

The results were submitted to analysis of variance (ANOVA), followed by the Tukey test, considering the level of 5% probability and using the program R (R Development Core Team, 2011).

RESULTS

Microbiological parameters of the soil

The use and management of the soil promoted significant alterations in its microbiological parameters. These parameters were mainly influenced by the addition of poultry litter after mineral fertilization. The MBC values were similar between the MF treatment and the treatments that received wood shavings at every dose and at 5.0 and 7.5 Mg ha^{-1} RS in collection A. In collection B, the treatment that did not receive any type of organic fertilizer presented the lowest value for this parameter. In addition, in every treatment in collection B, there was a significant decrease in MBC in comparison to collection A. As regards the variable total organic carbon (TOC), the lowest values and consequently the lowest reduction were found when analyzed between the collections (about 8 to 10%) in most doses of organic fertilizer and in the MF treatment. Comparison of the times of collections A and B in the microbial quotient (*q*MIC) analysis shows that these values increased in the treatments that received rice straw. The opposite occurred with the treatments that only received mineral fertilization and treatment with wood shavings. Analyzing soil basal respiration (SBR) in collection A, lower values for the MF, 2.5 RS and 2.5 WSH treatments are observed. This repeats in collection B, but only for the treatments with organic fertilization at low doses. In the MF treatment, there is a significant increase. This was reflected in the metabolic quotient (qCO_2), with the highest value in this treatment (Table 5).

The increase in MBC may be related to the larger quantity of organic waste, and can be attributed to the increase in the organic matter content and soil nutrients, which favor microbial growth (Lopes, 2001). Under natural conditions, microorganisms are found in equilibrium, where they depend on natural soil conditions. This equilibrium is frequently disturbed in cultivated areas (Silva et al., 2013).

The fall in MBC at the time of collection B may be related to the application of herbicide in the MF treatment. The application of herbicide promotes reduction in the quantity and even alters the composition of the SMB (Venzke Filho et al., 2008). Kaschuk et al. (2010) also reported that the application of herbicides in repeated harvests compromised the survival and the development of the microbial biomass and, in most cases, there is a decrease in the efficiency of the use of soil carbon resources; however, these agrochemical effects may be transitory (Silva et al., 2010). This is related to what has been observed by Ferreira et al. (2007), who demonstrated that a summer culture, when fully established, can stimulate the soil microbial community by means of effects on the rhizosphere.

Based on the results, there was direct influence of the SMB on the qCO_2 variations; however, there was an expressive increase in the metabolic activity of the microorganisms after application of herbicide (B), with the occurrence of greater stress in the treatments with mineral fertilization compared to the treatments with poultry litter. This may be related to cultural waste and/or recently-added material to the soil that acts in the aggregation of the OM to the soil particles, becoming a physical barrier protecting the SMB (Six et al., 2006).

Impact produced by soil management promotes deviation of energy from growth and reproduction to cellular maintenance, elevating the qCO_2 values. During stress in the SMB, energy routing occurs for cellular maintenance in the place of growth, so that a proportion of biomass carbon is lost in the form of CO_2 (Araújo and Monteiro, 2007). The results demonstrate that the treatments with poultry litter had a lower adverse effect on the microbial community than those that did not receive it, maintaining greater efficiency in the cycling of the nutrients present in the organic fraction of these materials. With low values of qCO_2, there are lower values of lost carbon.

High values of qCO_2 are indications of greater energy requirement by the SMB and consequent acceleration of the decomposition of the SOM. This could be reflected in a decrease in the stock and the quality of the organic matter (Baretta et al., 2005).

A tendency in the efficient use of total organic carbon was verified in this study. A higher rate of decomposition was observed in the treatments with rice straw in relation to wood shavings, because rice straw has a less lignified composition compared to wood shavings.

The microbial quotient (*q*MIC) has been used as an indicator of the quality of the organic matter present in the soil. This value expresses the efficiency of microbial biomass in the use of C (Baretta et al., 2005). Higher and lower values express accumulation or loss of C, respectively. The authors consider the value of 2.2% to be the level at which the soil presents equilibrium and is

Table 5. Mean of the values composed of four replications at two collection times, demonstrating the behavior of the microbiological parameters of the soil due to the doses of poultry litter used in the treatments.

