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Full Length Research Paper

Determination of the genus *Meloidogyne* species and study of their impact on the market gardening in the area of Bamako, Mali

Boubacar K. Touré^{1*}, Mohamed S. Maïga¹, Doulaye Dembélé² and Hammache Miloud³

¹Faculty of Sciences and Technics, USTTB, Bamako, Mali.

²IGBMC, CNRS UMR7104 - INSERM U1258 - University of Strasbourg, 1 rue Laurent Fries, 67400 Illkirch, France.

³Higher National Agricultural School, Kasdi Merbah, Algeria.

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The development of market gardening is faced with many problems among which there are the nematodes of the genus *Meloidogyne*. The aim of this study is twofold: (a) determining the *Meloidogyne* species that are crowned by the perineal plates of female and (b) studying the impact of the multiplication of these nematodes on the tomato cv Roma (*Lycopersicon esculentum* L). Root samples were collected at five sites around Bamako, Mali. In a trial, nematode larvae were extracted from the roots of culture and then inoculated on tomato plants. A second tomato plants non-inoculated was used as control. Using a maceration-filtration method, three *Meloidogyne* spp. were identified: *Meloidogyne arenaria*, *Meloidogyne javanica* and *Meloidogyne incognita*. All of the inoculated plants reacted by the presence of galls that were not observed on the non-inoculated plants.

Key words: Market gardening, genus *Meloidogyne*, galls, *Lycopersicon esculentum*, perineal plate, roots of culture, root samples.

INTRODUCTION

Market gardening for several reasons occupies a place of choice among irrigated crops. Among these reasons is their appreciable contribution to food self-sufficiency, the increase of the income of the farmers and especially of the women and young people who maintain them. Vegetable production in Mali has increased considerably in recent years to 1 900 173 tons on an area of 173 110 ha (Ministry of Agriculture of Mali, 2018). This production allows the farmers to diversify as well as to improve their diet thanks to the contribution of vitamins and mineral

salts, it ensures besides an increase of the monetary income of the actors. According to the results of a survey of the Planning and Statistics Unit of the Rural Development Sector of the Ministry of Agriculture of Mali (2016), the city of Bamako consumes about 22,932 tons of vegetables per year.

The development of vegetable crops is faced with many problems among which there is the scarcity of water and parasites. Of the parasites, nematodes are the most important group after insects. They cause a lot of

*Corresponding author. E-mail: boubakola@gmail.com. Tel: (00223) 75412629.

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damage resulting in lower yields (Nadine, 2015). Mokrini and Sbaghi (2017) reports that in nematode populations, species of the genus *Meloidogyne* are best known for their pathogenicity in vegetable crops. They are by far the most formidable, polyphagous that attack the majority of crops resulting in a significant decline in yield. This crop loss has been estimated globally at 14% per year (Groover and Lawrence, 2018), 15-25% or even 75% in some cases according to Jiang et al. (2018) great power of multiplication allowing them to quickly invade the roots of the plants on which they cause galls. In terms of money, Phani et al. (2017) have estimated the damage caused by these nematodes globally to 173 million Dollars by year. These parameters are currently posing serious problems for the market garden sites visited in Bamako and Ségou (Touré, 2017). The most sensitive crops are Solanaceae (tomatoes, eggplant, potato), Cucurbitaceae (melon, cucumber), Legumes (beans), Umbellifera (carrots, celery ...), Compounds (lettuce). In the present study, on one hand, *Meloidogyne* species on vegetable crops were characterized by perineal plaques and on the other hand their pathogenicity on tomato variety Roma (*Lycopersicon esculentum*) evaluated.

MATERIALS AND METHODS

Sites and sampling

Five permanent market gardening sites located near the Niger River were chosen for the collection of root samples: Samanko (12°31'41N; 08°04'92W), Sotuba (12°39'72N; 07°56'72W), Tiébani (12°32'80N; 08°02'34W), Daoudabougou (12°36'87N; 07°58'59W), and Baguinéda (12°37'97N; 07°47'50W). These sites were selected to be representative as possible of those used around the Bamako city, Mali (Figure 1). A systematic sampling was performed using a spreader. The collection of root samples took place on vegetable crops (tomato, eggplant, okra, lettuce, onion...). The roots collected were immediately placed in plastic bags marked with the site and crop names, and the date. They were then placed in a cooler to protect them from solar rays that can kill nematodes very quickly (Coyne et al., 2007). In the laboratory, the samples were kept in a refrigerator for analysis within two weeks at the latest.

Extraction and observation of the *Meloidogyne*

Extraction of larvae

Nematodes were extracted using the maceration-filtration method (Coyne et al., 2007). In brief, the roots were first gently washed under a trickle of tap water to clear the soil and much of the saprophagous nematodes. They are then cut into small pieces using a securateurs or a knife. The root pieces are then disinfected by soaking in a solution of bleach (1% active chlorine) for 4 to 5 min to lighten them.

They are then rinsed with tap water for 15 min, followed by maceration in a kitchen blender. The ground material is passed through a series of three sieves having holes diameter 150, 75 and 38 µm, respectively. This last sieve is used alone and tilted at about 45 degrees to maximize the retention of nematodes. The filtrate collected by the last sieve should contain larvae and nematode

eggs. In addition to this maceration-filtration technique, the Baermann technique (Tessier, 2010) was also used to extract root nematodes. The macerated roots were placed on small Baermann type sieves. The extraction of the eggs is done under stereoscope. In brief, the roots were disinfected with 1% bleach for 1 min and placed in a petri dish. Using a scalpel and a lanceolate needle, the gall was opened superficially. The eggs in mucus at the posterior end of the female under the bark is then torn off and placed on an optical microscope slide for observation.

Identification of *Meloidogyne* spp.

The fixation of the nematodes was made on roots carrying galls in good condition, by the method of sodium hypochlorite-fuchsin acid (Phani et al., 2017 ; Fayzia et al., 2018). The roots were cut into 2 cm fragments. These pieces of roots were then lightened with bleach for 4 min and then rinsed with tap water for 15 min to remove the after-effects of the bleach. They must be boiled for 30 s in 30 ml of distilled water plus 1 ml of a stock solution of fuchsin acid (0.35 g fuchsin acid, 25 ml acetic acid, 75 ml distilled water) and then refreshed for 30 min at room temperature. Then the roots were discolored in an acidified glycerol solution by adding 6 drops of nitric acid and boiled. The discolored roots will be freed of glycerol and placed in a petri dish containing lactophenol for temporary storage.

Female were dissected under stereoscope on a slide. The female nematodes were fixed with fuchsin acid (extraction) and were deposited on an object slide in a drop of distilled water. Then with entomological spines the female is pierced and emptied of its contents by slightly pressing on the nematode.

This identification was made from the perineal plates of nematodes (Rusinque et al., 2018). A drop of glycerol or lactophenol is then added. The set was covered with a coverslip object. The air bubbles were eliminated by slightly blazing the blade. The coverslip is then sealed with nail polish for a medium shelf life. These preparations are used for microscopic observation (400x). For a specific determination of nematodes, these perineal figures were compared to those published by Boros et al. (2018).

Study of the *Meloidogyne* pathogenicity on the cv Roma tomato

Nematode rearing was done in plastic, drilled at the bottom using a fire-heated needle. These pots were filled with pasteurized soil heat and were arranged on wooden boards. A 4 weeks old Roma tomato plant (*L. esculentum* L.) was transplanted into each pot. The objective was to study the impact of the multiplication of nematodes of the genus *Meloidogyne* on the development of tomato cv Roma VF which is known to be resistant to *Verticillium* and *Fusarium*. The Roma VF tomato variety is known to be vigorous and productive, but susceptible to nematodes.

The seed was disinfected by soaking for 3 min in 1% active chlorine sodium hypochlorite solution, rinsed twice with sterile distilled water and dried, treated with Apron star: a fungicide and insecticide (20% Thiamethoxam, 20% Metalaxyl and 20% Difenoconazole) and then dried. The transplanting pots were carefully washed with soap before filling them with pasteurized soil in the fire. Seeding was done in wooden trays filled with previously pasteurized potting soil. The substrate used was formed by a mixture of field compost (three volumes), fluvial sand (one volume); rice hull (5 kg); fertilizers (5 kg of compost, 120 g of NPK 6-10-20); water in adequate quantity. This substrate was used both in the germinating tanks and in the containers of the trial.

Four weeks after emergence, the young tomato plants are transplanted into 24 pots of 10 dm³ volume filled with pasteurized soil with 1 plant per pot. The trial design was two treatments

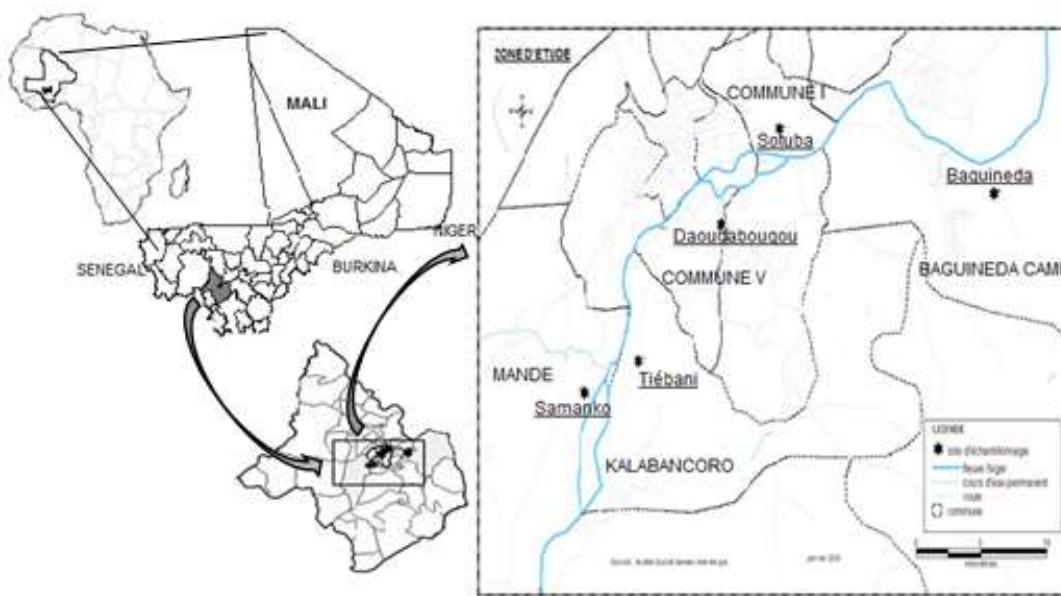


Figure 1. Localization of sites where samples were collected (Touré et al., 2019).

(inoculated and non-inoculated or control) and twelve pots per treatment. Three weeks after transplanting, the plants were inoculated with juveniles of the second stage (j2) nematodes by pouring a solution of 300 juvenile into a small gap dug around the roots.

The date of inoculation was marked on each pot. The inoculum rate was determined after counting on a box with a grid bottom. Other tomato plants were inoculated with the eggs obtained by dissection of the galls. The eggs carried in a tube with a plug or on a microscope slide was gently transferred to the roots of the tomato plants. The roots were then covered with soil. The success of the inoculation must be manifested by the formation of galls. The pots are watered once a day during the two months of the experiment. Phytosanitary treatments were applied one week after transplanting the tomato plants.

Two months after, the plants were removed and the roots were carefully washed, the rate of withered plants, the gall index of inoculated plants of nematodes, the dry weight of the plants cut at the neck were determined. The gall index was estimated using a scale of 0 to 5: 0 = no gall, 1 = traces of infection with some small galls, 2 = more than 25% of roots have galls, 3 = 25 to 50% of the roots have galls, 4 = 50 to 75% of the roots are galls; 5 = more than 75% of the roots have galls (Fayzia et al., 2018). The roots infested with galls are then ground in a kitchen blender and the ground material was filtered to extract *Meloidogyne* j2 confirming that the worms are at the origin of the galls.

RESULTS

Determination of nematodes

Microscopic observation of perineal plates revealed three types of tracks (Figure 2), which were compared to those published by Sasser et al. (1985). The first type corresponds to *Meloidogyne arenaria*, the second is to *Meloidogyne incognita* and the third to *Meloidogyne*

javanica. *M. arenaria* is the most abundant 43.1% of the samples, followed by *M. incognita* 33.8% of the samples. *M. javanica* is the least abundant 23.1% of the samples observed (Figure 3).

Pathogenicity

One month after the symptoms expressed by root gall indices were significant on all inoculated plants. The degree of infestation varied from one plant to another. Of the 12 infested plants, one had the index 5 (more than 75% of the roots carry galls), three plants had the index 4, three others the index 3. The index 2 was the most represented with 5 plants. No infested plants of index 0 and 1 were noted. Thanks to phytosanitary treatments no withered plants were observed (Figure 4).

According to the severity formula of the gall index rate: 1 plant had the index 5 or a severity of 100%, 3 plants had 80% of severity (index 4), 3 others had severity 60% (index 3) and 5 had severity 40% (index 2). Figure 4 shows the rate of gall index found on tomato roots.

The dried biomass did not vary significantly between the two treatments (Student t-test, p-value = 0.3), although the average weight of non-nematode infested plants (8.69g) was slightly higher than that of the infested ones (7.36 g). The *Meloidogyne* populations have not reached the sufficient level to influence the development of the tomato plants. Phytosanitary treatments also contributed to good plant growth.

Figure 5 shows the variations in the biomass of the dried plants of the two treatments and the twelve pots. However, the observation of Figure 5 shows a trend of

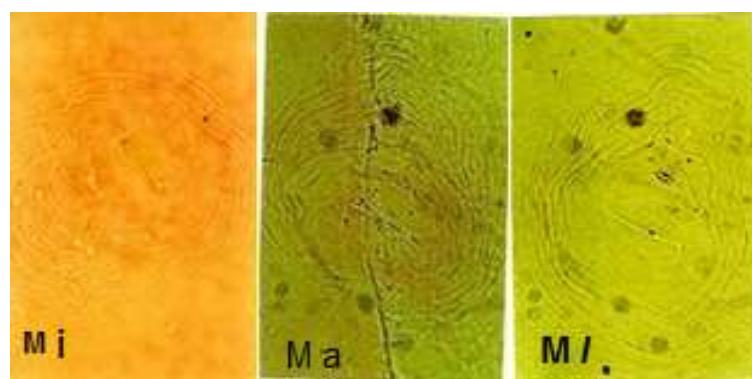


Figure 2. *Meloidogyne* female perineal patterns: Mj (*M. javanica*), Ma (*M. arenaria*), Mi (*M. incognita*).

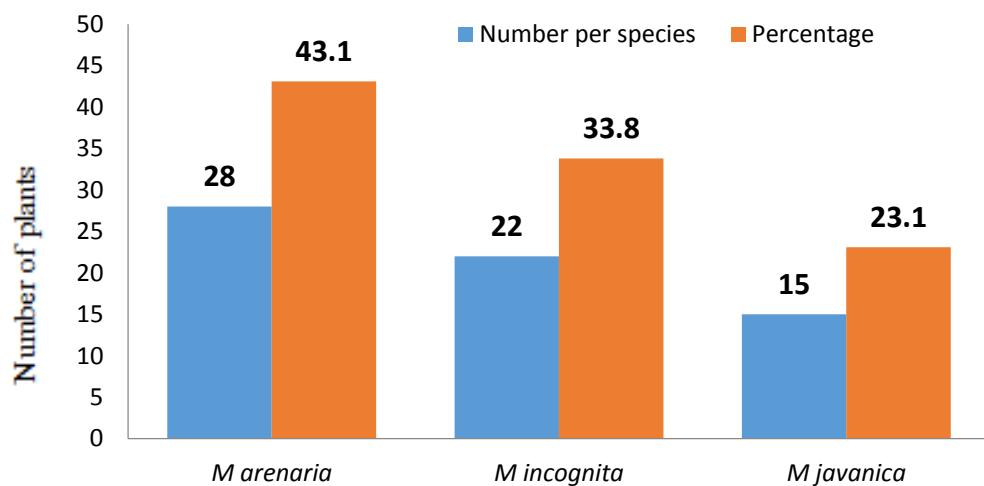


Figure 3. Number per species (blue) and percentage (orange) of *Meloidogyne* identified from female perineal plates.

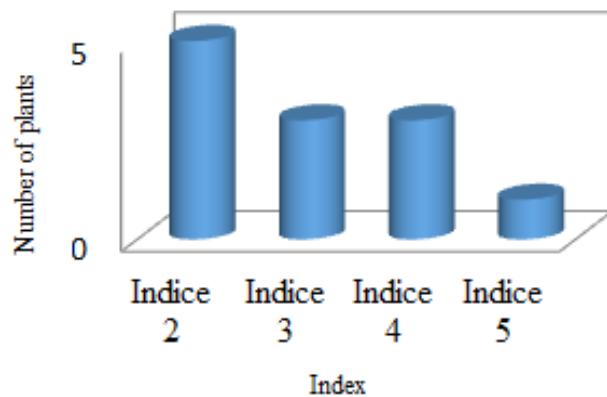


Figure 4. Gall indices observed on the tomato roots.

separation of the biomass values of the two treatments. This may be due to the fact that females of *Meloidogyne* by settling in the galls negatively influence the normal

growth of tomato plants. Signs of infestation (galls) of tomato plants were observed to varying degrees in all plants. These indices show that *L. esculentum* responded

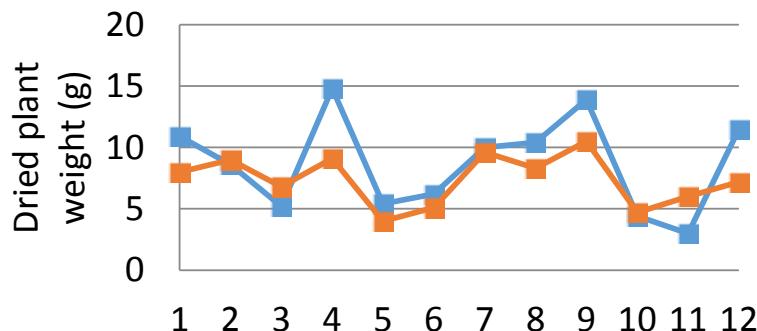


Figure 5. Dried biomass variation for inoculated (orange) and control (bleu) plants.

well to the attack of *Meloidogyne* larvae. The Roma tomato can therefore be considered as a host plant for nematodes of the genus *Meloidogyne*. The absence of significant variation may be due not only to the rate of inoculum (300 days per plant) which seems small compared to the threshold of 1000 j2/kg of soil (De Guiran, 1983), but also to the short vegetative period, 4 to 5 weeks, after transplanting.

DISCUSSION

The results obtained were compared to those for Mali neighbour countries. In this study, three of the major species of *Meloidogyne* were determined. These species are by far the most common in the inter-tropical zone (Powers et al., 2018; Rusinque et al., 2018). The present results show a difference with those of Alabama where Groover and Lawrence (2018) who found two species: *M. arenaria* with 3% of the observed samples and *M. incognita* 96%. The present results would also be different from those associated with tobacco in China reported by Zeng et al. (2018). There, the species *M. arenaria* occupies 61.9%; *M. javanica* 28.5% and *M. incognita* 9.5%. In these two countries, *M. javanica* is the most common species, 80% of the samples. The same species were reported by Mokrini and Sbaghi (2017) in Morocco in the orchard gardens confirm their presence around the world. The differences between the results obtained and those carried out elsewhere may lie in the fact that the samples were collected in a restricted area around the city of Bamako.

Perineal plates are most often variable within the same species, so they alone do not represent a sufficient method for a correct identification of the species of nematode galls. They do not distinguish *M. incognita* and *Meloidogyne mayaguensis* (Janete et al., 2002). It would

therefore be desirable to continue this work to elucidate the problem of the existence of other nematode species in Mali. Other techniques such as the differential host pathogenicity test (cotton, chili, melon, tomato) and morphometric measurements will of interest. Examination of the perineal plates is only a first step to achieve. In addition, in the process of identifying root-knot nematodes, it gives an idea of the variability of the *Meloidogyne* spp. population around Bamako.

This study also showed that tomato variety cv Roma is susceptible to nematodes of the genus *Meloidogyne* despite a low inoculum rate of 300 larvae per plant. Statistical analysis of the dry weight of the treatments did not show a significant difference (Student's t-test, $p = 0.3$). This result may be due to the low inoculum rate of 300 larvae. This rate is below the threshold of 1000 eggs reported by da Silva Rabelo et al. (2018), from Wubie and Temesgen (2019) on Solanaceae. The biomass values of the treatments tend towards those reported by Prabhu et al. (2018) in India who noted a gradual decrease in growth and yield of turmeric (*Cucurma longa* L) based on inoculum rate (0, 100, 500, 1000, and 10000).

Conclusion

The species encountered are mainly *M. arenaria*, *M. incognita* and *M. javanica*. These species are known in most African countries (Mokrini and Sbaghi, 2017). Other species could probably extend this list if research continues on other sites and with more precise identification techniques. The results of this study are only a rough sketch of the identification of root-knot nematodes. On the basis of this preliminary work, new research could be undertaken to confirm identification by more precise techniques such as differential hosts,

esterase electrophoresis and DNA-based molecular methods.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Genetic diversity of cassava (*Manihot esculenta* Crantz) varieties grown in Daloa district in Central-Western Côte d'Ivoire

Flora Yao¹, Mathurin Koffi^{1*}, Innocent Abe^{1,2}, Bernardin Ahouty^{1,2}, Siriki Simaro³, Ibrahim Konaté³, Barkissa Traore¹, Edwige Sokouri¹, Martial N'Djetchi, Thomas Konan¹ and Tidou A. Sanogo¹

¹Research Unit in Genetics and Molecular Epidemiology (URGEM), UFR Environment, Laboratory of Biodiversity and Sustainable Management of Tropical Ecosystems, Jean Lorougnon Guédé University, BP 150 Daloa, Ivory Coast.

²Laboratory of Genetics, UFR Biosciences, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Ivory Coast.

³Laboratory of agro valorization, UFR Agroforestry, University Jean Lorougnon Guédé, BP 150 Daloa, Ivory Coast.

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Cassava is an important staple food in Côte d'Ivoire produced in several areas including the Daloa district in Central-Western Region. However, this plant experiences biotic and abiotic constraints that strongly limit its productivity. Proper knowledge of genetic diversity is important to mitigate these constraints and select resistant and well adapted genotypes to increase the productivity. This study assesses the genetic diversity of four varieties of cassava (Bocou 1, Bonoua, Yace and Yavo) cultivated in Daloa. A total of 266 samples of leaves were collected and genomic DNA was genotyped with 5 simple sequence repeats (SSR) microsatellite markers. In all, 28 alleles were recorded from all the loci with an average number of alleles ranging from 2.6 to 2.9. The average heterozygosity obtained for all loci was higher than expected ($p = 0.008$) and significant genetic diversity was observed within all the varieties ($Fis = -0.43$, $p=0.02$) for Bocou1, ($Fis = -0.59$, $p = 6.10^{-4}$) for Bonoua, ($Fis = -0.32$, $p = 0.05$) for Yace and ($Fis = -0.38$, $p=0.02$) for Yavo. A strong genetic differentiation were also observed between varieties, except between varieties Bocou1 and Bonoua where differentiation was moderate ($Fst = 0.13$). Genetic structure of the population exhibited two or three clusters depending of the variety which might be due to the continuous exchange of plant materials among farmers, selection-base varieties, and use of several varieties in the same fields. This study provides improved understanding of the genetic basis of the varieties which can be exploited to fight against biotic and abiotic stresses in this area.

Key words: Cassava, Côte d'Ivoire, Daloa, genetic diversity, *Manihot esculenta*, microsatellite, population structure.

INTRODUCTION

Cassava is a Euphorbiaceous native to Central America. This plant is the staple food for more than 500 million people in the tropics and subtropics (EL-Sharkawy,

2004). According to FAO (2013), cassava is the world's fourth largest vegetable food behind maize, rice and wheat. In Côte d'Ivoire, cassava is produced mainly in the

south, west and center regions. Average annual production is about 2.41 MT, with an average yield of 6.5 t/h (N'zué et al., 2004, 2013); though of strategic importance for food security and poverty reduction, this crop experiences many abiotic and biotic constraints that limit its production in areas such as the district of Daloa located in Central-Western part of Côte d'Ivoire. In order to mitigate these constraints, cassava breeding programs are being considered (N'Zué et al., 2001, 2013; Kabeya et al., 2012). They consist of responding to the emergence of pests, adapting to abiotic conditions, increasing yields and reducing the level of cyanogenic compounds (Piero et al., 2015). To better understand the genes involved in traits of agronomic interest against pests and for selecting better genotypes that are resistant to pathogens, knowledge on the genetic diversity of cassava become crucial. This study aims to evaluate the genetic potential of four varieties of cassava; Bocou1, Bonoua, Yacce and Yavo, the most profitable and the most cultivated in the district of Daloa.

MATERIALS AND METHODS

Study site and sample collection

The study was carried out in the Daloa district located in the forest zone in Central-West region of Côte d'Ivoire ($6^{\circ} 53' N$, $6^{\circ} 27' W$). This area is covered with dense semi-deciduous forest, has ferralitic soil, a tropical climate with two rainy seasons and two dry seasons (Koffie-bikpo and Kra, 2013). Sampling was conducted throughout January 2018 and consisted of harvesting apparently healthy young cassava leaves belonging to four varieties at each selected site in the four cardinal points of the city area, taking into account the availability of the desired varieties. Samples were conserved in coded plastic bags and sent to URGEM laboratory for molecular analyzes. A total of 266 fresh leaf samples were collected, including 58 for Bocou1, 74 for the Bonoua, 70 for Yace and 64 for Yavo varieties as illustrated in Table 1.

DNA extraction

DNA was extracted using standard procedures according to Risterucci et al. (2000) with slight modifications as follow; 800 μL of MATAB were substitute by 400 μL of PBS and 400 μL of ASL. The concentration and DNA quality were check on 3% agarose gel in 0.5X of TBE buffer stained with ethidium bromide and visualized with a gel viewer before polymerase chain reaction (PCR) implementation.

Polymerase chain reaction analysis

PCR was carried out in thermal Cycler BIO-RAD T100TM using microsatellite primers listed in Table 2. These primers were chosen according to their good distribution on the cassava genome (Sraphet et al., 2011; Whankaew et al., 2011) and their high

polymorphism (Kawuki et al., 2013). The PCR amplification mixture (mix) was carried out in a final volume of 25 μL comprising 2.5 μL of DNA solution, 2.5 μL of 10X buffer, 1.5 μL of MgCl₂ (0.5 mM), 1.6 μL of dNTP (200 μM), 1.3 μL of each of forward and reverse primers (10 μM), 0.3 μL of Taq polymerase (5U/ μL) and 15.4 μL of ultra-pure water. PCR conditions included an initial denaturation of 45 sec at 94°C, followed by hybridization of 1 min at the annealing temperature defined for each primer used as shown in Table 2. The final extension included 1 min at 72°C completed in 35 cycles and final hold at 4°C. The PCR products were checked for amplification product on 3% agarose gel stained with ethidium bromide in 0.5X of TBE buffer at 100 V for 45 min and visualized under UV gel viewer.

Microsatellite markers reliability test

Quality and neutrality of markers used to evaluate the genetic diversity of different cassava varieties were calculated per locus (De Meeûs et al., 2007).

Genetic analysis

The data generated were analyzed for genetic diversity parameters. These parameters are estimated for each locus and the average on all the loci. The CREATE software made it possible to format the database for FSTAT and GENETIX software. The average number of alleles per locus reflects the richness of alleles in the studied population ($A = \Sigma(a/L)$, where a is the number of alleles at a given locus and L number of loci studied). The polymorphism (P) is the percentage of polymorphic loci in the study population. A locus is considered polymorphic when the frequency of the most common allele is less than 0.95. The heterozygosity observed (Ho) and expected (He) defined by Nei (1978) and the fixation index F_{IS} ($F_{IS} = (He - Ho)/He$) made it possible to evaluate within-population diversity. The allelic frequency and the number of rare alleles defined as those having a frequency less than 0.05 were also calculated for each locus. Genetic differentiation was examined with F_{ST} based on the formula: $F_{ST} = (H_T - H_S)/H_T$, where H_S is the expected heterozygosity of an individual within subpopulation and H_T the expected heterozygosity of an individual in the total population (Wright, 1978).

Genetic structure of cassava cultivars

Population structure of cassava cultivars were visualized based on dendrogram using Nei's method (Nei, 1978). This method is based on the dissimilarity matrix between individuals using Darwin software version 6.0. Similarity index is calculated between two individuals according to the following formula: $D_{ij} = \left(1 - \frac{1}{L} \sum_{l=1}^L \left(\frac{ml}{\pi}\right)\right)$, where D_{ij}, the dissimilarity between the individuals i and j; L, the number of loci; π = ploidy and ml the number of matched alleles per locus. In addition, a model-based approach was also applied to confirm the genetic structure. The number of groups (K) in each cassava cultivar was determined with the STRUCTURE software using the admixture model (Pritchard and Donnelly, 2001). The statistic ΔK , which is based on the rate of change in the log probability of the data between successive K-values, was then used to detect the true number of K populations in the dataset (Evanno et al., 2005). This test was performed using the correlated model of

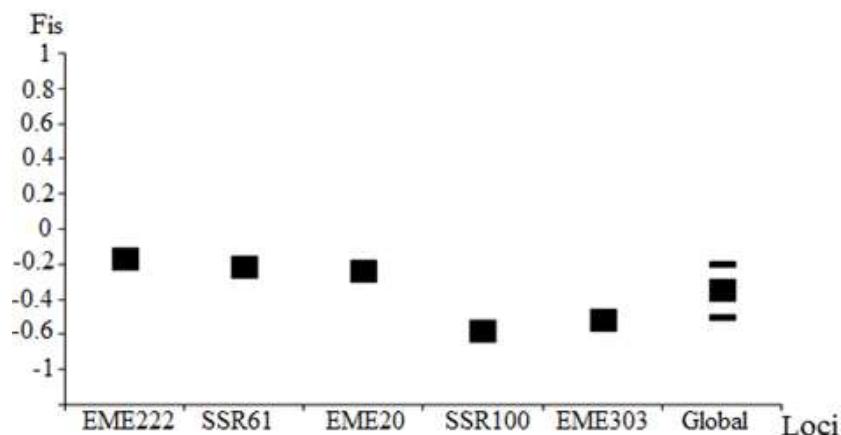
*Corresponding author. Email: m9koffi@yahoo.fr

Table 1. Sites where cassava samples were collected.

Site	Variety				Total
	Bocou1	Bonoua	Yace	Yavo	
Site 1	14	18	16	17	65
Site 2	14	19	19	15	67
Site 3	11	17	16	16	60
Site 4	19	20	19	16	74
Total	58	74	70	64	266

Table 2. Microsatellite markers used to assess genetic diversity.

Name	Type of repeat	Primers (forward and reverse)	Annealing temperature (°C)	Reference
EME20	(ATG)9	5'-CAG-CAC-CAG-TCA-ACA-TTC-CTG-3' 5'-CCT-TCT-GGC-AAT-GAG-CTC-ATG-3'	58	Sraphet et al. (2011)
EME303	(CT)11	5'-ATT-GGG-AAG-CAT-TGG-TGT-AGA-A-3' 5'-CAC-AAA-CAA-AAC-CCT-GTG-ACC-T-3'	58	Whankaew et al. 2011
EME222	(GAT)6	5'-CCC-ACT-CTC-TGT-CCA-CTT-C-3' 5'-CTT-CGA-CTC-TTC-TTT-ACG-GG-3'	58	Sraphet et al. (2011)
SSRY100	(CT)17TT(CT)7	5'-ATC-CTT-GCC-TGA-CAT-TTT-GC-3' 5'-TTC-GCA-GAG-TCC-AAT-TGT-TG-3'	58	Kawuki et al. (2013)
SSRY61	(CA)12	5'-GGC-TGC-TTT-ACC-TTC-TAC-TCA-A-3' 5'-CAA-GAA-CGC-CAA-TAT-GCT-GA-3'	58	Whankaew et al. (2011)

**Figure 1.** F_{IS} estimation per microsatellite locus.

allelic frequencies based on the clustering of individuals with similar allelic frequencies.

RESULTS

Microsatellite markers reliability test

The 5 microsatellite markers have good congruence and variances quite homogeneous regarding F_{IS} calculated per locus as illustrated in Figure 1. Therefore, all these

markers were used for subsequent analysis.

Genetic diversity parameters

Genetic diversity parameters were assessed with 5 microsatellites markers, and the results are presented in Table 3. All these markers were polymorphic for the four varieties. A total of 28 alleles were observed. The number of alleles varied from four to seven throughout loci with an average number of 5.6 alleles. The rate of rare alleles

Table 3. Mean of genetic diversity parameters across all loci.

Locus	No. of allele	Rare allele	H_o	H_e	F_{IS}	F_{ST}	P-value
EME222	7	2	0.752	0.645	-0.166	0.15	0,05
SSRy61	5	1	0.688	0.574	-0.199	0.10	0.05
EME20	5	1	0.721	0.597	-0.208	0.13	0.04
SSRy100	7	0	0.985	0.625	-0.576	0.19	0.004
EME303	4	0	0.909	0.599	-0.517	0.19	0.007
Mean	5.600	0.800	0.811	0.608	-0.333	0.15	0,008

H_e = expected heterozygosity, H_o = observed heterozygosity, F_{IS} = fixation index, F_{ST} = genetic differentiation index.

Table 4. Mean of genetic diversity parameters for each variety across all loci.

Population	No. of allele	Rare allele	H_o	H_e	F_{IS}	P-value
Bocou1	2.6	1	0.76	0.53	-0.43	0.02
Bonoua	2.75	1	0.92	0.58	-0.59	0.0006
Yacé	2.75	0	0.74	0.56	-0.32	0.05
Yavo	2.9	2	0.80	0.58	-0.38	0.02

Table 5. Estimated F_{ST} between pairs of the four varieties of cassava.

Population	Bocou1	Bonoua	Yace	Yavo
Bocou1	-	0.13	0.24	0.24
Bonoua	0.13	-	0.25	0.23
Yace	0.24	0.25	-	0.25
Yavo	0.24	0.23	0.25	-

in the overall population is 14.28%. The EME222 marker recorded the highest number of rare alleles as shown in Table 3. The mean number of alleles across all loci obtained for varieties Bocou1, Bonoua, Yace and Yavo were 2.6, 2.75, 2.75 and 2.9 respectively. Of the 28 alleles revealed by these 5 markers across loci and varieties, only one rare allele occurred in variety Bocou1 and Bonoua, and 2 occurred in variety Yavo. The average expected heterozygosity (H_e) across all the varieties and loci ranged from 0.574 in SSRy61 to 0.645 in EME222 with an average of 0.608, while observed heterozygosity ranged from 0.688 to 0.985 in SSRy61 and SSRy100, respectively, with an average of 0.811. The average heterozygosity obtained for all loci was higher than expected ($p=0.008$). A significant genetic diversity was observed within all the varieties ($F_{IS}=-0.43$, $p=0.02$) for Bocou1, ($F_{IS}=-0.59$, $p=6.10^{-4}$) for Bonoua, ($F_{IS}=-0.32$, $p=0.05$) for Yace and ($F_{IS}=-0.38$, $p=0.02$) for Yavo as illustrated in Table 4.

Genetic differentiation between varieties

F_{ST} values indicate good genetic differentiation between

varieties except between varieties Bocou1 and Bonoua where there is moderate genetic differentiation with a F_{ST} value equal to 0.13 as described in Table 5.

Population structure of cassava cultivars

Genetic structure within cassava varieties is described by radial style dendrograms based on dissimilarity matrices between individuals. These graphs shapes testify the existence of a spatial structuring of the diversity within these varieties. Thus, samples from different sites are grouped together to form distinct genetic groups within the four varieties as shown in Figure 2.

According to admixture model, varieties Bocou1, Bonoua and Yavo comprised of 2 clusters ($K = 2$) each, with a maximum likelihood for value $\Delta K = 696$, 106 and 98 for these varieties respectively. Variety Yacé comprised 3 clusters ($K = 3$) with a maximum likelihood for a value of $\Delta K = 16$. For variety Bocou1, there are 37 individuals (64%) in cluster 1 and 21 individuals (36%) in cluster 2. Variety Bonoua has 39 individuals (53%) in cluster 1 and 35 individuals (47%) in cluster 2. Concerning variety Yavo, individuals are distributed evenly over 2

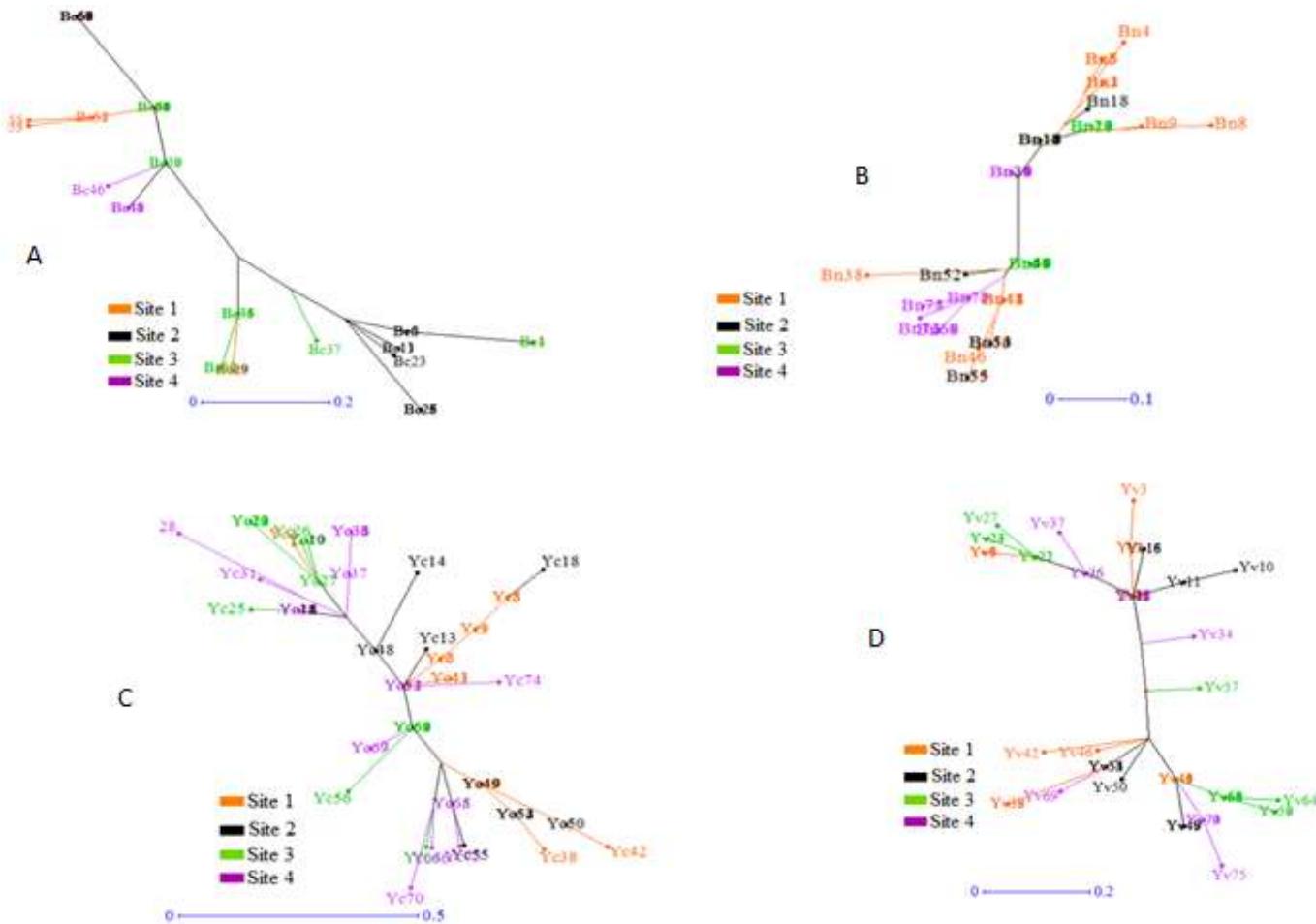


Figure 2. Dendrograms describing the genetic structure of cassava populations according to dissimilarity between individuals (A, B, C and D represent varieties Bocou1, Bonoua, Yacé and Yavo respectively).

clusters (50% each). Variety Yacé has 21 individuals (30%) in cluster 1, 35 individuals (46%) in cluster 2 and 14 individuals (24%) in cluster 3 as shown in Figure 3.

DISCUSSION

Genetic diversity and structure within and between 4 cassavas varieties cultivated in Daloa region were assessed with five microsatellite markers considered adequate to give reliable results on genetic parameters of cassava (Moyib et al., 2007). These markers were all polymorphic. Low levels of rare alleles were observed in these varieties, indicating the good quality of the markers (Meye, 2013).

Genetic diversity parameters

The average numbers of allele obtained in the different varieties analyzed are greater than 2 and reveal a good allelic richness within these varieties but less than an

average number of alleles of 4.76 observed by Kizito et al. (2005) in Uganda. The highest average number of allele from these authors may be due to the fact that their study was carried out on unselected traditional varieties with more diversity than selected variety used in our study. This mean that the varietal selection lost alleles and therefore genetic diversity contained in wild collections is higher than selected variety observed (Nassar, 1978). Low genetic variability among the cassava varieties was also observed by other authors in Nigeria using the same molecular markers (Kabeya et al., 2012; Afonso et al., 2019). This narrow variability is a drawback from the point of view of breeders, because they need high genetic variability to improve agronomic traits and the genotypes are selected only based on very few agronomic traits such as maturation time, height and yield. Increasing genetic variability is crucial for breeding programs (Kabeya et al., 2012). This shows the need to always maintain an ever more diverse wild core that is referred to when the varietal selection has led to the loss of some interesting trait in the wildlife species.

The average values of FIS are negative for all loci in the

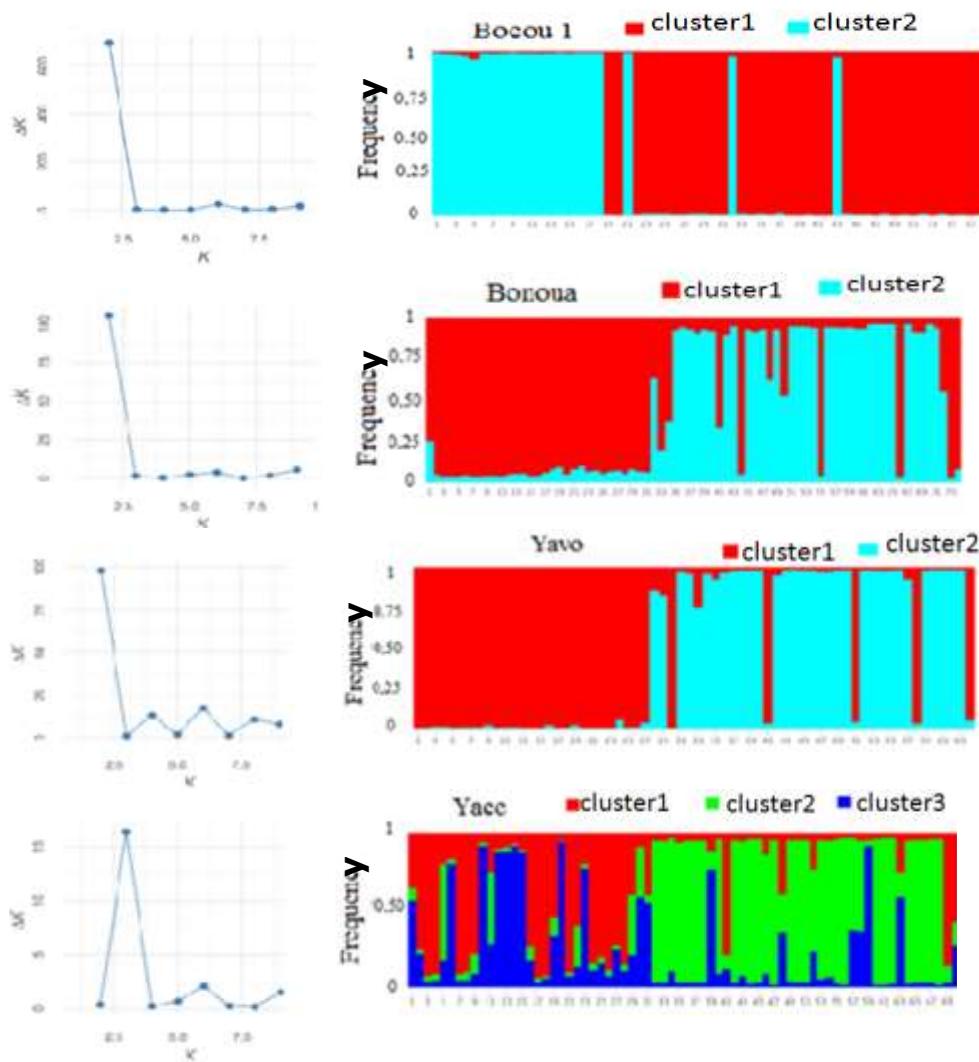


Figure 3. Clusters within cassava varieties using the admixture model (each color is representative of a cluster).

different varieties of cassava. There is therefore an excess of heterozygotes within the varieties that could be explained accordingly. One could think of the clonal reproduction of cassava which results in identical individuals to the original one. Otherwise, the excess of heterozygotes could be due to the natural sex-based reproduction systems of cassava. This argument is supported by Silva et al. (2003) who showed a pollen rate of cassava greater than 0.6 which reveals high levels of pollination with varying levels of selfing in this plant. The average number of alleles greater than 2 and the strong heterozygosity reflect the existence of genetic diversity within the varieties of cassava considered.

Let us note that the varieties studied (Yacé, Yavo, Bocou1 and Bonoua) are already improved (selected) varieties from the National Center for Agricultural Research (CNRA) and therefore from several crosses between landraces (N'Zué et al., 2013). This could explain the high number of heterozygosity and therefore

the no-equilibrium situation of Hardy-Weinberg (Beninga, 1992).

Chromosomal mutations due to environmental pressures might arise and thus contribute to genetic diversity. One of the factors behind this diversity is the continuous exchange of plant material with interesting agronomic traits among farmers in different localities (Missihoun et al., 2012). It could also be due to cultural practices based on the use of several varieties in fields that by gene exchange which creates genetic variability (Meye, 2013). In fact, the genetic diversity of cassava in a locality increases with varietal diversity (Adriano et al., 2013). In addition, when several varieties are grown by many households over large areas, they are minimally threatened and may simply be subject to in situ conservation of genetic diversity (Jarvis et al., 2000). Furthermore, it is noted that the clonal spread of cassava leads to an accumulation of pests, reducing yields. Thus, the higher the genetic diversity, the higher is the

resistance to pathogens (McKey et al., 2012).

However, many varieties are abandoned because of the adoption of higher-yielding varieties and only few are maintained in cultivation which could thus favor genetic erosion (Kombo et al., 2012). Indeed, genetic diversity is higher in Amerindian villages (Elias et al., 2000) because, farmers of these areas exploit seedlings from seeds to enhance the diversity of their plant material (Peroni et al., 2007). Seed multiplication is therefore source of new genotypes (Rival and McKey, 2008) but is unfortunately unknown in Côte d'Ivoire.

Genetic differentiation between varieties

Moderate genetic differentiation between cassava varieties observed ($F_{ST} = 0.19$) could be explained by a low gene flow between varieties because of few gene exchange between the improved varieties (Lokko et al., 2006). However, when the varieties are considered two by two, the genetic differentiation is strong except varieties Bocou1 and Bonoua which present a moderate genetic differentiation. We can say that gene flow is higher between these two varieties than between the others.

Population structure of cassava cultivars

The analysis carried out with STRUCTURE presented two main clusters for Bocou1, Bonoua and Yavo varieties and three genetic clusters for the Yacé variety. There is thus more diversity within these varieties which confirms their instability which could offer improvement possibilities for these varieties (Trochet et al., 2014).

Conclusion

Cassava is one of the income-generating crops grown in Côte d'Ivoire. This study evaluated the genetic diversity of four varieties of cassava cultivated in Daloa district. It comes up the existence of a moderate genetic diversity and differentiation within and between these varieties. Population structure defined two or three clusters depending on variety. This study is the first step in understanding the range of genetic diversity of cassava varieties grown in Daloa. The information generated is of paramount importance to justify and guide conservation strategies for cassava genetic resources in the area, to serve as a reference not only to the region but also to the country and a parental selection guide in the sense of varieties improvement.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Parent-offspring regression, correlation and genetic advance of drought and yield traits at early generation in groundnut (*Arachis hypogaea* L.)

Ousmane Sanogo^{1,2*}, Pangirayi B. Tongoona¹, Kwadwo Ofori¹, Samuel K. Offei¹
and Haile Desmae²

¹West Africa Centre for Crop Improvement, University of Ghana, P. M. B. 30, Legon, Accra, Ghana.

²International Crop Research Institute for the Semi-Arid Tropics, BP 320 Bamako, Mali.

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A study was carried out to estimate the parent-offspring regression and correlation and, to determine genetic advance of yield and drought related traits of groundnut at early segregating populations. All the experiments were conducted in the dry season 2015/2016 at the International Crops Research Institute for the Semi-Arid Tropics ICRISAT Samanko, Mali under full irrigation and drought stress conditions. The data were collected on plot basis on both water-stressed and fully irrigated plots in the F1, F2 and F2:3 generations of two populations. Data collected included chlorophyll concentration (SCMR), Specific Leaf Area (SLA) (cm²/g) and Pod Yield (PY)(kg/ha). Results of the parent offspring regression for the two populations evaluated both water regimes were low and revealed importance of non-genetic effects. Consequently, the genetic advances for the two crosses were mostly low to moderate irrespective of the generation and environment under study. Selection at early generation in groundnut could be slow under drought. Based on the findings, selection for drought tolerance would be inefficient to identify high yield and drought tolerant lines at early generation in groundnut. The highest heritability estimates for F1:F2 were 42% for SCMR 60 DAS and at 80 DAS under well-watered conditions, 20% ± 0.20 for SLA at 60 DAS and at 80 DAS under drought stressed conditions. The highest heritability estimates for F2:F3 progenies were observed from SCMR 60 DAS (22% ± 0.09) under well-watered conditions and SLA 60 DAS (22% ± 0.08) under water-stressed conditions.

Key words: Groundnut, heritability, drought stress, breeding.

INTRODUCTION

In a typical breeding methods such as pedigree, single-seed descent (SSD), bulk population and backcrossing, large number of genotypes are advanced through segregating generations (Ntare, 1999). These processes take a long time before identification of superior cultivars. Early Generation Selection (EGS) in self-pollinated crops involves the evaluation of F₂-or F₃-derived lines from a

cross between two homozygous parents (Bernardo, 2003). EGS may overcome the inability to identify superior yielding individual plants as early as F₂ and therefore speed up the process of developing new groundnut varieties following hybridization of diverse parents. Success in early generation testing was found with highly heritable traits (Rowe, 2009; Yang, 2009). In

*Corresponding author. E-mail: ouzbi777@gmail.com. Tel: +223 79180938.

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groundnut, Zongo et al. (2017) found that selection of agronomic traits (days to first flowering, 50% flowering, plant height) and early leaf spot disease at early generation selection was effective due to heritability recorded. Anderson et al. (1991) found that selection based on early generation family means was effective for improvement of both late leaf spot (LLS) and early leaf spot (ELS) disease resistance. In groundnut, Anderson et al. (1991) found that selection based on early generation family means was effective for improvement of both LLS and ELS disease resistance. Genetic advance of a trait is the product of narrow-sense heritability, phenotypic variation and the selection intensity. It is therefore a drawing force in selection, which measures the importance of the genes passed from parent to offspring. Sumathi and Ramanathan (1995) in using the parent-offspring regression method, reported moderate heritability estimates in groundnut for pod yield, while Ntare (1999) reported low to moderate heritability as well as correlation for yield and empirical traits such as crop growth rate, reproductive duration and partitioning. For traits characterized by low heritability such as yield, Songsri et al. (2008) proposed selection based on physiological criteria that are correlated with yield. These include traits such as the SPAD Chlorophyll Meter Reading (SCMR) and the Specific Leaf Area (SLA) sought as "surrogate traits" in drought (Nageswara-Rao et al., 2001; Upadhyaya, 2005; Songsri et al., 2008; Upadhyaya et al., 2011). These authors reported that both SPAD and SLA displayed additive effects; thus helping in selection for drought in plant crops. Globally, information on heritability of drought related traits such as SCMR and SLA in the groundnut breeding is lacking in Mali. Heritability estimates of drought-related traits SLA and SCMR and their genetic correlation with pod yield will be useful to formulate effective breeding strategies under drought.