Treatment	MBC[1]		TOC[2]		*q*MIC[3]		SBR[4]		qCO_2[5]	
	Collection		Collection		Collection		Collection		Collection	
	A*	B**	A	B	A	B	A	B	A	B
MIN	175.503^{aA}	63.809^{dB}	16.359^{bA}	14.995^{aB}	1.072^{aA}	0.431^{cB}	0.457^{cA}	0.719^{aB}	2.611^{eA}	11.356^{aB}
2.5 RS	130.249^{cA}	119.280^{bcA}	18.306^{abA}	14.022^{aB}	0.712^{cA}	0.850^{bB}	0.471^{bcA}	0.221^{cB}	3.613^{cdeA}	1.863^{dB}
5.0 RS	166.684^{abA}	166.184^{aA}	17.722^{abA}	14.411^{aB}	0.943^{abA}	1.157^{aB}	0.653^{abA}	0.366^{cB}	3.903^{bcA}	2.216^{dB}
7.5 RS	159.819^{abA}	139.205^{abB}	18.306^{abA}	14.801^{aB}	0.879^{abcA}	0.947^{bA}	0.606^{bcA}	0.573^{abA}	3.800^{cdA}	4.136^{bcA}
10 RS	143.189^{bcA}	133.714^{bcA}	16.943^{abA}	15.385^{aB}	0.849^{abcA}	0.868^{bA}	0.528^{bcA}	0.540^{bA}	3.696^{cdA}	4.053^{bcA}
2.5 WSH	169.350^{abA}	119.887^{bcB}	17.332^{abA}	14.216^{aB}	0.979^{abA}	0.809^{bB}	0.466^{bcA}	0.347^{cB}	2.759^{deA}	3.024^{cdA}
5.0 WSH	158.039^{abA}	109.552^{cB}	19.280^{aA}	14.411^{aB}	0.823^{bcA}	0.760^{bA}	0.665^{abA}	0.545^{bA}	4.207^{abcA}	4.251^{bcA}
7.5 WSH	171.352^{aA}	120.853^{bcB}	18.306^{abA}	14.995^{aB}	0.936^{abcA}	0.805^{bB}	0.834^{aA}	0.646^{abB}	4.869^{abA}	5.370^{bA}
10 WSH	169.910^{abA}	123.209^{bcB}	16.748^{bA}	14.995^{aB}	1.021^{abA}	0.823^{bA}	0.851^{aA}	0.573^{abB}	5.004^{aA}	4.683^{bcA}

Means followed by the same lower case letter in the columns and means followed by the same upper case letter in the rows do not differ according to the Tukey test (P<0.05). *collection A - Post-poultry litter; **collection B – Mineral fertilizer; [1]microbial biomass carbon of the soil; [2]total organic carbon; [3]microbial quotient; [4]soil basal respiration; [5]metabolic quotient.

better observed in soils with native forest (Wardle and Hungria, 1994; Baretta et al., 2005).

The elevation of this index is a favorable condition that produces an optimized environment for the microbiota of the soil. This may be related to the high continuous and varied input of substrates that influence the rate of decomposition of this material (Cardoso et al., 2009; Silva et al., 2010).

Fluctuations in the *q*MIC values reflect the entry of organic matter in the soil, efficiency of MBC conversion, carbon losses and the stabilization of organic carbon by the mineral fraction of the soil. This relationship indicates if the C is in equilibrium. This carbon is present in the labile part or in the recalcitrant part of the soil. The labile part acts directly in the C cycling and can respond more rapidly to change in the levels of entry and decomposition of organic matter (Balota et al., 1998). According to Silva et al. (2010), low relationships in the *q*MIC present a condition of stress, and under these conditions the rate of C use by the SMB is lower.

Productivity of the silage

The results as regards the productivity parameters of corn for silage high content of CP and EE was presented by the treatment with mineral fertilization with 15.36 g and 6.64 g DM^{-1} (per plant), respectively. However, the treatment with rice straw presented, in the ether extract, a value equal to the MF and WSH treatments. There was no significant difference between the treatments in the other attributes (Table 6).

The calculation of the diet of the animals is based on the percentage of DM present in the silage. Thus, the values of this attribute were among the most indicated for the conservation and production of the corn (28 to 35% of DM) (Cruz and Pereira Filho, 2001).

The higher production with mineral fertilizer compared to poultry litter is connected to the greater solubility of nitrogen (N) and phosphorus (P) in the chemical fertilizers, which results in the their greater initial availability to the plant (Lourenço et al., 2013). In the poultry litter, the water soluble fraction of these elements represents less than 0.25% of their total and the rest is in organic forms that need mineralization so that these nutrients are released and then absorbed by the plants (CQFSRS/SC, 2004).

Going against the literature, the test in this study presented the same percentages of dry matter between the treatments. Hirzel and Walter (2008), working with three treatments (control without any fertilization, treatment with only mineral fertilization and organic fertilization with poultry litter), and using 20 Mg ha^{-1} for three harvests,

Table 6. Mean values of the productivity of dry matter and the corn silage components in the fertilized dry mass with different types of fertilizer.

Type of fertilizer	%DM(%)[2]	DM(Mg ha^{-1})[3]	EE(g)[4]	CP(g)[5]	OM(g)[6]	MM(g)[7]
Mineral	28.23^{a}	13.22^{a}	6.64^{a}	15.36^{a}	230.46^{a}	9.88^{b}
Rice Straw	29.00^{a}	12.60^{a}	6.27^{ab}	14.06^{b}	221.12^{a}	10.89^{a}
Wood Shavings	28.92^{a}	13.88^{a}	5.72^{b}	14.07^{b}	227.06^{a}	10.49^{ab}
cv (%)[1]	7.40	7.40	11.81	8.26	7.59	1022

Means followed by the same letter in the columns do not differ according to the Tukey test ($P<0.05$). [1]coefficient of variation; [2]percentage of dry mass; [3]produtivity of silage in the dry mass; [4]ether extract; [5]crude protein; [6] organic matter; [7]mineral matter.

verified that there was no difference in the productivity of DM in any of the harvests, demonstrating that organic fertilization made nutrients available to plants effectively. In addition, the response is due to the larger quantity of nutrients available in the treatments with poultry litter (Cesarino, 2006; Graciano et al., 2006).

The following are advantages of the use of wastes of animal origin: improvement in the physical and chemical properties of the soil and in the supply of nutrients; increase in organic matter content, thus improving the infiltration of water and increasing the cation exchange capacity. Fertilization with poultry litter was shown to be an important alternative to the producer in unfavorable climatic situations in the production of silage, considering the benefits that organic fertilization brings to the soil (Hoffman et al., 2001).