The objective of this study was to estimate the parent-offspring regression, correlation and genetic advance of yield and drought related traits of groundnut evaluated under managed drought stress conditions.

MATERIALS AND METHODS

Experimental site and conditions

All the experiments were conducted at the experimental field of International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Samanko in Mali ($12^{\circ}54'N$ and $8^{\circ}4'W$, 330 m above the sea) in rain-free period in November 2014 to March 2015. The soils are poor in organic matter content, light and generally brown yellow, of tropical ferruginous wash type with pH of 4.5. The mean annual rainfall is 800 mm from June to October.

Irrigation water management and experimental design

The experiment was planted in split plot design using two environments as indicated below:

(i) Well-watered (WW) block - received full irrigation throughout the

life cycle of the crop (from sowing to harvesting period). Plants were irrigated one to two times per week with 20 mm of water until end-of-season (pod filling to pod maturity) at seven day interval depending on the prevailing weather conditions.

(ii) Water-stressed (WS) block - full irrigation was provided till 50 days after sowing (DAS). The plants were exposed gradually to end-of-season drought from the pod filling until maturity. This period started from the pegging to pod development and maturation. At 50 DAS, drought stress was imposed for 14 days and irrigation was resumed at the 15th day to bring the soil up to saturation. Then, drought stress was imposed for 10 days, followed by irrigation up to saturation. After that, drought stress was imposed for 7 days followed by irrigation up to harvest. This technique was supposed to mimic the end-of-season drought since water was withheld during the critical stage of the reproductive phase.

The two blocks (WW and WS) were separated by an alley that was 25.0 m wide to restrict lateral movement of water from the fully irrigated block to the drought stress block. Irrigation water was supplied with an overhead sprinkler irrigation system designed to dispense 40 mm of water twice per week. Except for the different irrigation treatments, all field management practices were uniform for both the well-watered and water-stressed experiments.

Basal fertilizer of 100 kg ha⁻¹ simple super phosphate was applied before hand-planting with one seed per hill. Standard cultural practices, including hand planting, hand weeding while the first as early as 16-20 days after sowing (DAS) were followed. The average ambient temperature during the trial period (November-March) was 26.07°C, with a standard deviation STDEV= 9.55%. The average relative humidity within the same period was 27.17% with a standard deviation STDEV of 16.56%.

Genetic resources and hybridization techniques

The material tested was part of an ongoing breeding program for tolerance to drought. The F₁, F₂ and F_{2:3} generations from the two populations ICGV 91317/ICGV 87378 and ICIAR 19BT/ICGS 44 were used.

Experiment 1

Sixty five groundnut genotypes comprising 20 F₁ and 45 F₂ for population five (ICGX-IS 13005 = ICGV 91317/ICGV 87378) on one hand, and 15 F₁ plus 45 for population twelve (ICGX-IS 13012 = ICIAR 19 BT /ICGS 44) on the other, were evaluated under managed drought stress conditions. The population 5 and the population 12 were named cross I and cross II, respectively in this study (Table 1). The experimental design was the split plot with two replicates (Table 2). An experimental plot consisted of two rows of 15 m long, with bulk plants within a row spaced 0.5 m. One groundnut seed was planted per hill.

Experiment 2

Ninety six groundnut genotypes comprising 45 F_{2:3} progenies for population 5 (ICGX-IS 13005 = ICGV 91317/ICGV 87378) and population 12 (ICIAR 19 BT/ICGS 44) were evaluated in a 9 x 11 alpha lattice with two replications. An experimental plot consisted of a 4 m single row with 0.6 m space. Two checks Fleur11 and 47-10 were used but excluded during the analyses because the evaluation of heritability parent-offspring regression and correlation were based on segregating materials.

Data collection

Data collected included Chlorophyll concentration (SCMR) at 60

Table 1. Number of F_1 and F_2 plants from the two populations used in the study.

Population	Entry	Pedigree	Generation	Number of plants
1	ICGX-IS 13005F1-B1	ICGV 91317/ICGV 87378	F_1	40
2	ICGX-IS 13012F1-B1	ICIAR 19 BT / ICGS 44	F_1	40
1	ICGX-IS 13005F2-B1	ICGV 91317/ICGV 87378	F_2	90
2	ICGX-IS 13012F2-B1	ICIAR 19 BT / ICGS 44	F_2	90

and 80 DAS, Specific Leaf Area (SLA) (cm^2/g) at 60 and 80 DAS and Pod Yield (PY) (kg/ha). Individual plants were harvested and their pods stripped from the plant for adequate sun drying. Individual pod weights were recorded for each F_1 and F_2 individual plant. For $F_{2:3}$ populations, data from progenies mean were used.

Data analysis

Separate analyses of variance (ANOVA) were done for each cross or population, generation and water regime using SAS (SAS Institute, 2009). Generations and individual families (genotypes) were considered to be random. Adjusted means from the lattice designs (Patterson et al., 1978) were used in the combined ANOVA across water regimes for each population. Parent-offspring regression (b) coefficients were obtained by regressing F_2 bulks on F_1 bulks and F_3 families or progenies mean on F_2 bulks using PROC REG in SAS. To quantify additive genetic variation (narrow-sense heritabilities) in intergeneration segregating, separate parent-offspring regressions for the population I and population II were performed for F_2/F_1 and F_3/F_2 progenies as described by Smith and Kinman (1965): $h^2 = b/r_{op}$. Where, b is the regression coefficient or slope and, r_{op} is the relationship of parent-offspring. Heritability estimates were grouped as high (>50%), moderate, (20 to 50%), and low (<20%) as suggested by Stansfield (1986). Linear regression coefficients (b) was calculated by regression of F_2 progeny means (Y_i) on F_1 plants means (X_i) and likewise, F_3 progenies means (Y_i) were regressed on F_2 plants means (X_i). Standard error (SE) for the slope of the regression was calculated according to the method of Ibrahim and Quick (2001). Since the bulked F_2 and their bulked F_1 parents were grown in the same experiment, they were expected to be environmentally correlated (Holland et al., 2003).

Estimate of genetic advance (GA)

The genetic advance was estimated as followed: $GA = i^*h^2*V_p$ (Falconer and Mackay, 1996); Where, i^* = selection intensity (1.76 for the top 10%), V_p = phenotypic variance and, h^2 = the narrow sense heritability. Negative estimates were considered equal to zero (Robinson et al., 1955; cited in Gusmini and Wehner, 2007) and were reported as suggested Dudley and Moll (1969). Derivations from negative estimates from another negative value were also considered to be zero and omitted (Gusmini and Wehner, 2007).

RESULTS

Mean squares of traits measured for the three generations

Results of analyses of variance (ANOVA) for the F_1 , F_2 and F_3 generations for cross I and cross II evaluated

under both water regimes were as presented in Table 3.

In cross I, variations among F_1 progenies mean squares were significant ($P < 0.05$) for SCMR at 60 DAS trait under water-stressed conditions. Under well-watered conditions, F_3 genotypes mean squares were significant for SCMR at 60 and 80 DAS, pod yield, SLA at 60 DAS and PY under well-watered conditions whereas F_3 genotypes were not significant for any studied traits evaluated under water-stressed conditions (Table 3). None of the three generations (F_1 , F_2 and F_3) was consistently alike when evaluated under well-watered and water-stressed conditions.

In cross II, the progenies showed high variation regarding generations and water regimes for some traits (Table 3). The mean squares for F_1 genotypes were not significant for any trait except for SLA at 60 DAS where very highly significant ($P < 0.001$) differences were found under both well-watered and water-stressed conditions. Under water-stressed conditions, no significant differences were detected among F_2 genotypes while significant ($P < 0.05$) differences were observed among genotypes for SLA at 60 DAS. Also, highly significant ($P < 0.01$) differences among F_2 genotypes were observed for the SLA at 60 DAS and pod yield traits (Table 3). F_3 genotypes showed significant ($P < 0.05$) differences for SLA at 60 DAS and very highly significant ($P < 0.001$) differences for pod yield under well-watered conditions. Mean squares for F_3 genotypes evaluated under water-stressed conditions showed highly significant ($P < 0.01$, $P < 0.001$) differences for SCMR at 60 DAS and pod yield, respectively.

Mean performance of the populations under the two water regimes

In cross I, the highest pod mean was found in F_3 progenies under well-watered conditions followed by the F_2 progenies whereas F_2 progenies exhibited the highest pod mean under water-stress conditions followed by F_3 progenies. There was a tendency for better pod yield harvesting in well-water than water-stressed, except for F_2 progenies as shown for the mean and the mean range (Table 4). The mean performance for the F_1 , F_2 and F_3 progenies were similar for the trait SCMR at 60 DAS. The F_1 progenies showed the highest mean for SCMR at 60 DAS under both well-watered and water-stressed

Table 2. List of the two populations comprising each 45 F_{2;3} progenies used.

S/N	Population I or cross I		S/N	Population II or cross II	
	Genotype	Pedigree		Genotype	Pedigree
1	ICGX-IS 13005F2-B1-106	ICGV 91317/ICGV 87378	1	ICGX-IS 13012F2-B1-105	ICIAR 19 BT / ICGS 44
2	ICGX-IS 13005F2-B1-11	ICGV 91317/ICGV 87378	2	ICGX-IS 13012F2-B1-114	ICIAR 19 BT / ICGS 44
3	ICGX-IS 13048F2-B1-12	ICGV 91317/ICGV 87378	3	ICGX-IS 13012F2-B1-115	ICIAR 19 BT / ICGS 44
4	ICGX-IS 13005F2-B1-132	ICGV 91317/ICGV 87378	4	ICGX-IS 13012F2-B1-130	ICIAR 19 BT / ICGS 44
5	ICGX-IS 13005F2-B1-14	ICGV 91317/ICGV 87378	5	ICGX-IS 13012F2-B1-140	ICIAR 19 BT / ICGS 44
6	ICGX-IS 13005F2-B1-167	ICGV 91317/ICGV 87378	6	ICGX-IS 13012F2-B1-15	ICIAR 19 BT / ICGS 44
7	ICGX-IS 13005F2-B1-171	ICGV 91317/ICGV 87378	7	ICGX-IS 13012F2-B1-156	ICIAR 19 BT / ICGS 44
8	ICGX-IS 13005F2-B1-182	ICGV 91317/ICGV 87378	8	ICGX-IS 13012F2-B1-20	ICIAR 19 BT / ICGS 44
9	ICGX-IS 13005F2-B1-185	ICGV 91317/ICGV 87378	9	ICGX-IS 13012F2-B1-207	ICIAR 19 BT / ICGS 44
10	ICGX-IS 13005F2-B1-187	ICGV 91317/ICGV 87378	10	ICGX-IS 13012F2-B1-24	ICIAR 19 BT / ICGS 44
11	ICGX-IS 13005F2-B1-189	ICGV 91317/ICGV 87378	11	ICGX-IS 13012F2-B1-268	ICIAR 19 BT / ICGS 44
12	ICGX-IS 13005F2-B1-19	ICGV 91317/ICGV 87378	12	ICGX-IS 13012F2-B1-276	ICIAR 19 BT / ICGS 44
13	ICGX-IS 13005F2-B1-198	ICGV 91317/ICGV 87378	13	ICGX-IS 13012F2-B1-281	ICIAR 19 BT / ICGS 44
14	ICGX-IS 13005F2-B1-205	ICGV 91317/ICGV 87378	14	ICGX-IS 13012F2-B1-29	ICIAR 19 BT / ICGS 44
15	ICGX-IS 13005F2-B1-222	ICGV 91317/ICGV 87378	15	ICGX-IS 13012F2-B1-297	ICIAR 19 BT / ICGS 44
16	ICGX-IS 13005F2-B1-252	ICGV 91317/ICGV 87378	16	ICGX-IS 13012F2-B1-312	ICIAR 19 BT / ICGS 44
17	ICGX-IS 13005F2-B1-262	ICGV 91317/ICGV 87378	17	ICGX-IS 13012F2-B1-319	ICIAR 19 BT / ICGS 44
18	ICGX-IS 13005F2-B1-287	ICGV 91317/ICGV 87378	18	ICGX-IS 13012F2-B1-381	ICIAR 19 BT / ICGS 44
19	ICGX-IS 13005F2-B1-301	ICGV 91317/ICGV 87378	19	ICGX-IS 13012F2-B1-40	ICIAR 19 BT / ICGS 44
20	ICGX-IS 13005F2-B1-359	ICGV 91317/ICGV 87378	20	ICGX-IS 13012F2-B1-431	ICIAR 19 BT / ICGS 44
21	ICGX-IS 13005F2-B1-37	ICGV 91317/ICGV 87378	21	ICGX-IS 13012F2-B1-475	ICIAR 19 BT / ICGS 44
22	ICGX-IS 13005F2-B1-381	ICGV 91317/ICGV 87378	22	ICGX-IS 13012F2-B1-491	ICIAR 19 BT / ICGS 44
23	ICGX-IS 13005F2-B1-388	ICGV 91317/ICGV 87378	23	ICGX-IS 13012F2-B1-50	ICIAR 19 BT / ICGS 44
24	ICGX-IS 13005F2-B1-40	ICGV 91317/ICGV 87378	24	ICGX-IS 13012F2-B1-518	ICIAR 19 BT / ICGS 44
25	ICGX-IS 13005F2-B1-404	ICGV 91317/ICGV 87378	25	ICGX-IS 13012F2-B1-520	ICIAR 19 BT / ICGS 44
26	ICGX-IS 13005F2-B1-411	ICGV 91317/ICGV 87378	26	ICGX-IS 13012F2-B1-525	ICIAR 19 BT / ICGS 44
27	ICGX-IS 13005F2-B1-425	ICGV 91317/ICGV 87378	27	ICGX-IS 13012F2-B1-528	ICIAR 19 BT / ICGS 44
28	ICGX-IS 13005F2-B1-450	ICGV 91317/ICGV 87378	28	ICGX-IS 13012F2-B1-534	ICIAR 19 BT / ICGS 44
29	ICGX-IS 13005F2-B1-46	ICGV 91317/ICGV 87378	29	ICGX-IS 13012F2-B1-537	ICIAR 19 BT / ICGS 44
30	ICGX-IS 13005F2-B1-470	ICGV 91317/ICGV 87378	30	ICGX-IS 13012F2-B1-554	ICIAR 19 BT / ICGS 44
31	ICGX-IS 13005F2-B1-481	ICGV 91317/ICGV 87378	31	ICGX-IS 13012F2-B1-561	ICIAR 19 BT / ICGS 44
32	ICGX-IS 13005F2-B1-488	ICGV 91317/ICGV 87378	32	ICGX-IS 13012F2-B1-562	ICIAR 19 BT / ICGS 44
33	ICGX-IS 13005F2-B1-49	ICGV 91317/ICGV 87378	33	ICGX-IS 13012F2-B1-563	ICIAR 19 BT / ICGS 44
34	ICGX-IS 13005F2-B1-494	ICGV 91317/ICGV 87378	34	ICGX-IS 13012F2-B1-566	ICIAR 19 BT / ICGS 44
35	ICGX-IS 13005F2-B1-498	ICGV 91317/ICGV 87378	35	ICGX-IS 13012F2-B1-571	ICIAR 19 BT / ICGS 44
36	ICGX-IS 13005F2-B1-5	ICGV 91317/ICGV 87378	36	ICGX-IS 13012F2-B1-576	ICIAR 19 BT / ICGS 44
37	ICGX-IS 13005F2-B1-50	ICGV 91317/ICGV 87378	37	ICGX-IS 13012F2-B1-586	ICIAR 19 BT / ICGS 44
38	ICGX-IS 13005F2-B1-559	ICGV 91317/ICGV 87378	38	ICGX-IS 13012F2-B1-600	ICIAR 19 BT / ICGS 44
39	ICGX-IS 13005F2-B1-586	ICGV 91317/ICGV 87378	39	ICGX-IS 13012F2-B1-62	ICIAR 19 BT / ICGS 44
40	ICGX-IS 13048F2-B1-591	ICGV 91317/ICGV 87378	40	ICGX-IS 13012F2-B1-69	ICIAR 19 BT / ICGS 44
41	ICGX-IS 13005F2-B1-65	ICGV 91317/ICGV 87378	41	ICGX-IS 13012F2-B1-75	ICIAR 19 BT / ICGS 44
42	ICGX-IS 13005F2-B1-85	ICGV 91317/ICGV 87378	42	ICGX-IS 13012F2-B1-78	ICIAR 19 BT / ICGS 44
43	ICGX-IS 13005F2-B1-90	ICGV 91317/ICGV 87378	43	ICGX-IS 13012F2-B1-84	ICIAR 19 BT / ICGS 44
44	ICGX-IS 13005F2-B1-91	ICGV 91317/ICGV 87378	44	ICGX-IS 13012F2-B1-93	ICIAR 19 BT / ICGS 44
45	ICGX-IS 13005F2-B1-93	ICGV 91317/ICGV 87378	45	ICGX-IS 13012F2-B1-98	ICIAR 19 BT / ICGS 44

conditions while the F₂ progenies exhibited the highest mean for SCMR at 80 DAS under water-stressed and

well-watered conditions. The mean range for the three generations F₁, F₂ and F₃ lines for SLA at 60 DAS and for

Table 3. Mean squares from ANOVA of measured traits for F_1 , F_2 and F_3 progenies from two populations evaluated under both well-watered and water-stress.

Trait	Gen [‡]	Population I		Population II	
		Well-watered	Water-stress	Well-watered	Water-stress
SCMRf	F_1	8.09	12.85*	6.59	4.78
	F_2	10.84	10.59	8.28	9.99
	F_3	6.28*	4.01	6.41	10.40**
SCMRz	F_1	5.56	5.67	14.44	7.75
	F_2	143.35	19.97	9.72	6.12
	F_3	12.88**	18.45	16.66	13.56
SLAf	F_1	3685.40	3122.1	2192.7	3499.50
	F_2	3016.03	3088.50	4335.7*	2372.10
	F_3	2910.4***	1874.40	2330.7*	2199.40
SLAz	F_1	2523.90	3372.50	5736.8**	3676.7**
	F_2	2574.90	2919.40	3334.4**	2286.10
	F_3	2914.70	2522.80	2220.7	2638.40
PY	F_1	53.78	19.90	29.99	30.90
	F_2	63.49	44.43	53.4***	50.40
	F_3	34.2**	14.00	50.5***	19.4***

*Gen= Generation. *, **, ***P<0.05, P<0.01 and P<0.001, respectively. PY= pod yield (kg/ha), SCMRf=SPAD meter reading at 60 DAS, SCMRz=SPAD meter reading at 80 DAS, SLAf= Specific leaf area (cm^2/g) at 60 DAS, SLAz=Specific leaf area (cm^2/g) at 80 DAS.

SLA at 80 DAS were close and similar across generations (Table 4). The mean of two generations F_1 and F_2 lines were similar for the trait SLA at 60 DAS and for SLA at 80 DAS under both well-watered and water-stressed conditions whereas the mean for the SLA at 60 DAS and for SLA at 80 DAS for F_3 lines were the highest under both well-watered and water-stressed conditions.

The coefficient of variation (CV %) and R square (R^2) for the pod yield for the F_1 , F_2 , F_3 generations ranged from 20.95 to 38.50% under well-watered and water-stressed conditions, respectively. The traits SCMR at 60 DAS and SCMR at 80 DAS showed low coefficient of variation across generations and water-regimes. The lowest CV (%) for the trial was 4.31% with $R^2 = 0.63$ while the highest was 38.50% with $R^2 = 0.84$. Across water regimes, the highest CV% for SLA at 60 DAS and SLA at 80 DAS were respectively 29.57% with $R^2=0.54$ and 35.31% with $R^2=0.48$ (Table 4).

In the cross II, the highest pod yield was found in F_3 progenies under across the two water regimes. Pod mean under well-water conditions was greater pod mean under water-stressed across the three generations. The mean performance for F_1 , F_2 and F_3 progenies were mostly similar for SCMR at 60 DAS and SCMR at 80 DAS. For the trait SCMR at 60 DAS, it ranged from 39.03 to 44.19 whereas for the trait SCMR at 80 DAS, it varied between 35.84 and 42.85 (Table 4). The highest mean

for SCMR at 60 DAS under both well-watered and water-stressed conditions was recorded on F_2 progenies. Likewise, the highest mean for SCMR at 80 DAS was recorded on the F_2 progenies under both water-stressed and well-watered conditions. The mean range for the three generations F_1 , F_2 and F_3 lines for SLA at 60 DAS and for SLA at 80 DAS were close and similar. The lowest mean for SLA at 60 DAS was observed on F_2 progenies with $146.39 \text{ cm}^2/\text{g}$ and the lowest mean for SLA at 80 DAS was recorded on $161.60 \text{ cm}^2/\text{g}$ on F_1 progenies under both stress and non-stress conditions. The mean of two generations F_1 and F_2 lines were similar and close for the traits SLA at 60 DAS and SLA 80 DAS under both well-watered and water-stressed conditions. The F_3 lines mean for SLA at 60 DAS and for SLA at 80 DAS were the higher than those observed for the same traits with the F_1 and F_2 lines irrespective of the water regimes (Table 4). The lowest coefficient of variation (CV%) for the trial was 5.28 with $R^2 = 0.66$ and the highest observed was 41.09% with $R^2=0.51$. The CV for the pod yield for the F_1 , F_2 , and F_3 generations ranged from 14.08% to 32.28% under well-watered and water-stressed conditions. The traits SCMR at 60 DAS and SCMR at 80 DAS showed low coefficient of variation across generations and water-regimes. Across water regimes, the highest CV% for SLA at 60 DAS and SLA at 80 DAS were respectively 41.09% with $R^2=0.51$ and

Table 4. Variability of traits of F_1 , F_2 and $F_{2:3}$ progenies of 2 groundnut populations evaluated under well-watered and water stress conditions.

Trait	Water regime	Population I			Population II		
		Mean \pm SE	R ²	CV (%)	Mean \pm SE	R ²	CV (%)
Generation F1	SCMR60DAS	WW	44.41 \pm 0.46	0.62	5.03	39.93 \pm 0.46	0.66
	SCMR60DAS	WS	43.96 \pm 0.46	0.74	5.21	41.78 \pm 0.46	0.52
	SCMR80DAS	WW	37.66 \pm 0.46	0.44	7.31	39.08 \pm 0.46	0.67
	SCMR80DAS	WS	40.83 \pm 0.46	0.38	7.68	40.92 \pm 0.46	0.44
	SLA60DAS	WW	212.28 \pm 10.43	0.74	19.72	166.59 \pm 10.43	0.37
	SLA60DAS	WS	158.50 \pm 10.43	0.63	29.33	175.38 \pm 10.43	0.65
	SLA80DAS	WW	166.82 \pm 9.22	0.48	31.41	161.60 \pm 9.22	0.83
	SLA80DAS	WS	167.26 \pm 9.22	0.76	23.72	161.81 \pm 9.22	0.79
Generation F2	PY	WW	10.27 \pm 1.24	0.61	29.16	11.93 \pm 1.24	0.37
	PY	WS	9.67 \pm 1.24	0.84	38.50	10.83 \pm 1.24	0.58
	SCMR60DAS	WS	43.05 \pm 2.35	0.49	8.29	44.19 \pm 1.93	0.60
	SCMR60DAS	WW	43.38 \pm 2.35	0.60	7.01	43.84 \pm 1.93	0.62
	SCMR80DAS	WS	43.94 \pm 6.25	0.57	26.07	42.13 \pm 2.73	0.59
	SCMR80DAS	WW	41.51 \pm 6.25	0.58	10.18	42.85 \pm 2.73	0.50
	SLA60DAS	WS	188.82 \pm 37.44	0.62	26.23	183.47 \pm 42.11	0.72
	SLA60DAS	WW	184.91 \pm 37.44	0.54	29.57	146.39 \pm 42.41	0.51
Generation F2:3	SLA80DAS	WS	162.47 \pm 38.01	0.48	35.31	171.60 \pm 29.72	0.78
	SLA80DAS	WW	178.93 \pm 38.01	0.58	29.23	164.29 \pm 29.72	0.62
	PY	WW	16.79 \pm 6.95	0.70	31.13	13.71 \pm 5.97	0.85
	PY	WS	16.64 \pm 6.95	0.59	32.95	11.83 \pm 5.97	0.60
	SCMR60DAS	WW	42.27 \pm 1.23	0.68	4.41	42.33 \pm 1.62	0.57
	SCMR60DAS	WS	38.43 \pm 1.23	0.63	4.31	39.03 \pm 1.62	0.69
	SCMR80DAS	WW	41.20 \pm 2.20	0.70	5.97	40.87 \pm 2.58	0.63
	SCMR80DAS	WS	35.59 \pm 2.20	0.63	10.05	35.84 \pm 2.58	0.58
Generation F2:3	SLA60DAS	WW	217.73 \pm 27.99	0.76	14.93	237.15 \pm 29.40	0.72
	SLA60DAS	WS	224.06 \pm 27.99	0.53	20.26	207.46 \pm 29.40	0.68
	SLA80DAS	WW	204.09 \pm 37.63	0.52	28.98	195.81 \pm 32.75	0.57
	SLA80DAS	WS	200.25 \pm 37.63	0.64	22.24	212.67 \pm 32.75	0.57
PY	WW	19.15 \pm 2.72	0.71	20.95	18.72 \pm 2.38	0.77	20.97
	WS	11.94 \pm 2.72	0.61	30.61	11.82 \pm 2.38	0.81	20.37

WW= well-watered, WS=Well-water-stress. PY (kg/ha), SCMR and SLA (cm²/g) = pod yield, SPAD Chlorophyll meter reading and specific leaf area, respectively.

26.80% with R²=0.62 (Table 4).

Genetic advance, parent-offspring regression and correlation of F_1/F_2 and F_2/F_3 progenies

In cross I, the parent-offspring regression for $F_1:F_2$ and F_3

progenies ranged from 0% for pod yield to 18% \pm 0.15 for SCMR 60 DAS under water-stressed conditions while under well-watered conditions, $F_1:F_2$ regression varied from 6 \pm 0.18% for SLA 80 DAS to 36% for SCMR 80 DAS. Regression of F_2 vs F_3 values ranged from 0.00 for SLA 80 DAS to 18 \pm 0.06% for pod yield. No significant relationship was detected between $F_2:F_3$ progenies and

Table 5. Genetic advance, parent-offspring regression (with their standard errors) and correlation of F_2 on F_1 , and $F_{2:3}$ on bulked F_2 mean from Cross I evaluated under well-watered and water-stress conditions.

Trait	ENV	Genetic advance (GA%)		Parent-offspring regression (<i>b</i>)		Parent-offspring correlation (<i>r</i>)	
		F_2	F_3	$F_1:F_2$	$F_2:F_3$	$F_1:F_2$	$F_2:F_3$
SCMRf	WW	0.00	1.58	0.14 ± 0.23	-0.02 ± 0.69	0.05	-0.02
	WS	0.63	0.66	0.18 ± 0.12	-0.10 ± 0.06	0.08	-0.08
SCMRz	WW	5.59	4.10	0.36 ± 0.26	0.06 ± 0.02	0.12	0.12
	WS	0.99	2.94	0.16 ± 0.16	-0.24 ± 0.09	0.05	-0.14
SLAf	WW	8.31	6.59	0.28 ± 0.15	-0.28 ± 0.08	0.16	-0.16
	WS	0.00	0.00	0.16 ± 0.11	0.02 ± 0.08	0.08	0.02
SLAz	WW	0.00	0.00	0.06 ± 0.18	-0.22 ± 0.11	0.02	-0.10
	WS	3.75	5.52	-0.16 ± 0.10	0.00 ± 0.09	-0.08	0.00
PY	WW	7.94	10.86	0.12 ± 0.20	0.18 ± 0.06	0.05	0.15
	WS	0.00	0.31	-0.20 ± 0.12	-0.16 ± 0.04	-0.09	-0.18

[§] ENV = environments (Well-watered WW and water-stress WS). SCMRf, SCMRz, SLAf, SLAz and PY = SPAD chlorophyll meter reading at 60 DAS at 80DAS, specific leaf area (cm^2/g) at 60 DAS and at 80 DAS and pod yield (kg/ha). GA (%), *b*, and *r* are genetic advance, regression coefficient and correlation coefficient, respectively.

almost all the regression coefficients were negative in cross I (Table 5). Similar trends of negative heritability values via regression of F_1 vs F_2 and that of $F_2:F_3$ were observed under both drought stress and well-watered conditions in cross II (Table 5). The highest heritability estimates for $F_1:F_2$ were 42% for SCMR 60 DAS and at 80 DAS under well-watered conditions, $20 \pm 0.20\%$ for SLA at 60 DAS and at 80 DAS under drought stressed conditions. The highest heritability estimates for $F_2:F_3$ progenies were observed from SCMR 60 DAS ($22\% \pm 0.09$) under well-watered conditions and SLA 60 DAS ($22 \pm 0.08\%$) under water-stressed conditions. Standard error values were often higher than regression coefficients. Inter-generation regression coefficients were lower and not significant for SCMR and SLA for F_1 vs F_2 while negative and non-significant correlations were mostly detected for F_2 vs F_3 for most of the traits in cross I (Table 5). For cross II, $F_2:F_3$ correlations were nonsignificantly negative for all traits studied under drought stress and well-watered conditions as well. Similar trends were detected for pod yield, SCMR 60 DAS and SLA 60 DAS under water-stressed conditions. A positive and significant correlation was found for F_1 vs F_2 with the trait SCMR 80 DAS under well-watered conditions (Table 6).

DISCUSSION

Performance of traits

The ability to maintain dense chlorophyll under water

deficit conditions is a drought resistance mechanism (van der Mescht et al., 1999; This et al., 2000; Arunyanark et al., 2008). Genotypic differences were found among lines and the chlorophyll content in the plants decreased as they reached their physiological maturity under water-stressed conditions. SCMR values at 60 DAS were 38.77 under water stress and 42.37 under well-watered conditions. Unlike SCMR at 60 DAS, the SCMR at 80 DAS of plants under stress and non-stress conditions were 35.87 and 41.10, respectively. Thus, genotypes tend to reduce their SLA from 60 DAS to 80 DAS and under drought conditions. SLA was decreased by drought stress and differed between genotypes. These findings were in agreement with the results of Liu and Stützel (2004) working on vegetable amaranth, Songsri et al. (2008) on groundnut, Zhang et al. (2015) on maize. Songsri et al. (2008) reported that groundnut genotypes having an ability to maintain higher SCMR and lower SLA under drought stress should be more tolerant to drought. This indicates that reduction in specific leaf area is a good indication of tolerance to drought and it is a water-saving mechanism where plants tend to reduce their transpiration by closing their stomata.

Parent-offspring regression, correlation and genetic advance

In the cross I, almost all the heritabilities values were low and were negative. Similar trends of negative heritability values via regression of F_1 vs F_2 and that of F_2 vs F_3 were

Table 6. Genetic advance, parent-offspring regression (with standard errors) and correlation of F_2 on F_1 , and $F_{2:3}$ on bulked F_2 mean from Cross II evaluated under well-watered and water-stress conditions.

Trait	ENV [§]	Genetic advance (GA%)		Parent-offspring regression (b)		Parent-offspring correlation (r)	
		F_2	F_3	$F_1:F_2$	$F_2:F_3$	$F_1:F_2$	$F_2:F_3$
SCMRf	WW	0.50	0.24	0.08 ± 0.21	0.22 ± 0.09	0.04	0.13
	WS	1.19	3.18	-0.12 ± 0.17	-0.10 ± 0.06	-0.06	-0.08
SCMRz	WW	0.00	2.90	0.42 ± 0.11	-0.16 ± 0.10	0.58	-0.09
	WS	0.00	0.00	-0.08 ± 0.16	-0.24 ± 0.09	-0.05	-0.13
SLAf	WW	5.63	7.15	0.10 ± 0.21	0.14 ± 0.07	0.04	0.09
	WS	0.00	2.97	0.20 ± 0.20	0.22 ± 0.08	-0.09	-0.13
SLAz	WW	8.44	9.77	-0.40 ± 0.15	-0.20 ± 0.10	0.03	-0.11
	WS	5.16	3.69	0.22 ± 0.21	0.00 ± 0.11	0.10	0.00
PY	WW	21.75	23.64	0.14 ± 0.12	-0.12 ± 0.07	0.11	-0.09
	WS	0.00	9.21	-0.24 ± 0.21	-0.12 ± 0.04	-0.20	-0.14

[§] ENV = environments (Well-watered WW and water-stress WS). SCMRf, SCMRz, SLAf, SLAz and PY = SPAD chlorophyll meter reading at 60 DAS at 80DAS, specific leaf area (cm^2/g) at 60 DAS and at 80 DAS and pod yield (kg/ha). GA (%), b, and r are genetic advance, regression coefficient and correlation coefficient, respectively.

observed under both drought stress and well-watered conditions in cross II. This result was in agreement with the findings of Ntare (1999) who reported non-significant $F_2:F_3$ regression for pod yield and physiological components such as dry matter, crop growth rate and the length of the reproductive period in groundnut. However, among the two crosses or populations, several valuable regression coefficients were detected with highest heritability estimates in $F_1:F_2$ were 42% for SCMR 60 DAS and at 80 DAS under well-watered conditions, 20% for SLA at 60 DAS and at 80 DAS under drought stressed conditions. The highest heritability estimates in F_2 : F_3 progenies were observed for SCMR 60 DAS (22%) under well-watered conditions and SLA 60 DAS (22%) under water-stressed conditions. These results probably involved additive genes for the SLA and SCMR traits. For both crosses, higher standard error values were obtained from parent-offspring heritability estimates, often higher than the regression coefficients. Some (2012) have had similar high value of standard errors of heritability in a study of F_1 orange-flesh sweet potatoes and he concluded that this revealed unexplained factors which were important and prevented a better understanding of the inheritance. Intergeneration coefficients were not significant and they were lower for SCMR and SLA for F_1 vs F_2 while negative and non-significant correlations were mostly detected in F_2 vs F_3 for almost all the traits in cross I. For cross II, $F_2:F_3$ correlations were negative and non-significant for all traits studied under drought stress and well-watered conditions as well. Similar trends were detected for pod yield, SCMR 60 DAS and SLA 60 DAS under water-stressed conditions. A positive and significant

correlation was found for F_1 vs F_2 with the trait SCMR 80 DAS under well-watered conditions. These results were in agreement with conclusions reached by other researchers (Halward et al., 1990; Ntare, 1999) who reported low and non-significant correlations between yields of F_2 : F_3 and F_3 and F_4 bulk populations in groundnut. They concluded that selection of pod yields in early generation could be delayed to later generations. Genetic Advance (GA) is a more reliable index for understanding the effectiveness of selection in improving the traits because the estimates are obtained from the product of heritability, phenotypic standard deviation and intensity of selection (Patil et al., 2015). It is therefore a drawing force in selection, which measures the importance of the genes that passed from parent to offspring. The observation of negative values from the variances biases the results of the estimates of heritabilities and genetic advance. This makes it difficult for the prediction. Except for the high genetic advance for SLA 80 DAS (83.75%) under both well-watered and water-stressed conditions, genetic advance for the two crosses were low to moderate irrespective of the generation and environment under study. The GA was low for SLA 80 DAS under water-stressed conditions (8.75%) in F_1 . This result is in accordance with results of Vishnuvardhan et al. (2012) who reported GA of 2.58% in genetic variability studies for yield attributes and resistance to foliar diseases in groundnut. Shukla and Rai (2014) reported low GA for pod yield (7.18%) and pod yield per plant (5.18%) in evaluating groundnut genotypes for yield and quality traits. Nath and Alam (2002) reported GA of 16.37% in groundnut.

In the two experiments, twenty individual populations were used at F_1 while forty five individual populations were used in F_2 and F_3 . Conclusions were then drawn based on one year evaluation with a small number of segregating populations in two replications. Walker (2012) stated that variance, heritability, and genetic correlations are often estimated in a single study and then considered representative of a population, and this is valid, to the extent that the population represented remains the one of interest. He concluded that if any of these parameters are estimated in a study of one set of genetic material, their application to another genetic material may be questionable depending upon the differences between them. Moreover, Conner and Hartl (2004) reported that twenty families should be considered an absolute minimum, and fifty or more is necessary for reasonable statistical power in quantitative genetic experiments of any design. These authors stated that the number of families that reflect the variance is of prime importance to the power of the analysis. They concluded that a finding of no significant additive variance with less than 50 families should be interpreted with caution, because the lack of significance could easily be due to a lack of statistical power rather than a real lack of variance. For low narrow-sense heritability, Resende et al. (2013) proposed an application of combined selection method such as selection indices. In plant breeding, flexible models or methods are available to help the breeder for selecting promising genotypes. Likewise, Cooper et al. (2013) suggested that because of the low estimates of heritability in yield trait in wheat, indirect selection could be more effective.

Conclusion

Parent-offspring regression for the two populations evaluated under well-watered and water-stress conditions were low to moderate and revealed importance of non-genetic effects. Parent-offspring correlations were also low and mostly showed negative and non-significant coefficients for traits studied under drought stress and well-watered conditions as well as for each of the studied populations. Consequently, the genetic advances for the two crosses were mostly low to moderate irrespective of the generation and environment under study.

Based on the reference populations, progress in selection at early generation in groundnut could be slow for a complex trait like drought. We suggested indirect selection with indices based on pod yield and drought related traits under contrasting drought conditions to identify promising lines since the heritability estimates were low.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

The trade of the cattle fair in alagoas' agreste:a description of this step of the comercialization process

José Adeilton da Silva Filho^{1*}, André Maia Gomes Lages¹, José Crisólogo de Sales Silva², Conceição Maria Dias de Lima² and Luciano Celso Brandão Guerreiro Barbosa¹

¹Faculdade de Economia, Administração e Contabilidade, Universidade Federal de Alagoas Universidade Federal de Alagoas – Ufal, Brazil.

²Departamento de Zootecnia, Universidade Estadual de Alagoas – Uneal, Brazil.

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This paper presents a descriptive study about the economic activity in cattle fairs from Alagoas' countryside, Brazil, through visits in loco, application of questionnaires and secondary data obtained from Adeil in the two main fairs on Alagoas' countryside, Brazil. The objective was to study the quantity of animals in the events, identifying the marketing channel and its segments, the negotiation volume and value, and also how to describe the working of this fair, the types of consumers and other agents. The sampling of secondary data was obtained through the animal transit guide (GTA) in the defense and farming system (Sidagro) to the Canafistula fair on the period of March to September of 2015. On this sampling, the total number of bovine that entered the fairs was 30,732 heads with 521 bovine negotiated in average, coming not only from Alagoas but also from Pernambuco and Sergipe, with 93, 04 and 03% being the respective percentages. The number of deals in average was 128 by fair, according to the data period collected in 47 fairs. The value negotiated per week, adding both fairs, is in average R\$474,711.04 or US\$131,135.65. The cattle fairs from Alagoas' countryside have three possible buyers' types, that are classified as for slaughter, trade and rearing. Thus it was possible to diagnose, in a way, the economic relevance of the event as also the confirmation of a distribution channel and consequently its macrosegments.

Key words: Cattle fair, economy, marketing channel, Alagoas.

INTRODUCTION

Brazil has as an important economic activity the production of bovine, and, according to data acquired by Brazilian Institute of Geography and Statistics (IBGE) (Brasil, 2006), since 2001 the quantity of bovine cattle is bigger than Brazilian individuals, reaching the mark of 215,199,488.00 bovine animals in 2015. The northeast region concentrates approximately 22% of the total herd of cattle, on which the state of Alagoas has a herd

of 1,255,696.00 animals.

Consequently, the counties from Alagoas' countryside have in the livestock one of its main economic activities, that according to Lira (2007) comes since the conception of the state, in 1817, when the mill lords need to rear animals, amongst them the bovine, to auxiliate the sugar production and in the livestock. "The mill lords, needing many animals to perform various tasks in the sugarcane

*Corresponding author. E-mail: adeiltonfilho@bol.com.br.

fields, began to rear horses, donkeys and bovine [...]” (Lira, 2007: 14, our emphasis).

The traditional animals fairs, known also as livestock fair, is a popular trade in a big number of the countryside counties from the state of Alagoas, that works as means to transact the exceeding animals produced in the farms to other activities such as rearing and slaughter. The cattle fair is a market of great significance to the population of northeast and consequently to Alagoas, be it economically as much as culturally, historically and socially. Since in every fair people from different social classes seek to socialize in the trade environment, this also happens in the cattle fair. Many people go there not only to commercialize, but also to seek leisure and entertainment.

“In the cattle fair [...] merchants, watchmen, bus drivers, doctors, lawyers and cattle ranchers, that have in common the ‘taste for cattle’, enjoy Sunday’s morning to meet, talk, ‘look at the cattle’ and also to do business” (MAIA, 2007: 22).

In order to know the economic activity of this important and popular mechanism of the agricultural trade, it was sought to describe its operation, the types of commercialization, the current values in reais moved in these activities, quantities of actives, types of consumers, distinguishing them through their buying finality, origin location of the animals, identifying the commercialization channel and the productive chain with its possible macrossegments according to Batalha (2009).

METHODOLOGY

The research is of descriptive and exploratory character with case study. In this frame, the study preserves some doses of pioneering in a local level. The application of the method is done through visits in loco, in the two most important cattle fairs of the state according to recommendation from the Agency of Defense and Farming Inspection of Alagoas (Adeal), the cattle fair from the village of Canafistula in the county of Palmeira dos Índios – AL and the cattle fair from Dois Riachos located in the county of same name. Primary data will also be used through the application of questionnaires and secondary data collected on the System of Farming Defense from Alagoas (Sidagro – AL).

The choice of primary data comes from the necessity of quantifying the price of the animals acquired in the negotiations, and the qualitative data relative to sex and age of these animals in months, as well as the type of consumers of these actives. The consumers were grouped according to the finality to the active (bovine) acquired in the negotiations. The secondary data from Sidagro – AL comes from the necessity of measuring the real number of negotiations that happened in the fair, being the negotiations the population studied and thus finding the feasible finite sample number for application of the quantitative questionnaire, and information on entrance and exit of animals and their locals of origin.

Sampling

The settlement of the sample to diagnose the proportion of each type of consumer on the fair will be given based on the estimative of the finite population proportion, through the formula:

$$n = \frac{Z^2 \times P \times Q \times N}{d^2 \times (N-1) + Z^2 \times P \times Q} \quad (1)$$

where: N = size of the population, Z = trust level, P = proportion of expected accuracy, Q = $(1 - P)$, d = sampling error.

The data collected with Sidagro are about the number of confirmed negotiations, that is, the quantity of sellers and buyers who closed a purchase agreement, as also the number of cattle that enters the fair and the origin of those who buy and sell at the fair. The period of data obtained on Sidagro to the Canafistula fair is from March 23rd to August 31st 2015, and to the fair of DoisRiachos the obtained data are from April 1st to September 23rd 2015. To get to the necessary sampling number to apply the questionnaire the average number of closed deals on both fairs was researched and then was made the utilization of 95% of trust level, 50% of expected accuracy¹ and 5% for sampling error.

According to the data collected on Sidagro and processed in the electronic spreadsheet, the average of confirmed negotiations, that is, the quantity of sellers and buyers that closed a purchase agreement in the period of approximately six months on the two main fairs from the countryside of Alagoas is 127.3617021, considerate 128 negotiations per fair, what asks for a minimum sample of 96 interviews.

CHARACTERIZATION OF THE OBJECT OF STUDY

Located in the left margin in the way Maceió – Santana do Ipanema of the highway BR – 316, in the village Canafistula of Frei Damiao on the county of Palmeira dos Índios/ AL, are the currals of the cattle fair of Canafistula. Located also in the left margin and of the same way of BR – 316, on the county of Dois Riachos, are the currals of the cattle fair of DoisRiachos.

The Canafistula fair takes place every Monday, and the Dois Riachos every Wednesday. The fairs begin at dawn, around 4:30 AM, when the sellers guide the animals to the fairs corrals where the negotiations happen. At first sunlight the day's negotiations begin, and they go as far as midday. However, according to the sellers, after 11 AM the chances of selling an animal drop highly. After the negotiations are finished the animals are conducted by the sellers and other workers, called “vaqueiros”, the cowboys, that have the role of guiding the cattle herding it from the corrals, making it climb a ramp, called “embarcadeiras”, to the cattle truck or heading by foot to their new or old places. The process makes it so that the movement on the fairs goes up to 1PM maximum. The peak of the fair is at 9 AM, when the flow of buying and selling is at its highest point. The sellers are the first to come and the last to leave, the cost to transport each animal is R\$ 20.00 or US\$5.52 a fixed price², there is also the expense of R\$ 1.00 or US\$0.28 per animal with the animal transit guide (GTA) and the fee to enter the fair of Dois Riachos of R\$ 2.50 or US\$0.69/animal. In Canafistula there is not this fee, although there is a monthly rent of R\$ 15.00 or US\$4.14 per corral. There is in Canafistula a community corral, if the seller does not want to rent an individual corral, that is free of charges.

The animals are negotiated in spot market, as in most fairs, although they can also be sold on credit. The credit according to some sellers is one of the most used modalities among buyers who negotiate also with cattle, therefore buyers who buy to commercialize, be it on the same day, in a process similar to the day trade of the financial market, or in another day or even another fair. The buyers according to the application of the questionnaire

¹ Because the possible values that would be found were unknown

² This value is only for destinations up to 40 km, that is to the route of most assiduous cattle Sellers.

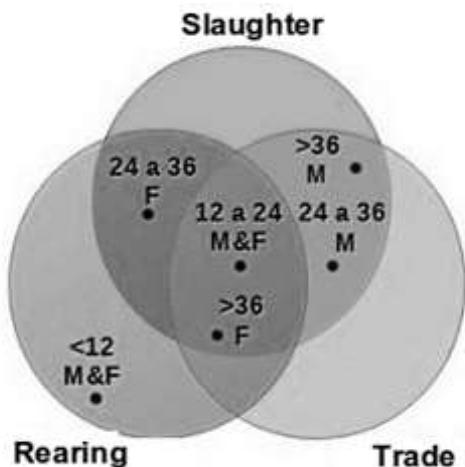


Figure 1. Buyers' groups and their demands.
Source: Authors (2015).



Figure 2. Cattle fair of Dois Riachos. (Alagoas – Brazil).
Source: Authors (2015).

are divided in three groups with distinct finalities: slaughter, trade or rearing. Even though each group wants animals with different specificities, some animals have the gender and age that answer to the three groups' needs, usually animals from 12 to 24 months both male and female or cows older than 36 months, as it is seen on the Figure 1.

The cattle fair is not only about bovine selling, there is also caprine, swine, ovine and equine commerce. At some moments of the fair, there are also some people that risk selling birds of many kinds, and on the surroundings of the fair, still very close, there is the sale of clothing and utensils for cowboys, as well as many other kinds of products that would be offered at any commerce. There are also snacks and meals, being so an event that moves the economy of both places a lot (Figures 2 and 3).

RESULTS AND DISCUSSION

According to the data found and processed from Sidagro – AL, the total number of bovine that entered the fair during the analyzed months was 30,732 heads, being in Canafistula 9,416 and Doisriachos 21,3016. The average of entrance in the fairs is 732 bovine, being made around

128 deals with approximately 4 animals sold on each deal, thus the average of bovine negotiated on the two main fairs of the countryside of Alagoas is 512 animals. The expressiveness of the bovine herd that moves to both fairs is noted. Animals that, according to the same data, come not only from Alagoas (AL), but also from Pernambuco (PE) and Sergipe (SE), the percentage represented on Figure 4.

The counties where the herds come from are 94 in total, belonging to the three previously mentioned states, being the fair of Doisriachos the meeting and commerce point of 66 counties, while Canafistula is of 28 counties located only on the states of Alagoas and Pernambuco. The furthest registered county is Santa Maria da Boa Vista – PE, that is 418 km away from the fair of Dois Riachos. Its presence was recorded only twice on the analyzed period (Table 1). The furthest county that destines cattle to the fair of Canafistula is Serra Talhada – SE, that is 327 km away from Canafistula's corrals. In average the counties are 93.97 km away from the corrals, even though this number does not show the everyday of the fairs, because the frequency of the furthest counties is very limited, being the counties of most merchants and animals 46 km away in average in both fairs (Table 2). The state of Alagoas, as shown, represents 93% of the total of sellers and their animals that entered the fairs during the period of data collected. The county of Palmeira dos Índios is the one that most destines animals to the fairs, followed by Santana de Ipanema and Estrela de Alagoas (Table 3).

The frequency is something very relevant on the fairs, sellers from distant counties do not go often. Although the average number of sellers by fair is 75, only 20 sellers are in 80% of the analyzed fairs, thus, most of the sellers are not dealers, are cattle ranchers that go to the fairs when they have surplus on their properties, according to the Table 4. To the environment accomplished in the cattle fair, askers and suppliers work within a system that can be denominated as a spot market, something very close, in theory, to denominated pure market. According to Batalha (2012) on the spot market it's possible to go back to the same fair and to the same seller and buy the same kind of product again. With the transaction being resolved in that instant, this commerce is also called physical market, being frequently used in transactions like commodities³. An important characteristic of the cattle fair is the considerable monetary movement that exists in this bovine commerce. The number of deals, in average, is 128 per fair, according to the data collected in 47 fairs, as it's shown by table 03. The average deal value⁴ is R\$3,708.68, or US\$1,024.50 that is, R\$ 474,711.04 per fair or US\$131,135.65 in average. In the year 2015 will happen 100 fairs, so we can assume a

³ Products that work as feedstock, produced in scale and that can be stocked without losing quality, such as petroleum, frozen orange juice, fat ox, coffee, soy and gold.

⁴ Data obtained through the applied questionnaire.



Figure 3. Cattle fair of Canafistula - district of the county of Palmeira dos Índios – Alagoas.

Source: ADEAL (2015).

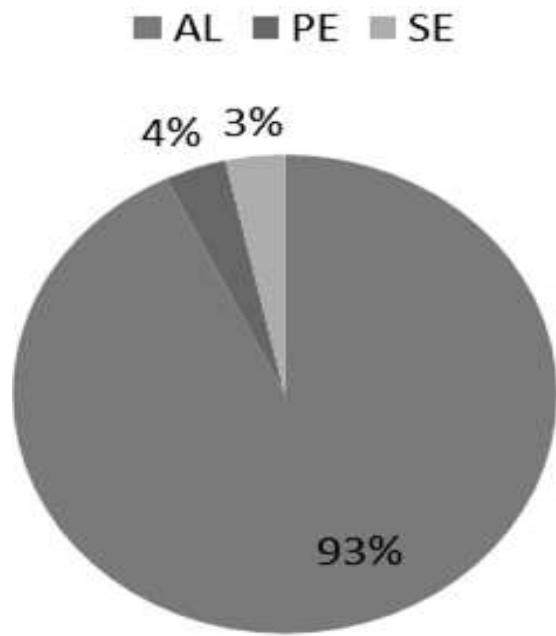


Figure 4. Percentage of bovine per state.

Source: Authors (2015).

Note: PE = Pernambuco, AL = Alagoas, SE= Sergipe, Brazil States.

value of approximately R\$ 47,471,104.00 or US\$13,113,564.64 for the referred year (Table 5). Before these results, it is known that the cattle fairs from Alagoas' countryside have three possible buyers, that are classified for purposes of slaughter, trade and rearing, and with this data it is possible to trace a draft of the commercialization

channel, where it is allowed to understand the markets and the development of this system, as Figure 5 shows.

Figure 5 shows one of the bovine commercialization channels on the countryside of the state of Alagoas, where the presence of the three types of characters diagnosed on the research can be verified, along with its

Table 1. Distance (in kilometers) from the counties of origin of the sellers and their animals to the corrals of the fair of Dois Riachos.