The decomposition velocity of wastes is influenced by their chemical characteristics and the environment where they are found. This difference in the decomposition time of manure in relation to cellulosic materials assures a continuous flow of nutrients in the soil and determines a slow and prolonged rate of release of the nutrients that are made available to the plants (Costa et al., 2009). As regards the OM component, the type of fertilizer did not influence the response, because all of the treatments were similar. As for mineral matter, the values were also similar, with higher values in the treatments with organic fertilization. This was also reported by Cesarino (2006), who observed high values of ashes for the treatments with organic wastes. They are more diluted in the plants probably due to these wastes.

The effects on the productivity of the silage, taking into consideration the sources and the doses of fertilization, are described in Table 5. There were no differences in the productivity results in percentage and per area of DM.

The different treatments did not influence the ether extract values of the plant. All of them were similar to the treatments that received only mineral fertilization; whereas, for the fertilization with wood shavings, the 10 WSH treatment had the highest content of this element (7.25 g DM^{-1}). On the other hand, the 2.5 WSH treatment presented the lowest content (4.35 g DM^{-1}). The ether extract value for rice straw in the 7.5 RS treatment was 7.21 g DM^{-1}. The highest content in grams of protein in the plant was observed in the 7.5 RS treatment (15.58 g DM^{-1}) and the lowest was found in the 5.0 RS treatment (12.27 g DM^{-1}). The other treatments did not present differences between the treatments (Table 7).

In the several studies carried out using mineral and organic fertilization, all obtained the same results. Gomes et al. (2005) and Konzen and Alvarenga (2003) did not observe differences in the yield of the corn culture with the chemical fertilizer and organic compound association at different doses, demonstrating that the nutrients, mainly the nitrogen that is found protected in the organic fertilizers (thus avoiding losses by volatilization), are effective in the fertilization of large cultures. Results were obtained in the study of productivity of diverse foragers like Marandú, Tanzania and Tifton-85 (Menezes et al., 2009).

Graciano et al. (2006), working with the production and analyses of components of arracacha (Peruvian carrot) in dystrophic RED LATOSOL, observed high contents of proteins, lipids, fibers and proteins of plants cultivated in soil with poultry litter fertilization (superior to mineral fertilization). The high yield in the production of corn by organic fertilization may be related to elevation of the dose of manure (animal excrement) (Silva et al., 2004; Cesarino, 2006). In the present study, the same result was observed in the treatment with fertilization with wood shavings, however, in 10.0 Mg ha^{-1} of this compound, the same yield was not observed.

The crude protein content in the foliar tissue is directly related to the nitrogen content, which is, on average, 16% of the nitrogen in the crude protein. Thus, its value depends on the extraction of nitrogen by the plant. Grasses generally extract elevated quantities of nitrogen and their response varies depending on the time of year, species, nutrient source, soil fertility and development stage of the plant (Vielmo, 2008).

According to the results obtained by Colussi (2013), who researched the development of the grass Tifton 85 using poultry litter as a source of fertilization in a series of four cuts, observed that the protein was superior in the third and fourth cuts, demonstrating the slower cycling of the element. In contrast, it was shown to be effective in the exchange of mineral fertilization for organic.

Organic matter and mineral matter did not differ

Table 7. Mean values of the dry mass productivity and the fertilized corn silage components with different sources and doses of poultry litter.

Treatment	%DM(%)[2]	DM(Mg ha^{-1})[3]	PLANT DM(g)[4]	EE(g)[5]	CP(g)[6]	OM(g)[7]	MM(g)[8]
MF [9]	28.23[a]	16.15[a]	240.35[a]	6.65[a]	15.36[ab]	230.47[a]	9.88[a]
2.5 RS	28.91[a]	14.94[a]	221.78[a]	5.96[ab]	13.59[ab]	210.86[a]	10.92[a]
5.0 RS	28.31[a]	14.63[a]	219.25[a]	5.92[ab]	12.27[b]	209.10[a]	10.15[a]
7.5 RS	30.28[a]	16.25[a]	249.66[a]	7.21[a]	15.58[a]	239.13[a]	10.53[a]
10 RS	28.51[a]	16.01[a]	241.49[a]	6.02[ab]	14.83[ab]	229.50[a]	11.98[a]
2.5 WSH	28.38[a]	15.56[a]	224.93[a]	4.35[b]	12.74[ab]	215.13[a]	9.80[a]
5.0 WSH	28.61[a]	15.67[a]	231.70[a]	5.36[ab]	13.94[ab]	221.64[a]	10.06[a]
7.5 WSH	29.01[a]	15.75[a]	241.52[a]	5.94[ab]	14.96[ab]	230.61[a]	10.91[a]
10 WSH	29.69[a]	17.37[a]	252.08[a]	7.25[a]	14.68[ab]	240.90[a]	11.19[a]
cv (%)[1]	8.22	11.97	10.17	13.59	9.27	8.46	9.40

Means followed by the same letter in the columns do not differ according to the Tukey test ($P<0.05$). [1]coefficient of variation; [2]percentage of dry matter; [3]dry matter; [4]dry mass of each plant; [5]ether extract; [6]crude protein; [7]organic matter; [8]mineral matter, [9]mineral fertilization.

between the treatments tested. According to Sbardelotto and Cassol (2009), the absence of significant response in the treatments is due to the soil presenting high initial fertility.