County of origin	UF	Km	County of origin	UF	Km
Anadia	AL	113	Ouro Branco	AL	49.9
Arapiraca	AL	74.5	Palestina	AL	65.9
Batalha	AL	63	Palmeira Dos Índios	AL	54.3
Belém	AL	78	Quebrangulo	AL	80.4
Belo Monte	AL	82.1	Rio Largo	AL	171
Cacimbinhas	AL	13	Santana Do Ipanema	AL	18.6
Campo Grande	AL	107	São José Da Tapera	AL	48
Canapi	AL	67.3	São Sebastião	AL	119
Capela	AL	139	Senador Rui Palmeira	AL	64.2
Carneiros	AL	55.4	Tanque D'arca	AL	90.9
Coité Do Nória	AL	81.2	Taquarana	AL	90.9
Craibas	AL	53.8	Traipu	AL	99.5
Delmiro Gouveia	AL	114	Itaíba	PE	121
Dois Riachos	AL	0	Águas Belas	PE	103
Estrela De Alagoas	AL	38.4	Caetés	PE	151
Girau Do Ponciano	AL	99.1	Santa Maria Da Boa Vista	PE	418
Igaci	AL	66.3	Serra Talhada	PE	335
Inhapi	AL	79.2	Caruaru	PE	221
Jacaré Dos Homens	AL	56.9	São João	PE	137
Jaramataia	AL	49.6	Garanhuns	PE	125
Lagoa Da Canoa	AL	87.4	Calumbi	PE	331
Major Isidoro	AL	27.7	Bom Conselho	PE	76.9
Mar Vermelho	AL	113	Capoeiras	PE	149
Maravilha	AL	43.6	Porto Da Folha	SE	88.6
Mata Grande	AL	98	Nossa Senhora Aparecida	SE	157
Minador Do Negrão	AL	42.7	Itabaiana	SE	197
Monteirópolis	AL	51.8	Tobias Barreto	SE	287
Olho D'água Das Flores	AL	37.2	Monte Alegre De Sergipe	SE	106
Olho D'água Do Casado	AL	100	Poço Redondo	SE	138
Olivença	AL	20.9	Nossa Senhora Da Glória	SE	135
Pão De Açúcar	AL	66.5	Canindé De São Francisco	SE	115
Piranhas	AL	94.6	Poço Verde	SE	311
Poço Das Trincheiras	AL	29.1	Aquidabã	SE	171

Source: Authors - adapted from ADEAL data (2015).

flows of the selling and buying of animals. The first important point is to show that the characters that use the animal fairs have in it their central role, that is, the fair is the core of every economic transaction and of the flows due to each market. Thus, it is about a representation, where the agents involved have only this commercialization routine, because there could be links in the chain, for example, between cattle rancher and slaughterhouse in a direct way, but that is not the case. The second important point is to notice that many times the figure of the middleman and of cattle rancher gets confused, because it can be the same individual in different time and situations, that many times alternate themselves, as there is also, between them, a bilateral market. The third and last point, to begin the analysis of the chain, is in the

presence of the slaughterhouse, that has only a unilateral flow⁵, being dependent of the dealers that buy on the fair with the intent of taking the cattle to be slaughtered.

The chain illustrated on Figure 6 shows four markets and their bilateral and unilateral flows rotating around the fair, where the rancher produces and sells the animal using either the institution that is maintained by the town hall of each county or selling to a middleman, that will use the fair to conclude the commercialization or sell directly to another rancher. Middlemen also rear animals, although

⁵ It is true that these agents can buy meat with the slaughterhouses, but they are a very small parcel before the vast number of other consumers that don't show enough representativity to the study of the productive chain, where they can't interfere with the prices.

Table 2. Distance (in kilometers) of the sellers and their animals' counties of origin from the fair of Canafistula.

County of origin	UF	Km
Palmeira Dos Índios	AL	11.1
Igaci	AL	21.2
Campo Grande	AL	85.7
Estrela De Alagoas	AL	5.8
Quebrangulo	AL	37.3
Minador Do Negrão	AL	20.5
Taquarana	AL	42
Batalha	AL	75.3
Craíbas	AL	30.8
Bom Conselho	PE	33.8
Mar Vermelho	AL	69.7
Belém	AL	34.9
Dois Riachos	AL	41.2
Paulo Jacinto	AL	53.7
Coité Do Nóia	AL	38
Cacimbinhas	AL	31.8
Viçosa	AL	61.4
Igreja Nova	AL	115
Atalaia	AL	84.7
Chã Preta	AL	77.2
Santana Do Ipanema	AL	61.7
Brejão	PE	57.4
Maravilha	AL	86.7
Limoeiro De Anadia	AL	61.2
Serra Talhada	PE	327
Ouro Branco	AL	93
Tanque D'arca	AL	47.8
Maribondo	AL	58.2

Source: Authors – adapted from ADEAL data (2015).

Note: PE = Pernambuco; AL = Alagoas; SE= Sergipe. Brazil States

Table 3. Total of bovine by counties from Alagoas in the fairs during the analyzed period.

Alagoas' counties	Total of bovine
Palmeira Dos Índios – AL	3,959
Santana do Ipanema – AL	2,929
Estrela de Alagoas – AL	2,600
Other counties from Alagoas	19,035

Source: Authors – adapted from ADEAL data (2015).

they dedicate, as main activity, the commercialization of bovine, that, as mentioned, sell to some rancher or use the fair. The slaughterhouse on the final end has only the unilateral role of receiving the animals acquired at the fairs by the dealers.

The channel could better represent the productive chain if complemented with the macrossegments

presented by Batalha (2009). When the cattle fair market is observed it is known that it includes other markets inside the sectors and their segments. The segment "before the gate", that represents the activities of support to the production such as fertilizers and agricultural inputs; the segment "inside the gate", where the activities like rearing of bovine happens; and the segment "after

Table 4. Percentage of sellers' presence.

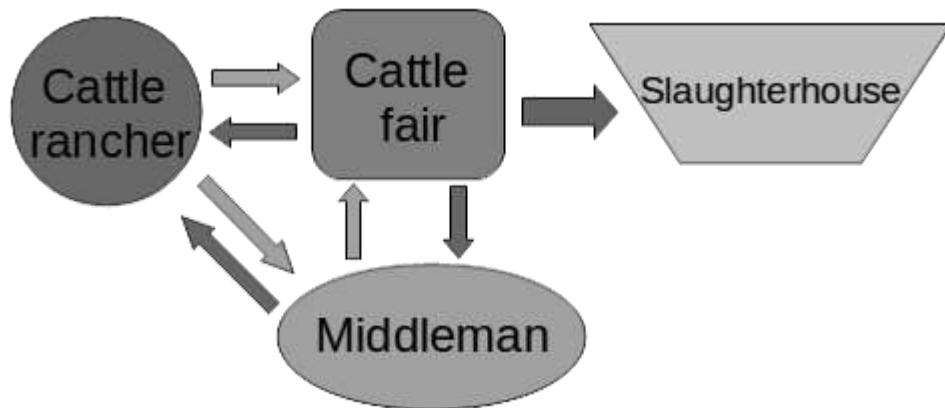
Presence (%)	Dois Riachos	Canafistula	Total
10	2,581	190	2,771
50	56	25	81
80	12	08	20

Source: Authors – adapted from ADEAL data (2015).

Table 5. Number of deals on the fairs on the analyzed period.

Month	Number of deals	Number of fairs	Average of deals
March	222	02	111.00
April	1.075	08	134.38
May	778	07	111.14
June	1.369	10	136.90
July	941	07	134.43
August	847	09	94.11
September	754	04	188.50
Total	5.986	47	127.36

Source: Authors – adapted from ADEAL data (2015).

**Figure 5.** Bovine commercialization channel on the countryside of Alagoas.

Source: Authors (2015).

the gate", where there are the processing industries and the distribution lines, that are the slaughterhouses and the meat fridges. To Batalha (2009) the structure of the productive chain and its macrossegments is the following: the first macrossegment being the initiatives for the formation of the farming activity, the production; the second corresponding to every transformation of feedstock from the agricultural sector to agroindustrial products; and the third being the actions of buying and selling done by the molds of retail or wholesale.

Therefore the process before the gate includes the production of inputs used by the ranchers, such as ration, cycling among other. Inside the rural macrossegment the

farmers are found, businessmen that in Alagoas, for example, use the cattle fair to commercialize with the other farmers and sellers, and also with the dealers. The latter buys cattle to slaughter, thus providing the animals that are negotiated in the fairs to the industrial macrossegment, where lies the transformation companies, like the private meat fridges and the main slaughterhouses from the State. The commercial macrossegment is at the end of the system, where the establishments like restaurants, steakhouses, buffets, supermarkets, hotels and other receptors are supplied by the slaughterhouse.

Along the chain could be shown ramifications with other

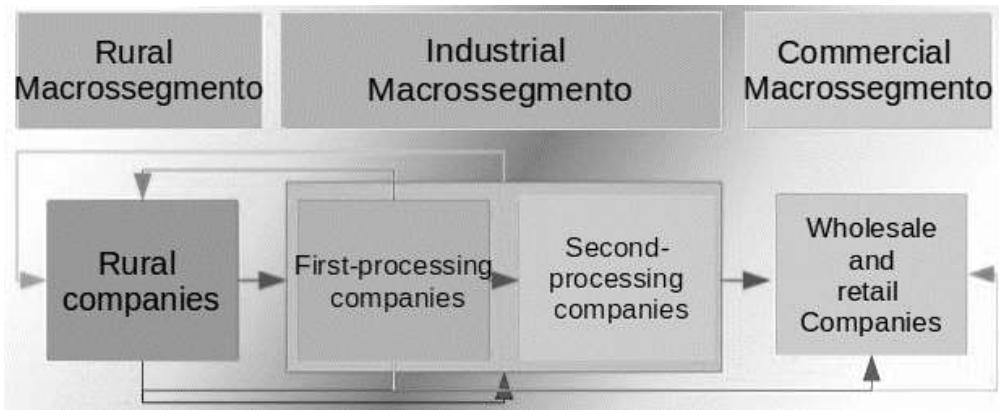


Figure 6. Agroindustrial system, its systems and supply flows.

Source: adapted from BATALHA (2009).

markets, for example the intense volume of the transportation system, or the many markets of goods and services in the fair, that exist in an indirect way around the event. One of the most demanded services for the working of the whole commercialization channel that includes the chains and their macrossegments is the transportation, that has been being developed in the countryside of Alagoas, and also, according to Mendes (1998), in all Brazilian agribusiness. [...]The transportation role of production factors, or products creates the possibility that these agribusiness goods are available in the place, time and quantity wished by the consumers, being clear its importance before the growing of the geographic distance between production and consumption, which economic development, globalization and other factors have been causing in Brazil for some time now (MENDES, 1998: 195).

Even with the development of the transportation means, mainly road transport with the cattle trucks, it is not unusual for the producers to go walking alongside the animals or by horse, guiding the animals to their place in the fair, in accordance with the animal transit guide (GTA). This modality is called "by foot", and is used by the producers or merchants that live up to 6 km away in average; the low costs, in some cases almost null, compensate the hard work, contrasting with the road transportation that charges R\$ 20.00 or US\$5.52 per head each 40 km, as it is seen in Figure 7. In the case of distances bigger than 90km this small extra group of producers doesn't spend with rented vehicles, but with private vehicles, be it vans or their own cattle trucks. Because of this peculiarity they are not found in the graphic.

The four markets in the chain: between rancher and fair; slaughterhouse and fair; middleman and fair and rancher and middleman show, sometimes, demands for a same group of animals, mostly females and males from 12 to 24 months of age, where each of the three types of buyers shows different payments for the same group of

animals, as it is presented by Table 6.

In order to understand the markets of the countryside and their complex price mechanisms, it's necessary to understand the theoretical aspects that compose part of the formation of offer and demand curves for the products. According to Callado (2011), the dispositions and disponibilities of the applicants and suppliers generate in the markets the prices and quantities of balance. Multiple variables, such as tastes and preferences, available income, climatic and technologic circumstances among others explain the price variation, in part. Thus, the difference of the average value paid by each kind of buyer happens because of many reasons, being the buyer with finality of trading (middleman) the one who pays more for the animals, and then slaughter and rearing. This is extremely important for it shows that the ones interested by the animals have distinct evaluations, where the pure market structure cannot make the right dissociation, like, for example, a rancher that invested in the genetic improvement⁶ of the 12 to 24 month old animal has the intention of finding another rancher who sees this quality, but this is very hard in the event, because of the randomness and low signaling of the fair. This way, they can end up selling to a middleman, and if they cannot find one, sell to a buyer with finality to slaughter. This implies a probable process of adverse selection, which results in animals with lessened genetic quality.

Conclusion

One of the main contributions of this descriptive study was the confirmation of the fair as an important

⁶ This kind of genetic enhancement is very limited in the fairs basically it can be a mixed race animal that shows crossing with breed animals, or any other form of genetic enhancement. This elimination comes from the commerce system itself.

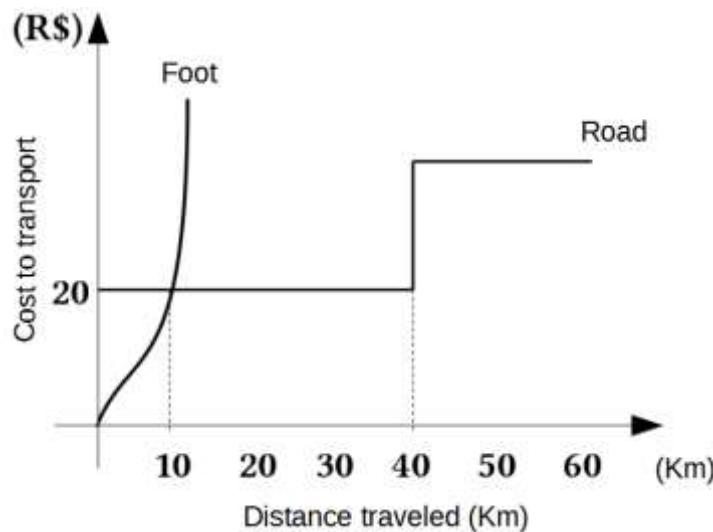


Figure 7. Relation between the distance (km) and the cost to transport the animals in the modality by foot and road for sellers without their own vehicle.

Source: adapted from MENDES (1998).

Table 6. Value paid in average by the three types of buyers.

Type of buyer	Age of the animal (in months)	Average of the value paid (in Reais)	Average of the value paid (in Dollar)
Finality: slaughter	12 a 24	R\$4,086.11	US\$1,128.76
Finality: trade	12 a 24	R\$7,707.78	US\$2,129.22
Finality: rearing	12 a 24	R\$2,405.88	US\$664.61

Source: Authors – adapted from ADEAL data (2015).

commercialization channel to the bovine farming of the state of Alagoas, and, consequently, its possible macrossegments, where the value inflicted only on the bovine negotiations shows an activity in the magnitude of dozens of millions per year, including in the negotiations importing and exporting up to three federative units on the border, and ninety four counties, thus showing to be a strong commercialization point. The descriptive study, in a succinct way, also approached the way these negotiations occur, and how each agent acts inside this commerce system, where it was possible to see the process' dynamic and the central participation of the fairs in the studied commercialization channel, formidable fact to the scientific knowledge of an important economic activity, never before studied in Alagoas.

Besides that, the peculiarities of these two fairs. The one located in the county of Palmeira dos Índios, Agreste, that represents a transition region between the biomes Mata Atlântica and Caatinga, sertão, northeastern semi-arid, a type of savannah existent only in Brazil. It is there where the fair of Dois Riachos is located. Besides that, the meat fridge mentioned has very

limited dimensions and roles more restricts than the ones located in other parts of the country, where farming shows bigger volume and development rate. Thus new papers can be done before the results found and discussed in this one, as much for economic studies as for geographic, social and historical studies, for governments, town halls and private sector, through confirmation of its relevance and economic and social relevance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Transpiration, water extraction, and root distribution of Tahiti lime (*Citrus latifolia* Tanaka) plant under different micro-sprinkler placements

Welson Lima Simões^{1*}, Eugenio Ferreira Coelho², Mauricio Antonio Coelho Filho², Miguel Julio Machado Guimarães³, Marcelo Rocha dos Santos⁴ and Edio Luiz da Costa⁵

¹Embrapa Semiárido, Petrolina, PE, Brazil.

²Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brazil.

³Universidade Federal do Vale do São Francisco, Juazeiro, BA, Brazil.

⁴Instituto Federal de Educação Ciência e Tecnologia Baiano, Guanambi, BA, Brazil.

⁵Universidade Federal de São João del Rei, São João del-Rei, MG, Brazil.

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Measurements of transpiration in cultivated plants are of utmost importance, especially in semi-arid regions where there is low water availability with this, the present work aimed to determine daily transpiration, root distribution, and soil water extraction of 'Tahiti' lime plant under different micro-sprinkler placements in semi-arid conditions of northern Minas Gerais state. We assessed soil water balance, root system and sap flow of plants irrigated by three different micro-sprinklers setups: T1 - a micro sprinkler with 35 L h⁻¹ flow rate located between two plants and along the plant row; T2 - a micro sprinkler with 70 L h⁻¹ flow rate watering and between two plants, along the plant row; and T3 - a micro sprinkler with 35 L h⁻¹ flow rate located 0.3 m from the plant. Treatments changed root distribution, soil water extraction, and transpiration of Tahiti lime. In T2 water loss was lower in upper soil layers than in the remaining treatments. Sap flow in T2 was higher than in T3 and T1, which indicates better water use in T2.

Key words: Sap flow, root, soil moisture.

INTRODUCTION

Transpiration measurements are of utmost importance, especially in semi-arid regions where low water availability is prominent as consequence of lacking and irregular rainfall. Climate change has medium to long-term effects on water resources and reduces water availability or the liability of water supply to numerous areas where water

scarcity is already faced (Consoli et al., 2017). Therefore, precision is to be prioritized in irrigation management so as to increase water-use efficiency.

Transpiration rates are directly related to leaf area (Lai, 2015) but canopy geometry and planting can also influence transpiration as these can affect the interaction

*Corresponding author. E-mail: welson.simoes@embrapa.br.

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between transpiration and other environment factors, such as relative humidity, temperature, wind, solar radiation, and soil water availability. Soil water content and climate alter water status of plants, gas exchanges, and leaf temperature, influencing growth, development, and overall yield (Santos et al., 2013).

Root-zone drying leads to stomatal closure, even in turgid leaves, to decrease transpiration water loss (Silva et al., 2015; Sampaio et al., 2010; 2014). Stomatal closure is linked to chemical signaling, especially abscisic acid (ABA), and other signals, such as pH and redistribution of inorganic ions from roots to shoot, as a response to water deficit. Lima et al. (2015) reported that water deficit as a result of alternate partial root-zone drying leads to higher ABA production in papaya trees. Santos et al. (2013) reported that either full or partial water deficit reduce photo-synthesis rates, transpiration, and stomatal conductance in 'Tommy Atkins' mangoes.

On the other hand, irrigation setup on the field changes water supply to plants and influences water-saving irrigation strategies, especially in warmer regions. Coelho et al. (2016) observed that using micro-sprinklers with different flow rates and wetting diameters affected leaf area, root distribution, and yield of 'Grand Nain' bananas.

Root distribution directly influences water and nutrient uptake by plants. According to Taiz and Zieger (2013), plants' roots are predominately superficial in well-watered soils; though, superficial roots decrease and deeper roots increase when water is depleted close to soil surface. Deeper roots growing towards soil water can be considered a second line of plant defense from incorrect irrigation management. Accordingly, knowing the root system distribution and its water-extracting patterns allows the use of more adequate crop practices, such as irrigation management and fertigation (Santos et al., 2016), which might provide increases in water-use efficiency and nutrients uptake by plants.

Several methods have been proposed to quantify water demand of plants. Among the alternative methods to determine transpiration of citrus plants, those that are based on heating plant stems (stem heat balance, thermal dissipation, and heat pulse methods) have advanced the knowledge of water relations and provided good transpiration estimates (Boehringer et al., 2013; Pinto Jr et al., 2013; Marin et al., 2008; Coelho Filho et al., 2005). As advantages, these methods are non-destructive (Hernandez-Santana et al., 2016), need no calibration, are easy to install, and allow monitoring numerous plants simultaneously.

A few studies have been done aiming at quantifying the transpiration of 'Tahiti' lime by using stem heat balance method (Vellame et al., 2012; Marin, 2008; Rojas et al., 2007; Coelho Filho et al., 2004). However, these studies have not addressed the relation between sap flow, water extraction by roots, and the irrigation system in place.

Therefore, the objective of this work was to determine daily transpiration, root distribution, and water extraction

patterns of 'Tahiti' lime under different micro-sprinkler placements and grown in semi-arid conditions.

MATERIAL AND METHODS

The work was carried out at an Experimental Farm of Mocambinho belonging to the Agricultural Research Enterprise of Minas Gerais (EPAMIG), located at the municipality of Jaíba, Minas Gerais state, at 15°32'S and 43°46'W. Soil at the experimental area is a Typic Quartzipisamment (90% sand, 2% silt, 8% clay, and mean density of 1.62 kg dm⁻³), in a BSwh climate (savanna hot climate, according to Köppen classification), with rainy summers and dry winters. 'Tahiti' lime, *Citrus latifolia* Tanaka, seedlings were grafted onto four-year old Rangpur lime *Citrus limonia* Osbeck) at a spacing of 5 × 7 m. Plant rows were east-west oriented and plants were irrigated daily.

The experimental design was randomized blocks in which each experimental unit consisted of three measurement plants. We assessed three micro sprinkler setups: T1 - a micro sprinkler with 35 L h⁻¹ flow rate located 2.5 m from the plant and along the plant row, following a plant-emitter-plant setup; T2 - a micro sprinkler with 70 L h⁻¹ flow rate watering two plants, located 2.5 m from one another, along the plant row, following a plant-emitter-plant-plant-emitter-plant setup; and T3 - a micro sprinkler with 35 L h⁻¹ flow rate located 0.3 m from the plant, along the plant row. Pressure-compensating micro sprinklers were used to avoid fluctuations in rate flows.

Crop practices for citrus were used when carrying out the experiment as recommended by Coelho et al. (2004). Irrigation scheduling was based on reference evapotranspiration (ET₀), computed by Penman-Monteith method using daily data of a weather station near the experiment site and crop coefficients to determine crop evapotranspiration (ET_c) (Doorenbos and Pruitt, 1977). Therefore, each tree was given the same amount of water, but it was applied differently. Cumulative rainfall over the study, from May to December, was 23.5 mm. Mean ET₀ was 4.4 mm day⁻¹ and its lowest and highest measurements were 2.1 and 7.0 mm day⁻¹, respectively. Mean solar radiation was 19.4 MJ m⁻² day⁻¹ and its lowest and highest measurements were 9.0 and 26.4 MJ m⁻² day⁻¹, respectively.

Water movement within the plant was measured by calculating sap flow using the stem heat balance approach, as recommended by Baker and Van Bavel (1987). The heat supplied at a constant rate (P_{in}) to sampled volume can be split into different heat fluxes (Equation 1).

$$P_{in} = Q_r + Q_v + Q_s + Q_f \quad (1)$$

where, Q_r is the radial heat loss from the sensor, Q_v is the heat transported axially by the stem both above and below the control volume, Q_s is the stored heat by unit time at heated section, and Q_f is the heat transported by convection through plant sap. Potency applied to the heating element was measured following Equation 2.

$$Pin = \frac{V^2}{R} \quad (2)$$

where, V is the voltage (volt) and R is the resistance (ohm) of heating elements. 111.9, 111.6, 90.8, and 60.6Ω corresponded to models SGB9, SGB13, SGB16, and SGB19, respectively. Radial outward flux (Q_f) was calculated from the thermal conductivity of a cork (K_{sh}), which consisted of a radial flow gauge, from the

difference in temperature (ΔT) adjacent to the heating element, and from the outer surface of the cork, calculated by a thermopile with alternate joints (flow gauge) attached to the heater (Equation 3).

$$Q_r = K_{sh} \Delta T \quad (3)$$

K_{sh} was calculated at dawn (4 to 5 a.m.) when sap flow was zero or close to zero. Axial fluxes (Q_v) were calculated with Equation 4.

$$Q_v = AK_{st} \frac{(\Delta T_c - \Delta T_b)}{\Delta z} \quad (4)$$

where, A is the cross-sectional area of heated section of the stem and K_{st} is the thermal conductivity of the stem, which is considered $0.42 \text{ W m}^{-1} \text{ K}^{-1}$ according to Steinberg et al., (1989), ΔT_c and ΔT_b are related to upstream and downstream temperature gradient from heated section of the stem, and Δz is the distance between two thermocouple junctions attached above and below the thermal sheath. According to Weibel and Vos (1994) and Trejo-Chandia et al. (1997) Q_s has little contribution to sap flow estimates of 'Tahiti' lime seedlings, so it can be disregarded without affecting estimates; therefore, flow rate of sap (SF) was calculated by Equation 5.

$$SF = \frac{Pin - Qv - Qr}{cp.dT} \quad (5)$$

where, cp is the specific heat capacity of sap ($cp = 4.186 \text{ kJ kg}^{-1} \text{ K}^{-1}$) and dT is the difference in sap temperature between the upper and lower limits of the heated section.

Sensors were installed in four branches of four measurement plants of each treatment, in the north, south, east, and west quadrants, at an approximate height of 1.5 m from the canopy, as recommended by Mars et al. (1994), to measure physiological parameters. Daily transpiration was calculated by dividing total sap flow by total leaf area of each plant. Total leaf area was estimated by multiplying the total number of leaves of each measurement plant by the mean leaf surface area. The latter was estimated by measuring the length and width of 10% of all leaves (Coelho Filho et al., 2005).

Water extraction by crops and percolation were measured at each treatment by soil water balance. Soil content was measured with time-domain reflectometry-TDR 100 (Campbell Scientifics) operating six multiplexers allowing the reading of 48 sensors simultaneously. TDR probes were installed in two trenches two months prior to readings. One trench was dug along the plant row and the other perpendicular to the row, with a plant in the center. Probes were positioned in these trenches so as to form a $0.25 \times 0.25 \text{ m}$ grid (profile) reaching radially a maximum distance of 2.5 and 2.0 m from the plant, longitudinal and perpendicular to plant row directions, respectively and at a maximum depth of 1.0 m. Data were collected every 10 min. The area occupied by the plant with the two trenches was covered with white plastic material to avoid soil evaporation.

Differences between water contents in the profiles one hour after irrigation and immediately before the next irrigation event were used to calculate, according to Coelho and Or (1996), the extracted water in the soil profile. Deep percolated water contents in the deepest monitored soil layer below 0.75 m were obtained every hour after irrigation up to right before the next irrigation event. Since intervals between measurements were the same, deep percolation occurring in the two profiles from one hour after irrigation up to right before the start of the next irrigation was measured by integrating numerically percolated water contents (Equation 6) during the time between two irrigation events.

$$P = \int_{t_1}^{t_2} q dt \quad (6)$$

where, q is the flow rate ($\text{m}^3 \text{ m}^{-3} \text{ h}^{-1}$); t_1 is the time immediately after irrigation and t_2 is the time before the next irrigation event. The flow rate q was determined by TDR redings in the deepest layer during time t , one-hour period (θ_{j-1} and θ_j), where θ is given in $\text{m}^3 \text{ m}^{-3}$ (Equation 7).

$$q = \frac{\theta_j - \theta_{j-1}}{t} \quad (7)$$

Soil samples were collected to assess roots with a soil auger made of galvanized iron measuring 0.1 m in diameter and 1.30 m in length. Sampling was done where TDR probes were installed in three plants at each treatment. Soil was rinsed off the sample with the aid of 0.5 and 1.0 mm sieves mesh, so that only roots remained. Then, roots were scanned at a resolution of 600 dpi, 100% scale, and intensity of 100 to 130% for thicker roots; and 43 to 62% for finer roots. These files were processed with the software Rootedge (Kaspar and Ewing, 1997) to determine root lengths and diameters. Roots were sorted in six diameter groups as described by Santos et al. (2014). Root lengths were added up to obtain total root length in the different soil layers. To do so, we used the average of three plants per treatments.

RESULTS AND DISCUSSION

Figure 1_{T1a} shows that in treatment T1 the highest percentage of water extraction by crop (WEC) was within the layer 0 - 0.375 m, with more than 70% of the total WEC. By adding up the percentage of WEC, we can observe that up to 0.625 m, more than 90% of water was extracted, both longitudinally (Figure 1_{T1a}) and transversally (Figure 1_{T1b}). The larger extraction of water in the shallower layer was due mainly because of soil water availability that was near 100% within the layer 0 to 0.375 m and reduced to 80% up to 0.60 m depth. Also 64% of very fine and fine roots (diameter smaller than 2 mm) were within the same soil layer and 82% within the layer 0 to 0.625 m. Figure 1_{T2b} shows that in treatment T2 the highest percentage of WEC, both longitudinally and transversally, was within the layer 0.125 to 0.375 m, extracting about 40% of the total. As for the total percentage of WEC in relation to depth, we can see that more than 85% of water was extracted up to 0.625 m deep. In treatment T2, the increase in the percentage of WEC in greater depths, compared to the remaining treatments, might be related to the distribution of total available water for this treatment in these deep layers. Soil water availability was about 100% within the layer 0.125 to 0.375 m and about 90% at 0.625 m depth. Most of roots, particularly, very fine and fine (81%) were within the layer 0 to 0.625 m. Both Figures 1_{T3a} and 1_{T3b} show that plants of treatment T3 exhibit higher WEC in longitudinal and transversal profiles, in 0 to 0.375 m, in which 70% of the total is extracted. Adding up the percentages of WEC in relation to depth, 90% of water

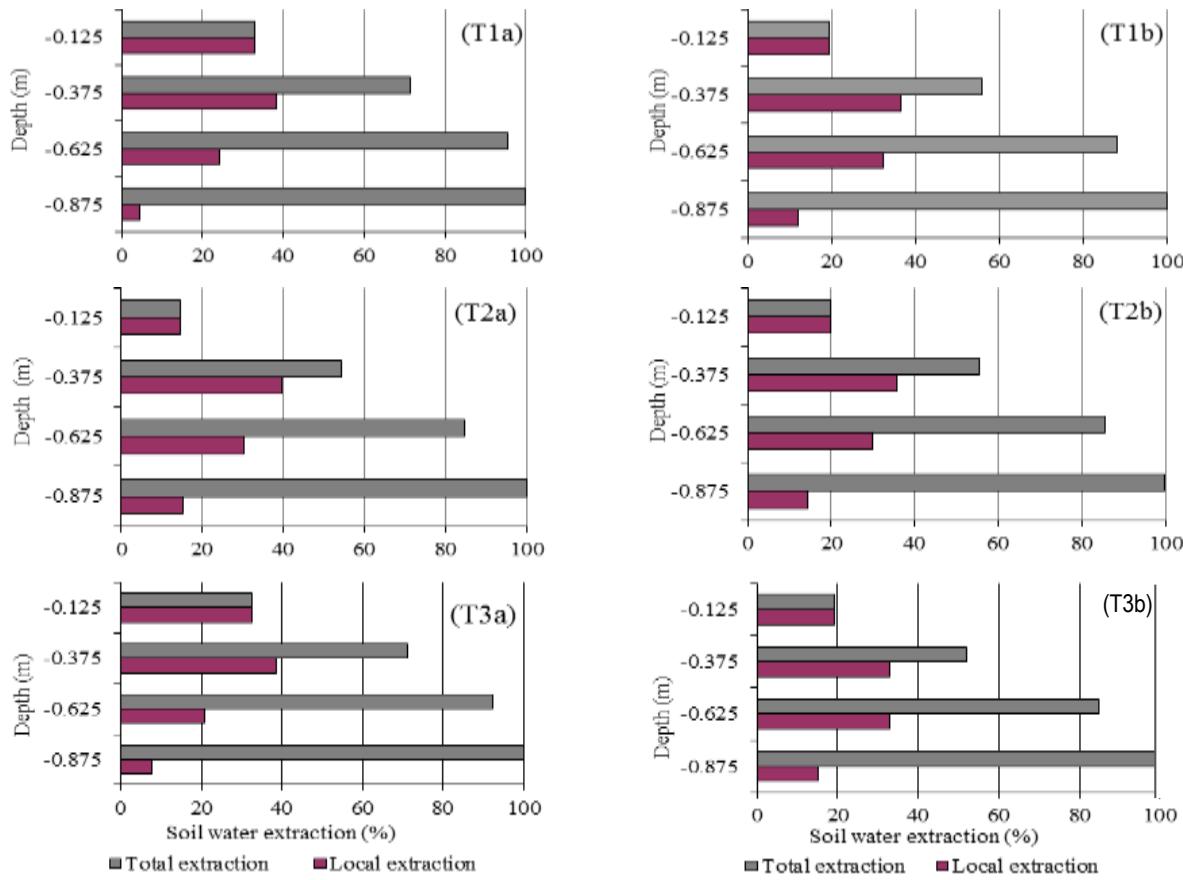


Figure 1. Total water loss in the profile, longitudinally (a) and transversally (b) to the plant row, in treatments T1, T2, and T3, as a function of depth.

was extracted up to 0.625 m, in longitudinal and transversal profiles. Results of this treatment did not differ from those of T1 and T2, mainly because of the root distribution, in which very fine and fine roots (80 %) were more concentrated within 0 - 0.625 m layer. Soil water availability was about 90% up to 0.375 m, but smaller than 80% below 0.625 m depth. Irrigation water depths were applied in order to minimize deep percolation and to keep soil water availability at levels above 70% in the 0 - 0.60 m layer. Santos et al. (2004) working with Tahiti lemon over a five year-old citrumelo swingle rootstock obtained most of the water extraction by roots at depths close to 0.25 m, that is, smaller depth than the ones in this work.

In treatment T1, the amount of percolated water was low both within and between plant rows, which was determined by integrating percolation measured hourly over the day (Figure 2a). Percolation peaked close to the plant and to the micro sprinkler within the row (2.5 m) and at 2.0 m from the plant in between plant rows. Figure 2b shows that as for T2, the amount of percolated water was somewhat higher than that of T1, both within and between plant rows. Percolation peaked at 0.25 and 1.0 m from the stem, within and between plant rows, which is

possibly due to the higher moisture found in these places after irrigation event. The amount of percolated water in T3 was also low (Figure 2c), both within and between plant rows, peaking close to the stem (0.25 m) as well as between 1.25 and 2.0 m from the plant, longitudinally and transversally to the plant. In general, these results were slightly higher than those found in treatment T1 and slightly lower than those of treatment T2, which supports the little difference in water distribution between treatments. The emitter flow rate of treatment T2 was double of the flow rates of other treatments and this favored percolation in this treatment mainly longitudinally between two plants wher the emitter was located.

The percentage of roots graded as to diameter (Tables 1 and 2) in the treatment T1 revealed that very fine roots represented 14.77% of root total. The highest percentages were in the upper layers of soil: 4.41% at 0.375 to 0.625 m. At a distance from the stem of 0 to 0.25 and 2.25 to 2.5 m, 4.78 and 2.55% of roots were observed, respectively, as the latter region corresponds to the area right below the emitter. Roots with diameter of 0.05 to 0.2 cm represented the majority of sampled roots, that is, 81.15% of the total. They were more prominent at

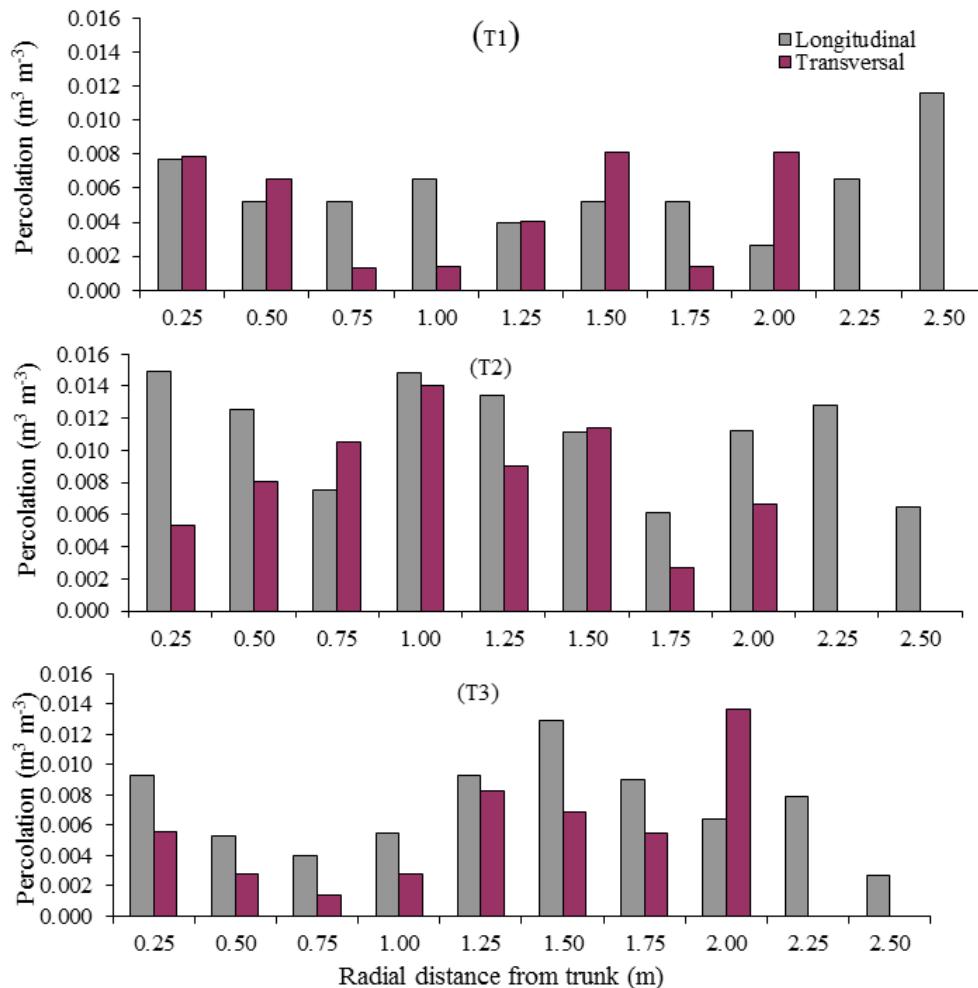


Figure 2. Daily mean water percolation in the soil profile, longitudinally and transversally to the plant row, in treatments T1, T2 and T3.

0.125 to 0.375 m deep (30.54%) and at 2.25 m from the stem (14.81%). Roots with diameter of 0.2 to 0.5 cm represented 3.6% of the total, concentrating at 0.375 - 0.625 m deep and at the distances of 1.0 and 2.5 m from the stem. The thickest roots (diameter > 0.5 cm) represented only 0.48% of the total, which were more abundant at 0.125 to 0.375 m and at a distance of 0.5 m away from the stem. In this regard, we observed that roots with a diameter below or equal to 0.2 cm were mainly found in upper layers of soil. As the diameter increases, so does root distribution at the depth to which roots can reach, which is evidence of root's function in anchoring and supporting the plant.

Roots with diameter lower than 0.05 cm represent 12.94% of root total found in treatment T2 (Table 1 and 2), concentrating in 0.375 to 0.625 m (7.83%) and at a distance of 1.50 m (4.21%) from the stem.

Roots with diameter of 0.05 - 0.2 cm were found at a depth of 0.375 to 0.625 m (7.83%) and at a distance of

1.50 m (4.21%) from the stem. Roots with 0.05 to 0.2 cm in diameter were the most abundant (83.42% of the total) and they were mostly found at a depth of 0 to 0.125 m (31.46%) and at a distance of 1.5 m from the plant (10.53%). Roots with 0.2 to 0.5 cm in diameter represented 3.12% of the total and were more prominent in the 0.875 to 1.125 m depth and at a distance of 0.25 m away from the stem. Thicker roots (diameter > 0.5 cm) represented only 0.53% of root total and were mostly found in the 0.125 to 0.375 m depth and 1.25 m away from the stem.

In the treatment T3 (Table 1 and 2), very fine roots (<0.05 cm) accounted for 14.70 % of root total. These roots were predominately found in the 0.375 to 0.625 m soil layer (4.23%) and distributed evenly in the 2.5-m range from the stem (1.88%). Roots with 0.05 to 0.2 cm diameter composed 81.32 % of root total and were predominantly found in 0 to 0.125 depth and at a distance of 0.75 m away from the stem. Roots with 0.2 to 0.5 cm

Table 1. Percentage of total root length (TRL) as to four diameter groups (< 0.5; 0.5- to -2.0; 2.0- to -5.0; and > 5.0 mm), at different depths, in the treatments T1, T2 and T3.

Treat	Depth (m)	Diameter (mm)			
		< 0.5	0.5 – 2.0	2.0 – 5.0	> 5.0
T1	0 – 0.125	4.03	25.56	0.14	0.04
	0.125 – 0.375	3.95	30.54	0.61	0.26
	0.375 – 0.625	4.41	14.09	1.20	0.03
	0.625 – 0.875	2.06	7.63	0.93	0.06
	0.875 – 1.125	0.32	3.32	0.72	0.09
	Total	14.77	81.15	3.60	0.48
T2	0 – 0.125	0.16	31.46	0.66	0.01
	0.125 – 0.375	1.16	27.38	0.70	0.34
	0.375 – 0.625	7.83	12.86	0.27	0.03
	0.625 – 0.875	1.92	7.94	0.66	0.08
	0.875 – 1.125	1.86	3.78	0.86	0.08
	Total	12.94	83.42	3.15	0.53
T3	0 – 0.125	1.57	35.75	0.41	0.01
	0.125 – 0.375	3.47	25.27	1.06	0.13
	0.375 – 0.625	4.23	9.73	1.09	0.12
	0.625 – 0.875	3.69	6.01	0.60	0.12
	0.875 – 1.125	1.74	4.55	0.33	0.10
	Total	14.70	81.32	3.49	0.48

diameter accounted for 3.49% of root total and concentrated in 0.375 to 0.625 m depth (1.09%) and at a distance of 0.5 m away from the stem (0.59%). Roots with diameter larger than 0.5 m constitute only 0.48% of root total and were mostly found in 0.125 to 0.375 m (0.13%) and at a distance of 0.5 m away from the stem (0.08%).

Roots with diameter between 0.05 and 0.2 cm, in the three treatments, accounted for more than 81% of the total and exhibited higher distribution in 0 to 0.625 m depth. There was a trend of a higher length density and root redistribution in treatments T2 and T3 possibly due to soil water distribution in these treatments. These results are consistent with Taiz and Zeiger (2013) who reported that lower soil water content in topsoil reduces superficial root development and increases the number of deep roots due to the higher water availability in deeper soil layers in treatment T2 in which the micro sprinkler close to the stem provided higher water percolation. It is worth noting that root growth into deeper soil layers towards wet soil can be considered a line of defense against drying topsoil. Results regarding root system's depth are consistent with those reported by Alves Junior et al (2011) who observed an effective rooting depth of 0.6 m in 30 and 48-month-old drip-irrigated 'Tahiti' lime grafted onto rootstock 'Swingle' citrumelo in Piracicaba, SP.

Average leaf areas of plants were: 184.62 m² (T1), 182.85 m² (T2) and 185.12 m² (T3). ETo and sap flow measurements recorded over the day are in Figure 3. Sap flow measurements were consistent over the three days on which evaluations took place; nonetheless, there is a difference between treatments as to the time at which sap flow reduced during the day. In T2, sap flow reduces in average after the most water-demanding time of the day, followed by a decrease during the afternoon. As for T3 and T1, sap flow decreases earlier as a result of low water availability in these treatments leading to premature stomatal closure, thereby decreasing plant transpiration.

Average sap flow measurements varied between branches with similar results reported by Oliveira et al. (2009) in mangoes, and the highest measurement was 0.686 L m⁻² day⁻¹ (Table 3). These are considered a low sap flow rate in comparison with studies carried out with lemons by Marin et al. (2008) and Rojas et al. (2007) reporting measurements varying from 1.00 to 1.83 L m⁻² day⁻¹ in young plants or plants having less leaf area than the plants used in this study. Cotrim et al. (2019) observed estimated values of sap flow ranging from 0.697 to 1.255 L m⁻² day⁻¹, under conditions that did not suffer water deficit during the three phases of development of 'Tommy Atkins' mango fruits. A more active plant metabolism might explain this lower rate at this early stage, as well as other factors mentioned by

Table 2. Percentage of total root length (TRL) as to four diameter groups (< 0.5; 0.5- to -2.0; 2.0- to -5.0; and > 5.0 mm), in different radial distance from the stem, in the treatments T1, T2 and T3.

Treat	Distance (m)	Diameter (mm)			
		< 0.5	0.5 – 2.0	2.0 – 5.0	> 5.0
T1	0.25	4.78	8.16	0.28	0.10
	0.50	1.89	5.55	0.30	0.13
	0.75	2.29	3.62	0.54	0.03
	1.00	0.37	7.06	0.65	0.03
	1.25	0.08	7.21	0.38	0.06
	1.50	0.09	8.35	0.15	0.03
	1.75	0.10	8.85	0.26	0.01
	2.00	0.57	12.25	0.10	0.01
	2.25	2.06	14.81	0.25	0.03
	2.50	2.55	5.28	0.70	0.05
T2	Total	14.77	81.15	3.60	0.48
	0.25	1.28	9.23	0.65	0.01
	0.50	1.13	6.17	0.51	0.01
	0.75	1.79	5.00	0.22	0.05
	1.00	0.35	9.77	0.54	0.11
	1.25	1.22	6.31	0.12	0.13
	1.50	4.21	10.53	0.09	0.05
	1.75	1.08	8.79	0.17	0.12
	2.00	0.35	10.08	0.44	0.01
	2.25	0.60	8.39	0.23	0.01
T3	2.50	0.93	9.16	0.18	0.01
	Total	12.94	83.42	3.15	0.53
	0.25	1.80	10.00	0.63	0.05
	0.50	2.20	7.80	0.67	0.10
	0.75	0.77	9.04	0.40	0.09
	1.00	1.90	7.59	0.30	0.05
	1.25	0.73	7.33	0.22	0.01
	1.50	1.41	7.71	0.21	0.01
	1.75	1.60	7.87	0.25	0.05
	2.00	1.28	8.50	0.45	0.08
T3	2.25	1.95	7.78	0.18	0.03
	2.50	1.07	7.70	0.18	0.01
	Total	14.70	81.32	3.49	0.48

Syvertsen (1982), such as lower water loss regulation than mature leaves since young leaves lack structural rigidity and have lower deposition of cuticular wax than mature leaves. Regarding plant size, according to Rojas et al. (2007), increasing leaf area may interfere with canopy net and lead to a decrease in mean net radiation per unit leaf area due to increasing leaf density and self-shading inside the canopy. When assessing the treatments as to averages of either sap flow/solar radiation (SF/SR) or SF/ETo, we can observe that treatment T2 exhibited the highest values followed by T3

and T1. This demonstrates that plants of treatment T2 are transpiring more than those of T3 and T1 in relation to incident radiation ($\text{MJ m}^{-2} \text{ day}$).

The above-mentioned difference can also be seen only when evaluating sap flow rates on days with similar ETo (4.27, 4.21, and 4.25). The same behavior is observed in analyses done using the proposed ratios (SF/ETo and SF/SR), that is, transpiration of plants in T2 is the highest, followed by T3 and T1.

If we consider the amount of water applied to the three treatments the same, then the water condition found in

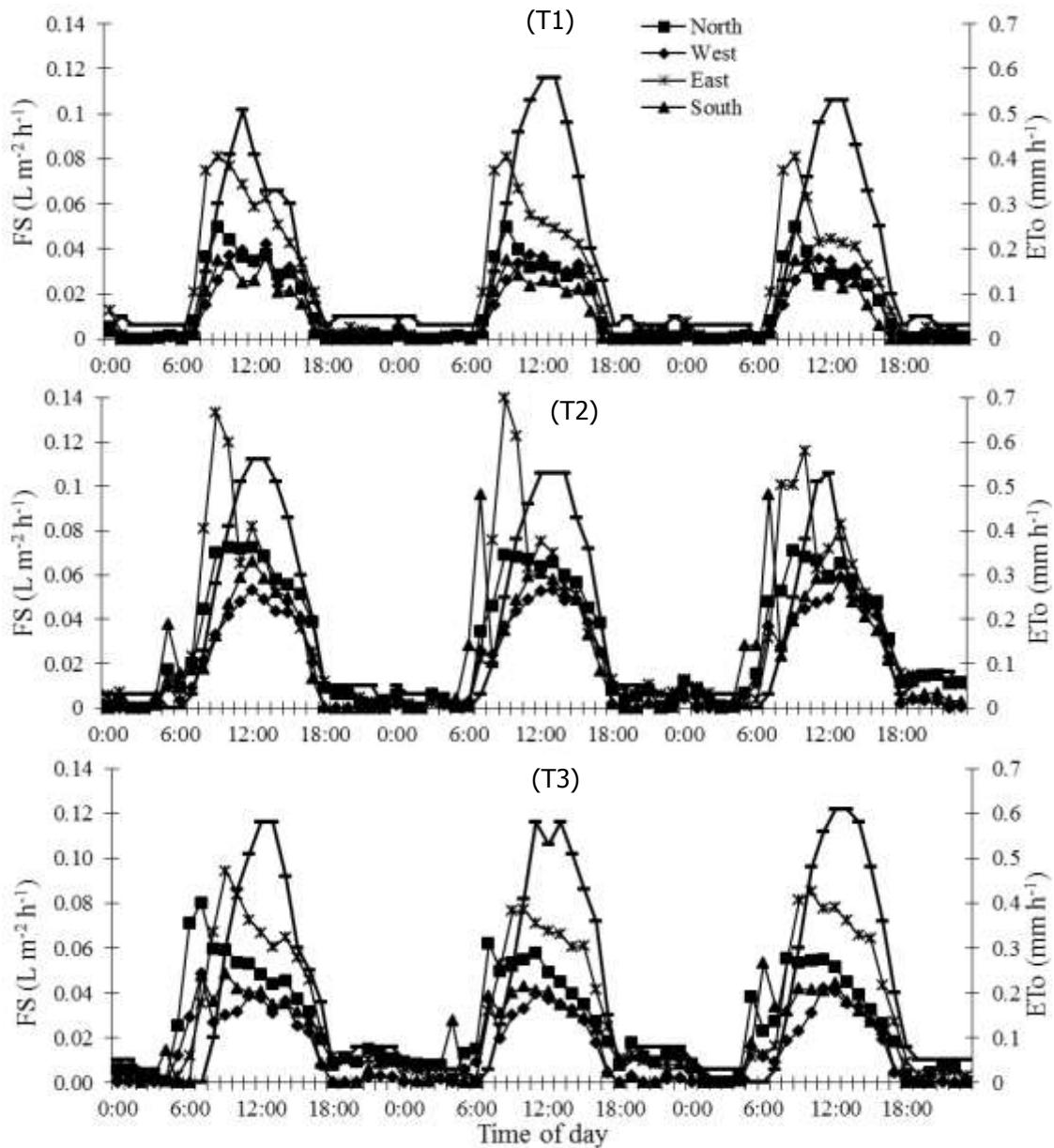


Figure 3. Daily variation of sap flow (FS) of 'Tahiti' lime tree and reference evapotranspiration (ETo) determined by Penman Monteith method, of treatment T1, T2, and T3 during the flowering stage.

treatment T2 resulted, possibly, in a system in which water is better stored and used.

Conclusion

Micro-sprinkler placements affected root distribution, water extraction, and transpiration of 'Tahiti' lime. Transpiration was higher using one micro-sprinkler of 70 L h^{-1} flow rate irrigating two plants, located at 2.5 m away from the stem, than using one micro-sprinkler per plant, either located at 0.3 or 2.5 m away from the stem, along the plant row.

Water extraction by lemon plants were more significant within the layer 0 to 0.375 m, but most of it, that is, about 85 to 90% occurred up to 0.625 m depth. The use of one microsprinkler per two plants instead of one plant is feasible concerning root water extraction, root development and plant transpiration as long as water supply be applied according to plant needs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

Table 3. Sap flow in each plant (FSp) determined by heat balance method, sap flow in branches (FSb) measured per quadrant, reference evapotranspiration (ETo) and solar radiation (RS) and ratio between sap flow and these factors, during flowering stage of 'Tahiti' lime.