Conclusions

Fertilization with poultry litter stimulated microbial activity in the soil, promoting greater mineralization and solubilization of organic matter nutrients, inducing better use of soil C. This was reflected in the production of corn silage. A 2/3 reduction of the mineral fertilization in the use of organic fertilization can be proposed, without loss in plant quality or production.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of climate variability on the choices of livelihood among farm households in Anambra State, Nigeria

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Climate variability has detrimental effects on the livelihoods of rural people who depend on agriculture. The situation becomes critical because of the significant contributions of agriculture to the economic and social well being of the rural people. The effects of climate variability could manifest in declining agricultural productivity and competitiveness, greater risks to human health, increased unemployment and poverty, declined food security and conflicts of resource use. The study examined the determinants of farmers' choices of livelihoods and perceptions of the effects of climate variability on choices of livelihoods in Anambra State, Nigeria. Data for the study were collected using structured questionnaire administered to 160 respondents drawn from four agricultural zones in the State. Data were analyzed using frequency, mean and Multinomial Logit Model (MNL). Results showed that household income, gender, marital status, household size, education level of household head and farm size were the major determinants of farmers' choices of livelihoods. Gender, education level and household income had a positive significant influence while marital status, farm size, and household size had a negative significant influence on the choices of livelihoods. Farm households perceived increase in precipitation; temperature; and rate of erosion; as well as decrease in agricultural yield as effects of climate variability. It is suggested that extension personnel should be trained and motivated in order to disseminate relevant information to farmers on how to diversify their livelihood in order to cope with climate variability.

Key words: Agriculture, farmers, precipitation, temperature, climate variability threat.

INTRODUCTION

Africa is vulnerable to impacts of climate change and variability (Lobell et al., 2011), although the area contributes only <3% of the world's total greenhouse gas emission (IPCC, 2007). Climate variability has been considerably impeded Africa's development and even as it is expected that climate variability will increase and climate extremes will become more intense or more frequent (DFID, 2004). According to Antwi-Agyei et al. (2014), climate variability poses a significant threat to many sectors of sub-Saharan Africa's economy and

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agricultural sector being dependent on rain fed cultivation is most sensitive to climate variability. Climate variability refers to a measure of the frequency of changes in the values of climate variables and their range over a given time period. Temperature and precipitation are the climate variables most critical to measure with regard to food systems, because not only does the range between high and low value matter, but also the frequency at which these extremes occur and intensity of the event (Zierovogel et al., 2006). Climate stress is exemplified in the presence of year to year variability, seasonality, uncertainty and patchiness of rainfall and extreme events such as droughts and flooding (McCarthy et al., 2001). According to IPCC (1990, 1997), anthropogenic green-house gas emissions are significantly altering the earth's climate. It is predicted from the study that mean annual surface temperature will increase by 1 to 5˚C by year 2100 and that the global mean sea level will rise by 15 to 95 cm with consequent changes in the spatial and temporal patterns of precipitation. In Sub-saharan African because of the deterioration in agricultural production due to climate variation, many households look for livelihood choices other than purely crop production and animal production. Livelihood choices are those employment options that the farm households can engage in so as to provide for their needs. Households engage in farm, and non-farm (non-agricultural) livelihood activities such as crop production, animal rearing, petty trading in order to generate additional income for survival and cope with this harsh and difficult environment (Gebru and Beyene, 2013; Kalinda and Langyintuo, 2014). Livelihood activities of the households' are related to their endowment of social, human, financial, physical and natural assets (Nkoya et al., 2004).

Some studies have shown the potential impacts of climate variability on agriculture in developing countries. Downing (1992) asserted that changes in global climate variables may present a precarious future for the households dependent on agriculture for their livelihoods because of shifts in temperature and precipitation. According to the study, climate variability could markedly affect income from agricultural production, increase costs to consumers and could also lead to scarcity. According to Eboh (2009), the effects of climate variability on agriculture are projected to manifest through changes in land and water regimes, specifically changes in the frequency and intensity of drought, flooding, water shortages, worsening soil condition, desertification, diseases and pest outbreaks on crops and livestock. Yields of some major staples such as maize, groundnut, millet, sorghum and cassava have been projected to decrease by 7 to 27% in parts of Sub-Saharan Africa by 2050 due to climate change and variability (Schlenker and Lobell, 2010). Zierovogel et al. (2006), worked on food security, climate variability and climate change in Sub Saharan West Africa. From the study, it was showed that crop yield is sensitive to variability in the time of onset and cessation of the rainy or growing season. Egbe et al. (2014) studied the rural peoples' perception of climate variability in Cross River State, Nigeria. However, the study did not cover the effects of climate variability on livelihood of farmers, though it is a known that increasing poverty in rural areas has a link with climate variability. Climate variability has been projected as a hindrance to achieving the Millennium Development Goal of halving the proportion of hungry people and improving the food security of the populace. In order to ameliorate the effects of climate variability, there is need to study its effects on the livelihood of farm households. This is especially important in south east Nigeria especially in Anambra State where climate variability has shown tremendous and visible impacts.

In Nigeria, it is a well known fact that climate has varied in time and space, and that it will continue to vary in future (Ojo, 1987). In Southeast Nigeria, droughts have been relatively less persistent, while rainfall is observed to be increasing and temperature increases and reduces moderately over the years compared with Northern Nigeria (Okorie et al., 2012). In Northeastern Nigeria, drought caused death of many animals and about 60% drop in crop yield (IPCC, 2007). In Oyo, Southwestern Nigeria, flooding caused 30 deaths and displaced nearly 2000 people (Nigeria Metrological Agency, 2008). In Anambra State, farmers depend on the natural environment for their livelihood due to poverty and paucity of resources. According to Nwalieji and Uzuegbunam (2012), in 2012, the rice farmers in the state suffered reduction in crop yield and grain quality, reduction of farm land by flood, high incidence of weeds, pests and diseases, decrease in soil fertility and the surge of human diseases such as meningitis, malaria and cholera. Extreme variation in climate variables has made these farmers vulnerable and helpless (Anayo, 2010). About 40% of the land areas in the State are severely gullied, while 27.8% are mildly gullied. The state accounts for 65% of gully erosion in Nigeria and there are over 780 active gully erosion sites in the State (Chinweze et al., 2013). The State was seriously affected by 2012 flooding in Nigeria. There were cases of displacement of communities, loss of rivers, loss of farmland, destruction of high ways, link roads and infrastructure in Nanka, Obosi, Ekwulobia and Abatete all in Anambra State. Huge amounts of money set aside for other purposes were used to ameliorate the effect of the natural disaster. These changes in the environment affected the composition of rural livelihoods through their impacts on agricultural production and income. This paper therefore analyzes the effects of climate variability on the choices of livelihood among farm households in Anambra State, Nigeria. The paper focused on:

(1) The identification of common livelihood choices of the farm households in the study area;
(2) The determination of the factors that influenced the

choices of livelihoods among the farm households in the study area;

(3) Ascertaining the perception of the farm household of the effects of climate variability on choices of livelihoods in the area.

MATERIALS AND METHODS

Study area and sampling

The study was conducted in Anambra State of Nigeria, with a population of 4.182032 million people (NPC, 2006). The State has a land area of about 4,415.54 square kilometers and lies between latitudes 5° 40' and 6° 48' North and longitudes 6° 35' and 7° 50' East. The State is bounded by Delta State to the West, Imo State to the South, Enugu State to the East and Kogi State to the North (Anambra State Government, 2007).

The State has twenty one Local government areas (LGAs) that are grouped into four agricultural zones namely: Awka, Onitsha, Anambra and Aguata. About 60 percent of the population is engaged in agricultural production such as food crops, tree crops, livestock and fisheries (Anambra State Government, 2007). Crops widely grown are yams, cocoyam, maize, okra, potatoes and amarathus. Tree crops grown include oil palm (*Elaeis guineensis*), mangoes (*Mangifera indica*), avocado pear (*Persea americana*), oil bean (*Pentaclethra macrophylla*) and paw-paw (*Carica papaya*) (Uguru, 1996).

Multi-stage sampling techniques were employed in the study. First, two LGAs most prone to climate variability were purposively selected from each of the four agricultural zones respectively, giving a total of eight LGAs, and these LGAs include Njikoka, Aniocha, Ogbaru, Idemili North, Anambra East, Ayamelum, Orumba North and Nnewi South . Second stage, two communities were randomly selected from each of the eight LGAs respectively making a total of 16 communities namely: Enugwu-Ukwu, Enugwu-Agidi, Agulu, Agu-Ukwu, Atani, Odekpe, Abatete, Eziowelle, Aguleri, Umueri, Ifiteogwari, Anaku, Nnaka, Oko, Ukpor and Osumenyi. In the last stage, 10 farm households were then randomly selected from each of the 16 communities giving a total of 160 respondents for the study.

Data collection and analysis

Data were obtained mainly from primary sources using structured questionnaire and interview schedule. The data focused on: socio-economic characteristics, choices of livelihood such as crop production, fishing, livestock production, Agro forestry and non-agricultural occupation, climatic variables including mean temperature and precipitation level within a period of one year. Climate data were obtained from the Nigerian Meteorological Agency (NIMET) database.

The data were used to identify the most common livelihood choices, the factors that influenced the choices of the livelihoods and ascertain the perception of the farm households of the effects of climate variability on choices of livelihoods. Descriptive statistics, such as mean, frequency distribution and likert type scale rating and multinomial logit model were used to realize the objectives. Multinomial logit has been employed in climate change studies by several authors. For instance, Kurukulasuriya and Mendelsohn (2006) used the multinomial logit model to see if crop choice by farmers is climate sensitive. Deressa et al. (2009) also employed Multinomial logit model to analyze factors that affect the choice of adaptation methods in the Nile basin of Ethopia.

Model

The multinomial logit was used in this study because of the various response categories. The livelihood choices were grouped into four categories, category 1, if the farm household chose crop production; category 2, if fishing was chosen; category 3, if livestock production was chosen and category 4 if the major livelihood choice was from agro-forest resources. The multinomial logit model was estimated with set of coefficients $\beta^{(1)}, \beta^{(2)}, \beta^{(3)}$ and $\beta^{(4)}$ as follows:

$$\Pr(Z=1) = \frac{e^{x\beta(1)}}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)}\ e^{x\beta 4}} \quad (1)$$

$$\Pr(Z=2) = \frac{e^{x\beta(2)}}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)} + e^{x\beta(4)}} \quad (2)$$

$$\Pr(Z=3) = \frac{e^{x\beta(3)}}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)} + e^{x\beta(4)}} \quad (3)$$

$$\Pr(z=4) = \frac{e^{x\beta(4)}}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)} + e^{x\beta(4)}} \quad (4)$$

To identify the model, one of the $\beta^{(1)}, \beta^{(2)}, \beta^{(3)}$ *and* $\beta^{(4)}$ was arbitrarily set to 0. When $\beta^{(4)}$ was arbitrarily set = 0, the remaining coefficients $\beta^{(1)}, \beta^{(2)}, \beta^{(3)}$ measured the change relative to the Z =4. The socio-economic characteristics of the farm household constituted the explanatory variables. By implication, after estimating the parameters, one can predict the probability that a sampled household with a specified set of socio-economic characteristics may chose crop production, fishing, livestock production or agro-forestry as their choice of livelihood relative to non-agricultural occupations such as trading.