Treatments		T1			T2			T3		
ETo	mm day ⁻¹	3.48	4.27	3.82	4.32	4.21	3.6	4.25	4.58	4.36
SR	MJ m ⁻² day ⁻¹	14.97	19.01	17.95	18.94	18.79	15.20	17.395	19.07	19.85
FSb (L m ⁻² day ⁻¹)	North branch	0.350	0.328	0.307	0.684	0.658	0.738	0.712	0.625	0.552
	West branch	0.284	0.275	0.252	0.417	0.462	0.501	0.389	0.341	0.314
	East branch	0.627	0.556	0.506	0.808	0.799	0.874	0.762	0.711	0.734
	South branch	0.245	0.221	0.215	0.514	0.577	0.630	0.473	0.415	0.436
	Mean	0.377	0.345	0.320	0.606	0.624	0.686	0.584	0.523	0.509
Ratio (FSb/SR)	North branch	0.023	0.017	0.017	0.036	0.035	0.048	0.040	0.0327	0.027
	West branch	0.018	0.014	0.014	0.022	0.024	0.032	0.022	0.017	0.015
	East branch	0.041	0.029	0.028	0.042	0.042	0.057	0.043	0.037	0.036
	South branch	0.016	0.011	0.011	0.027	0.030	0.041	0.027	0.021	0.021
	Mean	0.025	0.018	0.017	0.031	0.033	0.045	0.033	0.027	0.025
Ratio (FSb/ETo)	North branch	0.100	0.076	0.080	0.158	0.156	0.205	0.167	0.136	0.113
	West branch	0.081	0.064	0.065	0.096	0.109	0.139	0.091	0.074	0.064
	East branch	0.180	0.130	0.132	0.187	0.189	0.242	0.179	0.155	0.151
	South branch	0.070	0.051	0.056	0.118	0.137	0.175	0.111	0.090	0.089
	Mean	0.108	0.080	0.083	0.140	0.148	0.190	0.137	0.114	0.104
FSp	L day ⁻¹	76.39	70.42	65.95	110.75	114.12	125.3	110.10	99.01	97.43

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Full Length Research Paper

Prospects and challenges of agro-industry in Bangladesh: An agripreneur view

Dhanonjoy Kumar

Department of Management, Islamic University, Kushtia, Bangladesh.

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Agriculture sector plays an important role in overall economic development of Bangladesh. A plurality of Bangladeshis earns their living from agriculture. Agriculture is the largest employment sector in Bangladesh. Bangladesh is a fertile agrarian country where agriculture and its related branches have been acting as the main livelihood of major portion of citizen since independence. Agro-industry is playing increasingly important role in achieving sustainable development goal as well as faster economic growth in many developing countries like Bangladesh. To meet the domestic food demand and to create self-employment, young generation has diverted their profession to this potential sector by being an agripreneur. Considering the prospect and challenges of agro-industry, it is evident that agro-industry friendly attitude of the government as well as essential policies are necessary for the development and growth of this sector. Vibrant agro-industrial activities can expand the markets for primary agricultural products, add value by vertically integrating primary production and food processing system and minimize post harvest losses. With few exceptions, the agro-industrial sector of Bangladesh remains elementary, underdeveloped and largely without significant institutional, technical and financial support. However, the present study focuses on identifying the problems and prospects of agripreneurship in Bangladesh and the researchers also endeavored to provide some suggestions for the sustainable agripreneurship development.

Key words: Entrepreneur, agripreneur, agri-business, agro-Industry, Bangladesh.

INTRODUCTION

Bangladesh is an agrarian country. Agriculture has been the main occupation since thousand years due to fertility of the land of this country. The economic cycle of this country runs by agriculture. Most of the industries have established in this country, directly or indirectly, based on agriculture. Farmers harvest different types of crash crops based on characteristics as well as season. Paddy

is the largest harvested crops in Bangladesh. It is also main food in this country. Different part of this country has fame for different types of paddy. Governments as well as non-government research institutions have been innovating hybrid paddy by continuous research on agriculture to coherence between supply and demand of rice. Bangladesh is a populated country. The youth

E-mail: djoysk@yahoo.com.

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people ratio is 39.73% (male 30,094,014/female 32,614,286). Bangladesh's population reached 163.65 million people in June 2018. The country's Labor Force Participation Rate increased to 56.50% in December 2017. Bangladesh's unemployment rate increased to 4.37% in December 2017, from 4.35% in December 2016. Total dependence ratio is 52.6%. Agriculture is the main occupation of this country and this is one of the emerging sectors for attaining sustainable development. Entrepreneur in agriculture sector plays an important role in reducing unemployment problem. At present, agripreneur is a new term to define entrepreneur in agriculture sector (www.ceidata.com/bangladesh/unemployment).

LITERATURE REVIEW

Many researchers have conducted research works on different aspects regarding agro-based industry and policies for development. Several important empirical research findings have been taken into consideration.

Hock-Eam et al. (2015) in their study used a sample of 22 established agropreneurs and identifies 28 predisposed factors. Using the individual level or self-fulfillment factors, interest and satisfaction being entrepreneur, are ranked the most important. The social network and soft skills (creativity and innovation, problem solving) are also ranked among the top. On the other hand, the institutional factors such as degree of entrepreneur is ranked bottom. Finding of this study suggests that the individual level factors are the most important predisposed factors, and it can be complemented by the social and institutional level factors. Using a sample of fresh graduates, it is found that there is a substantial amount of graduates who intended to be entrepreneurs. Results also reveal that there are around one third of salaries graduates and around half of unemployed (or economically inactive graduates) are miss-intended to be entrepreneurs. Thus, government is able to increase the number of graduate agropreneur by facilitating or smoothing the realization of this intention.

Owoade (2017) found that agropreneurs need a system of mentorship, handholding, and bridging support to launch them into higher-value food production using modern agriculture and agribusiness methods. The paper posited that bolstering food security and sustainability is not just a matter of helping existing rural small-holder farmers but also recruitment of innovative new ones. The paper also identified challenges to agropreneurs development to include restricted market access, poor management practices, low entrepreneurial skills, poor infrastructural facilities, non access to information, land, money and capital market, etc. To promote agropreneurs development, the paper recommended creation of an enabling agribusiness environment, adoption of consistent and enduring government protection policies

and risk sharing strategies, mentoring and establishment of agricultural parks and incubation centers.

Rahman (2017) study sketches a brief scenario of agriculture sector in Bangladesh. The agricultural sector remains an irreplaceable driving force for economic growth of the country. Based on secondary data, the study intends to describe the role of agriculture in the economy of Bangladesh with a focus on problems and challenges of the sector. The main reason behind the loss of agricultural land in Bangladesh is the growth of rural housing followed by urbanization and industrialization. Residences of increasing population of the country are expanding at the cost of agricultural land. Despite many prospects of agriculture sector, some challenges are still present there. In order to address the challenges, a number of collaborative and coordinated steps should be initiated. As the food security is a major concern for Bangladesh, necessary steps should be taken to conserve agricultural land from its shifting to non-agricultural utilization.

Quddus (2009) mentioned that agro industry contributes a significant portion of national income and the prospect of employment generation is increasing at the higher extent for the sectors food processing, tanning and leather finishing, leather industry, saw milling and wooden furniture. Food processing, fish processing, tanning and leather finishing, leather fabrication, livestock, and poultry were the key sectors of the Bangladesh economy. Agro-industry and high value-added agricultural sector is proposed as the key sector to improve inequality problems, smoother employment transformation, generate high growth and induce high output production. The study showed that some of the agricultural production sectors and most of the agricultural processing sectors have better potential to generate more income to different households to create better income distribution and to induce more savings in the country. The high value of multipliers in the case of agro-industries indicates that the knock on effects are relatively large compared with the initial impact of increased output.

Sharma (2014) in his study concluded that the study was pursued to ascertain the existing internal and external factors, alternative strategies and priorities of the strategies applied in enhancing beef cattle agribusiness at Pabna and Sirajganj districts in Bangladesh. The primary data and present study were collected by employing various techniques such as survey, FGD, KII and observation methods. The total sample size was 180 which were selected through convenience sampling technique. The following analytical tools used were employed (i) IFE-EFE analysis, (ii) SWOT analysis, (iii) SWOT matrix, and (iv) QSPM model. By analyzing all the factors from SWOT four strategies were developed to determine the beef cattle development. The best strategy was selected by using QSPM matrix. The results IFE is 2.610, EFE is 2.438 and the total weighted score is 5.833

indicating that beef cattle sub-sector agribusiness are opportunity to explore their strengths and minimize their weaknesses. The beef cattle agribusiness development through the implementation of the integrated or contract farming that supported to backward and forward linkage and support services.

Mahboob and Islam (2014) in their study concluded that Bangladesh cannot sustain long-run economic progress without having a strong agricultural sector accompanied by a dynamic agribusiness sub-sector. At a general level, the paper recommends various structural, institutional, and market-friendly policy reforms accompanied by infrastructural developments in order to encourage entrepreneurship, innovation, and investments along with better and more effective strategic management of this sector. Such reforms are expected to promote better utilization of scarce resources to promote a strong, dynamic, and sustainable agribusiness sector that would be able to contribute substantially to industrialization and economic development of the country.

Olusada et al. (2018) examined the new trends in innovation and entrepreneurship for sustainable development. These trends were specifically examined using the Nigerian context. Also, the advantages and shortcomings of these new trends were looked at and solutions proffered were necessary. Entrepreneurship has come to stay and it is a great way of reducing poverty if not eradicating it; it is also a way of empowering young people and women and other people in the economy that are disadvantaged. Subscribing to entrepreneurship by any economy can improve greatly such economy.

Objectives of the study

- (1) To identify the prospect of agro-based industry in Bangladesh in the view point of Agropreneur.
- (2) To understand the challenges faced by the agro-based organizations in Bangladesh.
- (3) To provide some policy implication for development of agro-based organizations in Bangladesh.

METHODOLOGY

The study followed a quantitative approach to achieve the objectives of this study, which was descriptive in nature. Both primary and secondary data have been collected for the purpose of the study. The primary data have been collected through personal interview from the agro based industry entrepreneurs and workers. In this regard, one set of interview schedule prepared in the light of objectives of the study. The interview schedule has been filled up directly by the researcher. Secondary data is the data that have been already collected by and readily available from other sources. The secondary data have been collected from published economic review of Bangladesh, related books, journals, articles, seminar paper, publications from national and international research institutions, report of different financial institutions, public records and statistics, different research reports, etc. Purposive sampling

and judgment sampling have been used under non-probability sampling techniques for sample design. So the sample selection has been chosen as per the following ways. A total of 40 agro industry was visited for collected the data, 10 located in Kushtia district, 04 located in Jhenaidah district, 09 located in Chapainawabgong district, 12 located in Naogaon district and 05 located in Bogura district. The descriptive statistics like frequency distribution and SWOT analysis method used to analyze the data.

EXPLANATION OF THE TERMS ENTREPRENEUR AND AGRIPRENEUR

An entrepreneur is an individual who, rather than working as an employee, found and run a small business, assuming all the risks and rewards of the venture. According to Timmons and Spinelli (2003), entrepreneur is an innovator or developer, who recognizes and also seizes opportunities, converts those opportunities into a workable or marketable idea, adds value through time, effort, money or skills, assumes the risks of the competitive marketplace to implement these ideas and realizes the rewards from these efforts. Based on this, they classified entrepreneurs into four types:

- (1) Innovating entrepreneurs: Innovating entrepreneurs are those who introduce new goods, inaugurate new method of production, discover new market and reorganize the enterprise. It is important to note that such entrepreneurs can work only when a certain level of development is already achieved, and people look forward to change and improvement.
- (2) Imitative entrepreneurs: These are characterized by readiness to adopt successful innovations inaugurated by innovating entrepreneurs. Imitative entrepreneurs do not innovate the changes themselves, they only imitate techniques and technology innovated by others. Such types of entrepreneurs are particularly suitable for the underdeveloped regions for bringing a mushroom drive of imitation of new combinations of factors of production already available in developed regions.
- (3) Fabian entrepreneurs: Fabian entrepreneurs are characterized by very great caution and skepticism in experimenting any change in their enterprises. They imitate only when it becomes perfectly clear that failure to do so would result in a loss of the relative position in the enterprise.
- (4) Drone entrepreneurs: These are characterized by a refusal to adopt opportunities to make changes in production formula even at the cost of severely reduced returns relative to other like producers. Such entrepreneurs may even suffer from losses but they are not ready to make changes in their existing production methods.

Beside the previously discussed type of classification,

entrepreneurs are classified into different types based on different classifications as mentioned in the following.

Based on the use of technology

(1) Technical entrepreneur: The entrepreneurs who establish and run science and technology-based industries are called 'technical entrepreneurs.' Speaking alternatively, these are the entrepreneurs who make use of science and technology in their enterprises. Expectedly, they use new and innovative methods of production in their enterprises.

(2) Non-technical entrepreneur: Based on the use of technology, the entrepreneurs who are not technical entrepreneurs are non-technical entrepreneurs. The forte of their enterprises is not science and technology. They are concerned with the use of alternative and imitative methods of marketing and distribution strategies to make their business survive and thrive in the competitive market.

Based on ownership

(1) Private entrepreneur: A private entrepreneur is one who as an individual sets up a business enterprise. He/She is the sole owner of the enterprise and bears the entire risk involved in it.

(2) State entrepreneur: When the trading or industrial venture is undertaken by the state or the government, it is called 'state entrepreneur.'

(3) Joint entrepreneurs: When a private entrepreneur and the government jointly run a business enterprise, it is called 'joint entrepreneurs.'

Based on the type of business

(1) Trading entrepreneur: As the name itself suggests, the trading entrepreneur undertakes the trading activities. They procure the finished products from the manufacturers and sell these to the customers directly or through a retailer. These serve as the middlemen as wholesalers, dealers, and retailers between the manufacturers and customers.

(2) Manufacturing entrepreneur: The manufacturing entrepreneurs manufacture products. They identify the needs of the customers and, then, explore the resources and technology to be used to manufacture the products to satisfy the customers' needs. In other words, the manufacturing entrepreneurs convert raw materials into finished products.

(3) Agricultural entrepreneur: The entrepreneurs who undertake agricultural pursuits are called agricultural entrepreneurs. They cover a wide spectrum of agricultural activities like cultivation, marketing of agricultural produce, irrigation, mechanization, and technology.

Agripreneur

Agriculture is the main occupation of this country and this is one of the emerging sectors for attaining sustainable development. Entrepreneur in agriculture sector plays an important role in reducing unemployment problem. At present agripreneur is a new term to define entrepreneur in agriculture sector. Agripreneur defined as "entrepreneur whose main business is agriculture or agriculture-related" Agripreneur = Agriculture + Entrepreneur.

Agripreneurship

Agripreneurship defined as "generally, sustainable, community-oriented, directly-marketed agriculture. Sustainable agriculture denotes a holistic, systems oriented approach to farming that focuses on the interrelationships of social, economic, and environmental processes". Agripreneurship is an employment strategy that can lead to economic self-sufficiency of rural people. Agripreneurship development through training is a key elements for the promotion of Micro, Small and Medium Enterprises (MSMEs), particularly, the first generation agripreneurs. These can result in improved performance of an individual that can contribute to employment generation, poverty reduction and Human Resource Development (Nagalakshmi and Sudhakar, 2013).

ROLE OF AGRICULTURE AND AGRO-INDUSTRY IN BANGLADESH

Agro-industry can play a strategic role in pro-poor growth strategies, particularly in developing countries, like Bangladesh where major percentage of the poor live in rural area. The development of agro-industry has an important impact on the local agricultural sector as well as the livelihoods of small holder farmers.

GDP from agriculture in Bangladesh increased to Tk.10117.30 million in 2017 from Tk. 9922.80 million in 2016. GDP from agriculture in Bangladesh averaged Tk. 8747.38 million from 2006 until 2017, reaching an all-time high of Tk. 10117.30 million in 2017 and a record low of Tk. 7017.10 million in 2006 (Figure 1).

Figure 2 shows that agricultural growth is constant in recent years. It was high in 2009 to 2010 due to some positive factors. In comparison with other two sectors,



Figure 1. Bangladesh GDP from agriculture.
Source: Trading economics.com/Bangladesh Bureau of Statistics.

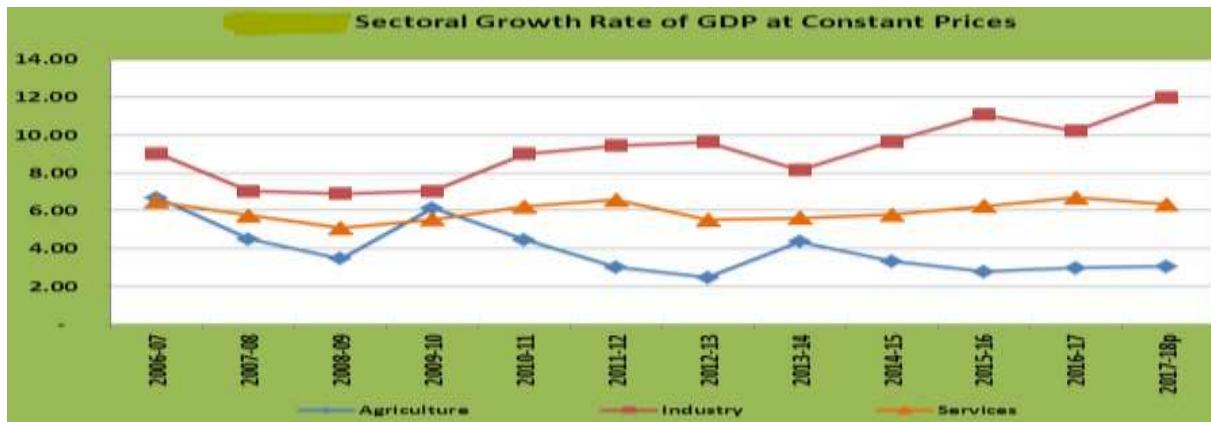


Figure 2. Sector wise share of GDP at constant price.
Source: National Accounts Statistics (2016-2016).

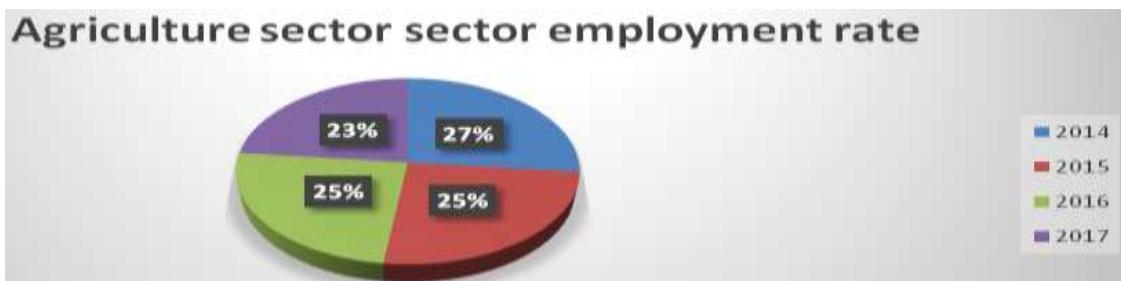


Figure 3. Distribution of employment by economic sector of Bangladesh
Source: Share of total employment from (<https://www.statista.com/statistics/438360/employment-by-economic-sector-in-bangladesh/>).

though it provides lower GDP growth but the industrial and service sector's growth is high. So it is high time to collaborate agriculture with industrial sector by establishing balanced agro-industries all over the country.

Figures 2 and 3 show that agricultural sector contributes a lot of employment since 2014 in our country. It happens for generic ability of farming knowledge of large labor forces and rapid expansion of

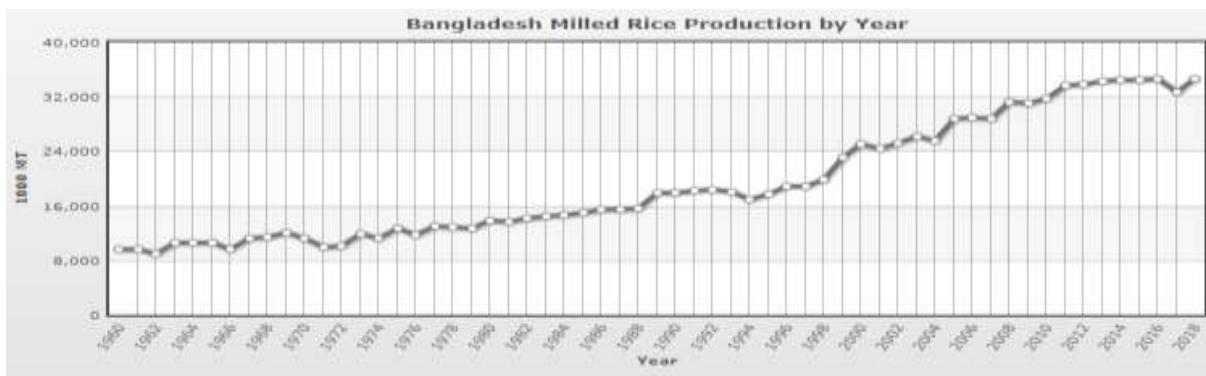


Figure 4. Bangladesh milled rice production by year.
Source: <https://www.indexmundi.com/agriculture/country>

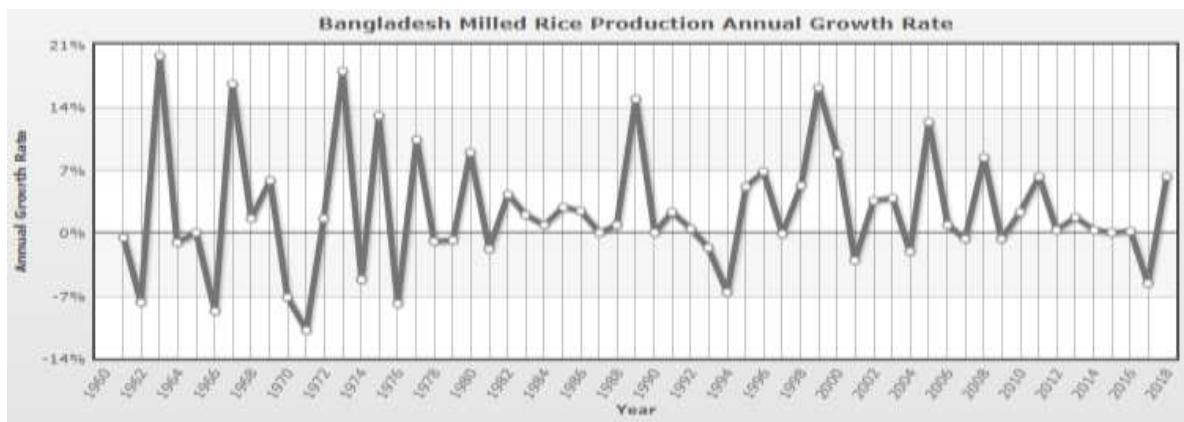


Figure 5. Bangladesh milled rice production annual growth rate.
Source: <https://www.indexmundi.com/agriculture/country>

agripreneur and agro based industries.

RICE MILLING INDUSTRY IN BANGLADESH

Paddy is the largest harvested crops in Bangladesh. It is also main food in this country. Different part of this country has fame for different types of paddy. Governments as well as non-government research institutions have been innovating hybrid paddy by continuous research on agriculture to coherence between supply and demand of rice. Twentieth century adds new technology in harvesting (e.g. soil testing, seed producing, cutting paddy, processing, using insecticides, using internet to detect disease of crops, etc.) before 2006 to 2007 farmers used to follow traditional procedures to make rice from paddy to sell commercially after fulfilling their own family demand. Where rice production and selling were totally dependent on season. By the advancement of science and technology as well as globalization, the production of paddy to rice conversion, everywhere it has seen tremendous changes.

Nowadays, it has become an industry with home; it creates new employment as well as investment. Traditional and semi auto rice mills create jobs, particularly for women. Rice husking mills have roles in building stocks at local levels. Over the last few years, hundreds of automatic and semi-automatic rice mills were in different rice producing zones. Naogaon, Ghapainawabganj, Dinajpur, Kushtia and Noapara of Jessore are some districts that have attracted investment to set up big automatic rice mills. At present more investments are coming up to set up automatic rice mills (Figures 4 and 5). In 2005, there are only 200 semi-automatic and automatic rice mills. The number has tripled to more than 600; now a body of about almost 17,000 mills. This sector has attracted many large investors to set up big automatic rice mills as demand for rice processed at automatic mills has risen. Industry insiders link the growth in numbers of automatic rice mills to changing consumer preferences. The ultimate consumers want better quality rice, a longer shelf life, less broken qualities and rice that is almost free from inedible substances, such as stones.

Table 1. Prospects and challenges of agro-industry in Bangladesh SWOT analysis.

SWOT analysis
Strengths:
Suitability and availability of raw materials
Low labor cost
Rice is the main food for Bangladeshi inhabitants
Market demand of this finished product is very good
Opportunities:
Rice mills also create a large number of employment opportunities
It has potential market demand
Develop technology and create important by product
Increase gross domestic product (GDP)
Weakness:
Capital problem
Lack of research and technology and technical support
Seasonality of crops
Sub-optimal use of processing facilities and equipment
Poorly trained personnel and a lack of qualified food technologists
A lack of proper hygiene and sanitation practices
Weak or non-existent market development
Threats:
Inappropriate or obsolete processing and ancillary equipment
Inappropriate packaging materials
Absence of good management of the processing facility once commercialized
Poor and inconsistent quality of processed products
Backdated technology and dependency on other countries
Lack of proper control on imported product
Natural calamities

Source: Personal Survey

DISCUSSION AND ANALYSIS OF THE STUDY

Ownership style: The ownership style out of the 40 organizations; 03 was sole proprietorship, 21 was partnership, and 16 was private limited company.

Types of Organization: From this 36 was an auto rice mill, 03 was semi auto rice mills, and only 01 was rice bran oil.

Capital: Only 01 entrepreneur performs their business in own capital. 11 entrepreneurs own and loan capital ratio was 75:25. 18 entrepreneurs own and loan capital ratio was 50:50. And 10 entrepreneurs own and loan capital ratio was 25:75.

Raw materials: 100% entrepreneurs think that their raw materials are available.

Supply and demand relationship: Out of 40

entrepreneurs, 36 realize that their product supply and demand relationship was positive and only 04 realize that their product supply and demand relationship was negative.

Business environment: Out of 40 entrepreneurs, 35 provide positive attitudes toward their business environment. Only 5 entrepreneurs face negative impact from the business environment.