Therefore, using four category response as in the model for this study and setting $\beta^{(4)}$ = 0, the equation becomes

$$\Pr(Z=1) = \frac{e^{x\beta(1)}}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)}} \quad (5)$$

$$\Pr(Z=2) = \frac{e^{x\beta(2)}}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)}} \quad (6)$$

$$\Pr(Z=3) = \frac{e^{x\beta(3)}}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)}} \quad (7)$$

$$\Pr(Z=4) = \frac{1}{e^{x\beta(1)} + e^{x\beta(2)} + e^{x\beta(3)} + e^{x\beta(4)}} \quad (8)$$

The relative probability of Z = 1 to the base category is

$$\frac{pr(Z=1)}{pr(Z=4)} = e^{x\beta(1)} \quad (9)$$

This is called the relative likelihood and X and $\beta_k^{(1)}$ are vectors and are equal to (X1, X2,…, Xn) and $\beta_1^{(1)}, \beta_2^{(1)}, \beta_3 k^{(1)}$ respectively, the ratio of relative likelihood for one unit change in X1 relative to the base category is then stated as:

Table 1. Frequency distribution of the respondents according to choices of livelihoods

Livelihood Choices	Number of respondents	Percentage
Crop production	80	50.0*
Fishing	13	8.13
Livestock production	58	36.3
Agro forestry	9	5.57
Non-agricultural occupation	18	11.25*

* = Multiple responses were recorded, Source: Field survey, 2014.

$$\frac{\ell^{\beta_1^{(1)}} x_{1+ \ldots + \beta_1^{(1)} (x_{1+}1) + \ldots + \beta_k^{(1)} xk}}{\ell^{\beta_1^{(1)}} x_{1+ \ldots + \beta_1^{(1)} x_1 + \ldots + \beta_k^{(1)} xk}} \quad (10)$$

Enete (2003) citing StataCorp (1999) reported that, the exponential value of a coefficient is the relative likelihood ratio for one unit change in the corresponding variable. As pointed out, the dependent variable "choices of livelihood" have four (4) possible values; value 1, 2, 3 and 4 if it is crop production, fishing, livestock production and agro-forestry respectively.

X1 = Age of households head (in years)
X2 = Gender of the household head (if male 1; 0 if female)
X3 = Marital status (married 1, otherwise 0)
X4 = Household size (number of individual in the family.)
X5 = Education of household head (years)
X6 = Farming experience (in years)
X7 = Access to credit (Access = 1, 0 otherwise)
X8 = Household income (In Naira)
X9 =Farm size (in hectares)
X10 = Membership of farmers organization (if any 1, otherwise 0)
X11 = Precipitation (Annual mean precipitation level in mm)
X 12= Temperature (Average temperature of the area in degree celcius)

In addition, a 4 point likert type scale rating of "very severe, severe, moderate and no effect" was also used to ascertain the perception of climate effects among the farm households. The mean was 2.5 and the interval scale was 0.05. Mean score above 2.55 was considered very severe while below 2.45 was considered moderate and between 2.45 and 2.55 were considered severe.

RESULTS AND DISCUSSION

Livelihood choices of the respondents

People make livelihood choices according to the level of their household assets or availability of infrastructure in their community (Gebru and Beyene, 2012). The frequency distribution of respondents according to their choices of livelihood is shown in Table 1. The Table shows that 50% (half) of the respondents chose crop production as their major source of livelihood, 36.3% chose livestock production, while 8.13, 5.57 and 11.25% of the respondents chose fishing, agro forestry and non- agricultural livelihood respectively. The communities sampled had very limited livelihood options as most of them indicated to have little or no significant secondary livelihood sources. The implication is that the communities will have reduced resilience to the effects of climate variability due to lack of wide range of livelihood options. This is line with the work done by Oni and Fashogbon (2013) which showed that in Nigeria that farming is the predominant livelihood activity. In addition, in Ogun state, Nigeria, majority of farm households engage in fishing and fishing related activities as their occupations (Olawuyi and Rahji, 2012). However, it is evident that rural households in Nigeria engage in multiple livelihood activities such as trading, small scale business enterprises and processing of agricultural goods and arts and craft in order to supplement earnings from agriculture (Matthews-Njoku et al., 2007; Ekong, 2003; Adepoju and Obayelu, 2013).

Factors influencing Choices of livelihood among the respondents

Table 2 summarizes the multinomial logistic regression analysis of the socio-economic factors that influenced livelihood choices adopted by the respondents. The base category in the model is $\beta^{(4)}$ and the model was estimated with maximum likelihood procedure. The Chi square result was highly significant (p< 0.0000), suggesting that the model has a strong explanatory power. The pseudo R^2 was 27.85%, thus confirming households' choice decision making process could be attributed to fitted covariates. In terms of consistency with *a priori* expectations on the relationship between the dependent and the explanatory variables, the model appeared to have performed well.

Gender coefficient was positive and significantly (p< 0.01) related to the probability of the male headed household choosing fishing as a major livelihood source as compared to crop production. This implies that male headed households are more likely to choose fish production as a livelihood option while the female headed households are more likely to choose crop production. Also in a traditional African society, serious fishing activities are always done by male folks. It is also believed that male headed households have ready access to information about new technologies and may

Table 2. Multinomial logit regression results of factors influencing the choices of livelihoods among the respondents in the area.

Variables	Fishing (2)	Livestock production (3)	Agro forestry (4)
Gender	2.5289*** (0.9525)	0.3314 (0.4624)	-1.0216 (0.9030)
Age	0.0057 (.0473)	0.0460 (0.0342)	-0.0342 (0.0633)
Marital status	-3.1588** (1.3391)	-1.9673** (0.8848)	14.0730 (1176.587)
Household size	-0.2146 (0.1875)	-0.2577** (0.1177)	-0.6769** (0.2636)
Education	-0.6869 (0.0789)	0.1245** (0.0613)	-0.0916 (0.0954)
Farm size	-1.2944*** (0.3856)	-0.0808*** (0.2624)	-0.5983 (0.4424)
Farming Exp	0.1068 (0.0673)	-0.0016 (0.0471)	0.1026 (0.0771)
Household income	0.0001*** (3.05e-06)	6.91e-06*** (2.13e-06)	6.95e-06*** (3.28e-06)
Credit access	-0.7762 (0.6938)	-0.2479 (0.4375)	0.7176 (0.8862)
Membership of org	1.2527 (0.9530)	-0.1816 (0.4911)	0.2367 (1.0154)
Precipitation	-0.0015 (0.0023)	-0.0033 (0.0019)	0.0007 (0.0032)
Temperature	-0.5528 (0.4021)	0.3014 (0.2409)	-0.1508 (0.5669)
Intercept	12.9375 (9.8807)	-2.4041 (6.6004)	-10.1960 (1176.656)

Statistics: chi^2 (36) = 98.54, prop > ch^2 = 0.0000; Pseudo - R^2 = 0.2785; number of observation= 160. Note: (1) Crop production is the comparison category. The figures in parenthesis are standard errors. ***p ≤ 0.01; **0.01 < p ≤ 0.05.4.4, Source: Field survey, 2014.

not be confronted with traditional social barriers as in the case of female headed households. Hence, they make their livelihood choices more freely than their female counterparts (Asfaw and Admassie, 2004).

Age coefficient was not statistically significant in all the livelihood options, but was positively related to the probability that the household will choose fishing or livestock production and negatively related to the probability of the household choosing agro forestry as a livelihood strategy as compared to crop production. This could mean that agro forest might be far from home, hence older household heads may not have the strength to trek to forest for their livelihoods. This agrees with the findings of Jacobs (2000) in which older household heads left the tedious jobs to the younger ones and adopted the easier jobs.

The coefficient of marital status was negatively and significantly (p<0.05) related to the probability of the household choosing fishing and livestock production as their major sources of livelihood in comparison with crop production. However, marital status was positively related to the probability that the household head will choose agro forestry production. Implying that married household heads could have bigger household size which could mean more family labour for crop production and agro forest activities.

Household size was negative and significantly (p<0.05) related to the probability that the household chooses livestock production or agro forestry as their major sources of livelihood in comparison with crop production. This means that households with bigger sizes are more likely to choose crop production as their major source of livelihood. This could be because bigger household sizes mean more available family labour for crop production activities (Okon and Enete, 2009). This finding is also in line with that of Hassan and Nhemachena (2008), who observed that household with bigger sizes were more likely to choose crop production as their choice of livelihood.

Educational level of the household head was positive and significantly related to the likelihood of the household head choosing livestock production in comparison with crop production. This implies that educated household heads are more likely to practice livestock production in comparison with crop production. Education is expected to impact positively on farmer's decision making, since educated households are expected to be more informed and knowledgeable on the best livelihood choices to make in combating the effect of climate variability. This finding is in line with that of Birkmann and Fernando (2008), who noted that education and skills up grading are powerful adaptive strategies for individual families and communities. In addition, Adi (2007) identified education as one of the determinants of livelihood choice in Eastern Nigeria.

Farm size had a negative and significant (p<0.01) relationship with the probability that the household chooses fishing or livestock production as their major source of livelihood as compared to crop production. The implication of this finding is that households with large land size are more likely to choose crop production as their major source of livelihood.

Farming experience was positive and not statistically significant in fishing and forestry, but was negative in livestock production, compared to crop production. This could mean that households with more years of experience could chose fishing and agro forestry as their major sources of livelihood.

Household income was positive and statistically significantly ($p<0.01$) in all choices of livelihood. This is to be expected because income is the major determinants of livelihood options. There is every tendency of the household choosing a livelihood source that will generate more income in other not to be crushed by the depressed economic situation. More income got from a livelihood source, the greater the probability of a household choosing it as their major livelihood option. This finding is in line with Kinsella et al. (2000), who observed that financial resources such as cash, credit and other economic assets are essential for pursuit of livelihood strategies.

Credit access was not statistically significant in all the livelihood choices but was positive in agro forestry option and negatively signed in both fishing and livestock production option as compared to crop production. This could mean that household heads that had access to credit facilities most likely chose agro forestry production as their major livelihood choice.

Membership of farmer's organization was not statistically significant and was positively signed in both fishery and agro forestry as livelihood choices, but negatively signed in livestock production. The implication of this finding is that household heads that are members of farmer's organization are more likely to choose fishing and agro forestry production as their major livelihood sources.

The coefficient of precipitation was not statistically significant and was positive in agro forestry as a livelihood source but negatively signed in both fishing and livestock production as livelihood choices in comparison with crop production. This could mean that increase in precipitation will more likely increase the probability of the household heads choosing agro forestry production as their major livelihood source. Also, increase in precipitation will decrease the probability of the household choosing fishing or livestock production as their major source of livelihood.

Temperature was not statistically significant but was negatively related to the probability of the household heads choosing fishing and agro forestry as livelihood options. It was however, positively related to the probability of the household heads choosing livestock production as compared to crop production.

The perceptions of the effects of climate variability on choices of livelihoods by the respondent

In Table 3 the overall mean value (Summation across the 20 items) on the perception of the farm household on the effects of climate variability on choices of livelihood in the study area was 3.06 and the standard deviation was 0.864. The overall perception on the effects of climate variability on choices of livelihood shows that the farm household in all the communities sampled perceived the effects of these elements of climate variability which had adverse effects on their choices of livelihood. This finding is in line with Jallow et al. (1999), who noted that climate variability through sea level, storm and flood frequency, impact on the physical capital of the households or of entire communities, leading not only to decrease harvesting capacity but also to disrupting of public infrastructure and services that support livelihood. Gworgwor (2008) stated that the uncertainty on the magnitude of change make awareness imminent at all level. He also suggested that the present solution to man's survival on the earth's environment sustainably hinge on the option of knowledge of climate variability and adopting mitigation and adaptation measures as widely recognized as vital components or approaches to reducing climate variability. The table observed that fourteen (14) out of twenty (20) effects of climate variability perceived by the farm households were above 2.55 indicating very severe (VS) (with a mean score of 3.18-3.49). These perception on the effects of climate variability that were assessed on four likert scale include: increase of precipitation (3.49), increase in temperature (3.48), decrease in soil fertility (3.18), loss of crop due to flood (3.36), loss of income (3.43), increase of pest and disease (3.30), depletion of household assets (3.41), increase in rate of erosion (3.39) poor supply in market (3.23), decrease in agricultural yield (3.44), land degradation (3.26), high food price (3.47), loss of infrastructure (3.31) and poverty (3.43). The table also shows that only two perceptions on effects of climate variability on choices of livelihood were recorded severe (S) by the respondent. These were migration (2.46) and lack of access to the market (2.48).

This finding was similar to Okorie et al. (2012) who noted that in the southeast state, drought have been relatively less persistent, while rainfall is observed to be increasing and temperature increases and reduces moderately over the year compare with others states in northern part of the country. In addition, in South Africa, Gbetibouo (2008), 91% of the farmers surveyed perceived an increase in temperature over the past 20 years. In contrary, Apata (2011), noted that in southwest, Nigeria that 58% of the investigated farmers perceived decreasing rainfall over the past 10 years.

Conclusion

Conclusively, household income, gender, marital status, household size, educational level and farm size were the major determinants of households' choice of livelihood sources in the study area. Households with large land sizes chose crop production as their major livelihood choice. However, male headed households especially in riverine areas chose fishing as their choice of livelihood, perhaps because they had no access to land. Educated

Table 3. Mean Ratings of the perception of the effects of climate variability on the choices of livelihoods by the respondent. (N=160).

S/N	Perception on the effects of climate variability on the choices of livelihood.	$\overline{X}$	Std.Dev
1	Increase in precipitation	3.49***	0.604
2	Decrease in precipitation	2.34*	1.263
3	Increase in temperature	3.48***	0.582
4	Decrease in temperature	2.09*	1.045
5	Decrease in soil fertility	3.18***	1.007
6	Loss of crop due to flood	3.36***	0.740
7	Loss of income	3.43***	0.749
8	Increase in frequency of drought	2.28*	1.309
9	Increase of pest and disease	3.30***	0.725
10	Migration	2.46**	1.033
11	Depletion of household assets	3.41***	0.873
12	Increase in rate of erosion	3.39***	0.691
13	Poor supply in the market	3.23***	0.833
14	Decrease in agricultural yield	3.44***	0.670
15	Land degradation	3.26***	0.820
16	High food price	3.47***	0.624
17	Changing from farming to non-farming activities	2.36*	1.174
18	Loss of infrastructure such as school, road &hospital	3.31***	0.663
19	Poverty	3.43***	0.650
20	Lack of access to the market	2.48**	1.233
	Mean (Overall)	3.06	0.864

***Very severe (SV), **Severe(S), * Moderate (M), Source: field survey, 2014.

household heads chose livestock production as their major livelihood choices. Gender, education level and household income had a positive and significant influence while marital status, farm size, and household size had a negative but significant influence on the choices of livelihoods. The farm household equally perceived increase in precipitation, increase in temperature, decrease in soil fertility, loss of crop due to flood, loss of income, increase in pest and disease, depletion of household assets, increase in rate of erosion, decrease in agricultural yield, poverty, high food price as very severe effects of climate variability while decrease in temperature, decrease in precipitation, increase in frequency of drought were moderate on their choice of livelihood. Based on the findings of this study, the extension personnel should be trained and motivated in order to disseminate relevant information to farmers on how to diversify their livelihood in order to cope with climate variability.

Conflict of Interest

The authors have not declared any conflict of interest.

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