Government co-operation: Out of 40 entrepreneurs, 17 find out government co-operation for the growth and development of agro-industry is less than required level; on the other hand, 14 entrepreneurs think moderate co-operation from government in this issue. Only 9 entrepreneurs get spontaneous government co-operation for their venture. It summarizes subjacent co-operation from government.

Government support: From 40 entrepreneurs, only 17

provide positive reply in this issue; on the other hand, differently 23 entrepreneurs figure out negative support for this sector. This result implies negative support from government.

Trading system: Among 40 entrepreneurs, 36 perform only domestic trading, 02 entrepreneurs are related with import and export simultaneously. It identifies that domestic trading is the main trading system in the present agro-industry.

Products market potentiality: Out of 40 entrepreneurs, 14 show satisfactory, 13 entrepreneurs think high, 5 entrepreneurs find out moderate and only 8 entrepreneurs denote low satisfactory level of market potentiality of their products.

Opportunity of the agripreneur: Out of 40 entrepreneurs, 6 foresight high opportunity, 6 perceive very little opportunity and 28 entrepreneurs claim very little opportunity for the entrepreneurs of the agro-industrial sector.

Creation of new product: Out of 40 entrepreneurs, 7 realize high opportunity, 12 figure out limited opportunity and 21 of 40 entrepreneurs think very little opportunity for creation of new product for the expansion of this agro-industrial sector.

Demand of customers: Out of 40 Entrepreneurs, 34 face no impact on changing demand of customers and consumers; on the contrary, only 6 entrepreneurs face moderate impact on constant changing demand of customers and consumers of this agro-industrial sector.

Government and industrial law: Out of 40 entrepreneurs, 23 face no hazardous impact on government and industrial law but 17 entrepreneurs think it partially impact on the business functionality.

Competition: Among 40 entrepreneurs, 7 face some problems due to low existence of unhealthy competition. Among them, 30 entrepreneurs find out no existence of unhealthy competition; differently, only 3 entrepreneurs incur loss due to high existence of unhealthy competition.

PROSPECTS OF AGROPRENEURSHIP IN BANGLADESH

(1) Paddy is the only raw material of rice industry. Easy collection of paddy is one of the major forces of recent success of this industry. As Bangladesh produces huge amount of paddy, so the cost of collection of raw material is easy.

(2) In the last decade, there was commonly three variety of paddy. But by the development of science and

technology in harvesting and research field now farmers are producing a large variety of paddy almost all over the year. Rice research institutes as well as agriculture universities have invented new variety of rice every year.

(3) In Bangladesh, rice is the staple food, so it has high production as well as demand. It is hard for traditional system of rice mills to meet the domestic demand of rice, as a result noticeable number of auto rice mills has been established each district. An auto rice mill can produce a large quantity of rice by using low land as well as labor and paddy.

(4) Rice mills create a large number of employment opportunities. Though the machine is automatic but to input paddy to machine and to transport and marketing rice mills employ large number of labor and staffs. It helps entrepreneurs of rice mills to create self-employment.

(5) Most of the auto rice mills produce more than 4 types of rice like Minicate, Shorna, Najirshail, Basmati sugondhichaul, etc. As consumers consumption pattern has changed so different consumers demand different types of rice. So consumer's increasing demand helps to rapid expansion of agro industries. It has potential market demand.

(6) At present, new rice milling machines are available in our country. Most of the rice mills use China and Indian technology as it is cheap and very easy to install and also easy to replace. Different parts of China's machines are available. These machines also produce God quality rice.

(7) Finance is the most vital and challenging part for an entrepreneur of agro industry. In our country it is almost easy to get necessary capital from bank. Government has declared single digit interest based loan for agro-industries which implementation is under processing.

(8) Electricity is the main energy source of rice mills. As electricity is available, so the number of traditional rice mills are being converted to automatic rice mills. Government also provides uninterrupted electricity supply to agro-industry areas.

(9) Availability of labor with a cheap wage rate also foster the growth of agro-industries like garment sector. As most of the rural side labor of this country has farming experience, so they can work easily with a little training. Besides, youth employed educated personnel works in rice mills. It is very easy for agro-industries to maintain a healthy number of human resources.

(10) Government support for this industry is spontaneous. They provide as much support as they can from establishment to maintain of agro-industries. Government provides infrastructural support to less developed rural areas to ensure balanced agro based industrial development.

(11) Quality is maintained and controlled by proper authority. BSTI frequently visits ago-industries to examine quality, besides peripatetically court frequently check the retailer rice to ensure quality.

(12) Law and enforcement authority plays a very helpful

role in this sector. For any kind of criminal activities like snatching of transport, rough-neck, local violence, etc., they provide utmost help to industrialist.

(13) Processing of input and marketing of finished goods is essential for proper establishment and growth. Rice millers use variety of channels for collection of raw material as well as, marketing. After collection of raw materials (paddy); it was processed to rice by using modern technology after that it was sent to market through using supply chain.

(14) Rice mill not only produces rice but also it produces various kinds of byproducts such as broken rice, polish, rice bran, etc. which are used as raw material of other industries like animal feed industry, rice bran oil industry, etc., rice bran oil is a faster growing industry. It helps consumer by providing better quality edible oil. Rice bran oil has huge domestic as well as international demand. This by product makes a large portion of profit of agro-industries.

Besides a large number of prospect factors, there are some weaknesses and threats faced by the entrepreneur and established agro-based company in Bangladesh. These challenges are common in almost all kind of agro-industries in Bangladesh.

CHALLENGES OF AGROPRENEURSHIP IN BANGLADESH

(1) To establish and run an agro-industry, the entrepreneurs need to arrange a large number of capital. Most of the agro-industries are dependent on external source of financing, rather internal financing. It mostly depends on government and commercial banks. To arrange capital from external source, they have to bear a large amount of cost of capital. The interest rate also fluctuates time to time. Though government has declared single digit interest based loan for industrial sector but the implementation is under processing. When market falls or decreases in market demand of rice due to imported rice, it becomes difficult for industrialist to repay the installment on time. Sometimes they are declared as bankrupt.

(2) Maintaining market share and creating potential market is one of the big challenges for agro-industry. Consumers are more aware and health conscious than previous in selection of consumer goods. Most of the consumers demand better quality; as a result industry needs to ensure good quality product within the budget of consumer. As there are lot of competitor are continuously trying to inaugurate new variety of product so having qualified marketing agent and maintaining a competitive marketing channel is a must. But most of the industries do not have excellent marketing channel and they are less bothered of rapid expansion of existing market.

(3) Trading system faces great challenges. Most of the

industry has to sell product to wholesalers or retailers on due basis. It is almost impossible to make all transaction in cash basis as a result creation of bad debt is unavoidable and the number of bad debt rate is high in this sector. Sometimes, there is a problem of trustworthiness of the customer and retailer.

(4) Technology is a great challenge to small scale agro-industries. Though there is availability of modern rice milling machines and technology but almost all the technology installment expenditure is high. We do not have our own technology for auto rice mill and other agro-industry. So we have to purchase manufacturing and service providing technologies for this sector from abroad paying a high value for patent issue. Though some Bangladeshi technologies are used in semi auto rice mills, but there is no Bangladeshi technology in the market for automatic rice mills. Import tax is high for purchasing machines. Spare parts are not available; as a result it causes shut down of factory for shortage of machine parts or little troubles. It takes high investment for small and medium scale firm to adopt technology with high production capacity as well as low production cost like England, Japan technology so they use India or China technology.

(5) Lack of managerial knowledge is the main challenge. As most of the automatic rice mills, are converted from traditional small scale rice mills to auto rice mill, as a result, the executives have lack of knowledge and management ability to us; their industrial resources efficiently and effectively. They follow self-assumptions and previous business experience to deal with recent and upcoming challenges. There is noticeable number of system loses which could eliminate by using effective management practices.

(6) Very few agro-industries are connected with research institutions but almost all the agro-industries has no connection; even they do not feel the need of connection with research organization. An industry can prosper if it continues research for generating or upgrading product and business procedures. Though there are various consulting agencies and agro-based government and non-government research institutions, universities who work on development of new variety of raw material, finished goods, human resource development, entrepreneurial development, marketing, accounts maintaining, operation development, etc., but they are reluctant in connecting with medium and small scale agro-industries. So connecting with this research institution and maintaining co-operative relation is a challenge.

(7) Industrial law, environment law and labor law are not properly followed by the firm. So appropriate implementation of this law is mandatory for proper growth of this sector. But most of the industry do not practice all the law related with agro-industry. Millers follow self-created payment structure for labor and employees. Most of the time, they do not follow the proper environment

law; they haphazardly establish factory beside populated area. There prevails poor drainage system, smoke emission system, storage system in almost every firm. Bangladesh standard and testing institute (BSTI) though visit occasionally to ensure better quality but their presence and proper steps for quality assurance was not found in the survey.

(8) Illegal competition prevails in this agro-industry in case of government procurement of rice and other agro-based products. Powerful industrialist gets more allocation than their capacity as a result small scale firm's faces challenges.

(9) Lack of proper control on imported product creates threat for domestic agro-based industries. When natural calamities destroy crops the time government reduce taxes on imported product but government do not demotivate import on the time of surplus production of agro-product like rice.

(10) Transport is a vital factor for the success of marketing of the agro-industries. Though very few of the well-established industries have its own transportation system but most of the agro-industries have to depend on rented transport which increase the cost of product on the other hand this transport agencies are not very trustworthy.

(11) There is deficiency in services of law and enforcement authority. In case of transport snatching, local miscreants, product snatching in road and highway, etc., industrialist infrequently gets support from law and enforcement authority.

(12) Lack of infrastructural support is one of the biggest challenges for these agro-based industries. Most of the industries situated far away from the town or locality. In rural site, there is lack of infrastructural support like road is narrow, lack of central drainage system, lack of high speed internet facilities, etc.

(13) Natural calamities are one of the biggest challenges. It causes scarcity of raw materials as a result production cost becomes higher than as usual. It also hampers each and every elements of supply chain as our country is a riverine country and has seasonal impact so unexpected flood, storm, hailstorm, and river corrosion are common natural calamities which is a challenge of this agro-industries.

RECOMMENDATIONS TO FACE THE CHALLENGES

(1) A complete package for the benefits of young agripreneur programs needs to be introduced which provides technical advice, land management ideas, easy access of funding, continuous guidance/monitoring, and services on marketing.

(2) Single digit loan from government as well as other specialized and non-government organization and attractive foreign investment are needed to boost up the agro-industrial sector.

(3) Government should ensure proper infrastructural facilities to rural areas to create overall employment opportunity and to stop rapid migration by establish agro-industries.

(4) Law and enforcement authorities (industrial police) need to perform their duty properly to ensure free and fair trading domestically and in abroad.

(5) Government should provide realistic and agro-industrial friendly national as well as international trading policies, to ensure market stabilization.

(6) Research organizations need to be connected with agro-industries to ensure sustainable industrial growth, employment creation, introduce varieties of product line and to explore exclusive managing process.

(7) Environment friendly and renewable energy based agro-industries need to be established as it is a national as well as international demand.

(8) Millers could provide employment opportunity for both men and women of the rural areas. Compared to other major industries, employment scope in the agro-industries was observed high at the national level.

Conclusion

Bangladesh is still an agro-based economy where real economic development depends on the improvement of this sector, which is why the government always patronizes this sector. The agricultural activities in the country are pursued intensively for the crop as well as allied sectors and in conditions of scarce natural resources. The performance of this sector has great impact on macroeconomic situation like employment generation, poverty alleviation, food security and nutritional attainment. Rice is the staple food in the everyday diet of Bangladeshis. The production of rice, which can be harvested 2 or even 3 times a year. However, due to weather conditions, the production of rice and wheat fluctuate greatly, forcing Bangladesh to import food from the international market or turn to international aid. The agro-industrial sector remains elementary, underdeveloped and largely without significant institutional, technical and financial support. Finally, in general agripreneur, the agro-industrial sector of Bangladesh remains elementary, underdeveloped and largely without significant institutional, technical and financial support.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Farmers' perception of the parasitic weed *Alectra vogelii* Benth. and their cowpea varietal preferences in Burkina Faso

DIENI Zakaria^{1*}, BATIENO T. Benoit Joseph¹, ILBOUDO Dieudonné¹, TIGNEGRE Jean-Baptiste De La Salle^{1,2}, BAMA B. Hervé¹, ZIDA M. W. Felicien Serge¹, ZONGO Hamadou¹, SIDIBE Hamadou¹, PODA S. Léandre¹, TRAORE Karidiatou¹ and OUEDRAOGO T. Jeremy¹

¹Institut de l'Environnement et de Recherches Agricoles, Burkina Faso.

²AVDRC-World Vegetable Center, Mali.

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Burkina Faso was the third largest cowpea [*Vigna unguiculata* (L.) Walp.] producing country in West Africa in 2017. Cowpea is the fourth leading staple grain legume crop produced in the country. However, its production is hampered by several constraints, among which parasitic weeds are some of the most devastating. *Alectra vogelii* Benth. and *Striga gesnerioides* Wild Vatke are the main parasitic weeds in cowpea production in Burkina Faso. This study aimed at determining farmers' awareness about *Alectra vogelii* and their cowpea varietal preferences. To achieve this goal, information was obtained via a semi-structured questionnaire and focus group discussions in three districts (Koupela, Tenkodogo and Toussiana) of Burkina Faso, where *Alectra vogelii* occurs. The results showed that farmers in Toussiana district were more aware of this weed than in the other districts. Cowpea yield loss attributable to *Alectra vogelii* was up to 100%. Farmers' preferred traits were short-season varieties, with large size, rough and white grain. However, erect varieties were selected in Koupela and Tenkodogo districts; prostrate varieties were preferred in Toussiana district.

Key words: *Alectra vogelii*, awareness of *Alectra*, parasitic weed, preference, *Vigna unguiculata*, yield loss.

INTRODUCTION

Cowpea is the fourth most important food crop after sorghum, maize and pearl millet in production in Burkina Faso. It also serves as an important cash crop for the producers. The crop is an important source of protein in both human and animal diets. However, the average yield of cowpea remains low (500 kg/ha) (FAOSTAT, 2016). Several production constraints, including parasitic weeds,

are responsible for this low yield. The main parasitic weeds in cowpea production are *Striga gesnerioides* and *Alectra vogelii*. Hundred percent (100%) yield losses attributed to *S. gesnerioides* are often times recorded, with an average of 44.2% (Muleba et al., 1997; Tignegre, 2010). In contrast to *Striga*, yield losses related to *Alectra vogelii* in Burkina Faso have not been evaluated yet. In

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

other countries, such as Kenya, Malawi, Nigeria and Tanzania, more than 50% yield losses have been reported (Mbawaga et al., 2009; Omoigui et al., 2012; Karanja et al., 2013; Mbega et al., 2016).

A survey conducted in Malawi revealed that both agricultural extension agents and farmers were not well aware of the noxiousness of *A. vogelii* (Kabambe et al., 2013). Unlike *S. gesnerioides*, *A. vogelii* has green leaves like a normal photosynthetic leaf but they lack functional chlorophyll (Parker, 2012). The normal weed like appearance of *Alectra* could contribute to the ignorance by farmers of its noxiousness. This ignorance facilitates the dissemination of the weed, which is reproductive, within and across zones. An individual *Alectra* plant can produce up to 600 000 seeds that can remain viable in the soil for 15 to 20 years (Visser, 1978), making its eradication almost impossible. Nevertheless, control measures can be taken to reduce the effect of *Alectra* on cowpea if all the concerned actors are sensitized about this threat. Many years of efforts were deployed to develop new technologies for several constraints of production. Unfortunately, these technologies were rejected by end-users because they did not meet their needs and preferences. That constitutes a waste of time and resources (Tignegre, 2010).

To bridge the gap between breeders' objectives and the stakeholders' needs and preferences, a new research approach including all the partners was necessary. One of the popular and effective alternative proposed to address this issue is the participatory breeding approach through Participatory Rural Appraisal (PRA) (Cavestro, 2003). The PRA involves identifying with stakeholders the principal constraints in their cropping system and their desired varietal characteristics. This collaborative research is a powerful way for technology adoption and dissemination. It allows a clear communication and understanding of stakeholders' expectations and strengthens the collaboration among stakeholders, researchers and policymakers (McEntee, 2013). In fact, independently of scientists, farmers continuously experiment with new technologies and innovate sometimes on their own. Therefore, involving them in a collaborative research program will greatly contribute to the development and the sustainability of agriculture (Leitgeb and Vogl, 2010). Studies have shown that when stakeholders are associated with the selection process, the adoption rates of the products of breeding, goes up, thereby optimizing efforts deployed by breeders. In Burkina Faso a study implemented to evaluate the socio-economic impacts of cowpea technologies, showed that the income generated by cowpea at Donsin (Central Burkina Faso) had increased from 0.0% (1990) to 14.1% (2001). Within the same period it had increased from 15.7 to 49.9% at Bik Baskoure (Eastern Burkina Faso) (PRONAF, 2003). Cowpea consumers from Ghana and Cameroon are even willing to pay a premium for their

preferred grain types (Langyintuo et al., 2004).

Cowpea consumers' preferences are more or less specific to each country and even within areas of a country. However, in West and Central Africa, white color grain is widely preferred (Langyintuo et al., 2003). Other authors reported that white large-sized seeds are the most preferred in these areas (Tignegre, 2010; Saidou et al., 2011; Batieno, 2014; Ibrahim et al., 2014; Horn et al., 2015). In southern Benin, yield performance and resistance to insect pests in this order were reported as the most important criteria for choosing varieties (Gbaguidi et al., 2013).

Participatory Rural Appraisal (PRA) studies reported that low soil fertility, climate change, drought, *Striga gesnerioides*, inaccessibility to markets and lack of inputs were some of the main constraints to cowpea production in Burkina Faso (Tignegre, 2010; Batieno, 2014). However, the importance of some threats and farmers' preferences were specific to the targeted area. In addition, farmers can neglect the importance of a threat. The previous studies did not cover farmers' preferred cowpea varietal growth habits and cropping cycle. Therefore, it was important to determine farmers' preferences for these traits in combination with grain quality for the sake of further capturing their preferred varietal characteristics. It was also necessary to assess farmers' knowledge on the parasitic weed, *Alectra vogelii* in areas where it occurs.

The objectives of this study were to (i) determine the awareness among farmers about the parasitism of *A. vogelii* on cowpea and (ii) identify farmers' preferred varietal characteristics.

MATERIALS AND METHODS

Study sites

The Participatory Rural Appraisal (PRA) was conducted in two regions of the country: the West and the Central-east. These regions are located in the Sudanian and Sudan Sahelian climate zones, respectively. The annual rainfall range is over 900 mm for the former and from 600 to 900 mm for the latter regions. Two districts (Koupela and Tenkodogo) in the central-east region and one from the western region (Bobo-Dioulasso/Toussiana) were selected for this study. In each district, three villages where *Alectra* infested fields have been observed were targeted. Details of these sites are presented in Table 1.

Questionnaire administration

A questionnaire was randomly administrated to 10 to 15 farmers per village making a total of 112 interviewed farmers. Gender ratio was considered as much as possible in each village. Respondents were asked about their personal information, their crop production systems and problems they encountered in cowpea production as well as their varietal preferences. It was further necessary to understand how far farmers know the relation between the parasite and its host. For this purpose, they were asked to identify *A. vogelii* host crops they knew. It was also important to know farmers' perceptions about the effects of *Alectra* on cowpea yield. To

Table 1. Geographical description of the study sites.

Region	District	Village	Longitude (N)	Latitude (W)	Altitude (m)	Rainfall (mm)
Central-east	Koupela	Boantenga	12° 12,8'	0°20	330	600-900
		Zano	11°42,5'	0°22, 4'	325	
	Tenkodogo	Dabare	11°33, 3'	0°20, 59'	323	
		Laylay	11°34,3'	0°23, 44'	328	
Haut Bassin	Bobo-Dioulasso (Toussiana)	Wempea I	10°52,9'	4°33, 59'	339	>900
		Wempea II	10°51,52'	4°18, 56'	339	
		Tapoko-deni	10°52,44'	4°32, 44'	339	

facilitate this estimation, farmers were told to assume that they harvest 100 bags of 100 kg if *Alectra* does not occur. Subsequently, they estimated the number of bags they lost due to the prevalence of *Alectra*.

Focus group discussion

Focus group discussions (FGD) were held with 10 to 15 farmers in November 2015 in the selected villages about the issues encountered in cowpea production in general and particularly about the parasitism of *A. vogelii* on cowpea as well as their expectations in terms of cowpea varietal improvement. Heads of farmers' local organizations and/or extension agents were involved in the organization of the FGD. A group discussion was organized in each village. A multi-disciplinary team, composed of a breeder, a social scientist and a weed scientist had full day discussions with farmers in each village.

Data analysis

Data collected were analyzed with Sphinx Lexica version 4.5.0.28. Chi-square tests were run to check for similarities and dissimilarities of responses. Computation was done for each district or as a combination of the three districts. Factorial analysis and principal component analysis were also used. Results are presented in tables and graphs. The yield loss estimates were grouped into three classes as follows:

- Class one: Yield loss of less than 50%;
- Class two: 50% yield loss and
- Class three: More than 50% yield loss.

RESULTS

Farmers' awareness on *A. vogelii* as a parasitic weed to cowpea production

A total of 112 farmers were interviewed, among them 23.2% were women. The sample was distributed as follows: 33 farmers from Koupela, 32 farmers from Tenkodogo and 47 farmers from Toussiana (Table 2). Most of the respondents (72%) have seen *Alectra* before the survey but some of them did not recognize it as a parasite (Figure 1).

The results of the study also showed that nearly sixty

percent (59.8%) of the interviewed farmers knew *Alectra vogelii* as a parasitic weed. However, a large variability was observed in farmers' awareness across regions (Table 3). The results of the factorial analysis of farmers' awareness on *A. vogelii* showed that 66% of this variability was explained by the two first principal components (Figure 2). The district of Toussiana was associated to farmers' awareness. The chi-square test for awareness between districts was highly significant ($P<0.001$), in contrast it was not significant for gender ($P=0.34$) (Table 3).

Eighty-nine percent (89%) of the interviewed farmers from Toussiana knew *Alectra* as a parasitic weed. This locality accounted for 62.7% of the global farmers' knowledge about *Alectra*. On the other hand, 89.9% of the farmers who ignored its noxiousness were from Koupela and Tenkodogo (Table 3). No significant difference was observed between men and women awareness of *Alectra vogelii* (Table 3).

Farmers' identification of *Alectra vogelii* host crops

The results of farmers' identification of *A. vogelii* host crops are presented in Table 4. 65.2% of the respondents identified cowpea as host crop of *Alectra* followed by groundnut (48.2%). Three cereal crops: Pearl millet, sorghum and rice were identified as hosts of *Alectra* by 16.1% of the respondents. These farmers thought that *S. gesnerioides* and/or *A. vogelii* change into *S. hermonthica* and vice versa depending on the crop grown (legume or cereal). They did ignore that *Alectra* and *Striga* are different species of parasitic weeds parasitizing crop plants from different classes. The remaining legume crops (soybean and Bambara groundnut) were not well known by farmers as host crops as was expected because they were produced by few respondents.

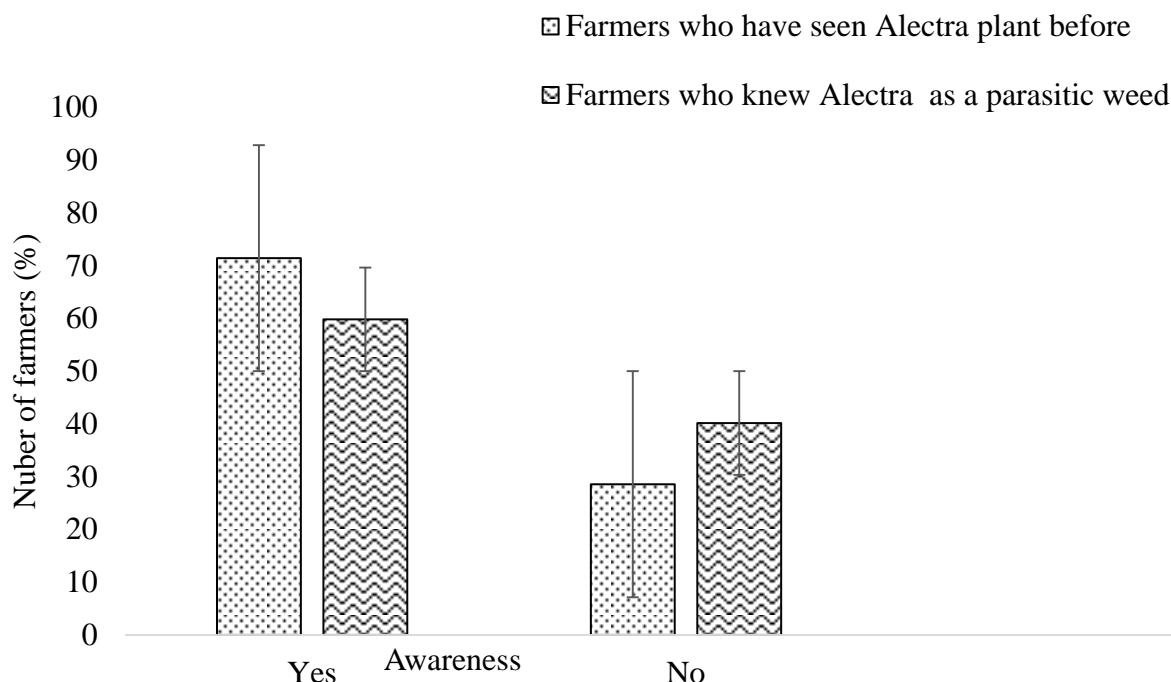
Farmers' identification of cowpea production constraints

Five biotic constraints to cowpea production were ranked

Table 2. Number of interviewed farmers per district and per sex.

Gender	District			Total	p-value
	Koupela	Tenkodogo	Toussiana		
Female	6 (23) [†]	10 (38.5) [†]	10 (38.5) [†]	26 (100) [†]	
Male	27 (31.4) [†]	22 (25.6) [†]	37 (43) [†]	86 (100) [†]	0.19 ^{ns}
Total	33	32	47	112	

[†]Percentage in brackets; ^{ns}not significant.

**Figure 1.** Proportion of farmers for their awareness on *Alectra vogelii* in the study areas.**Table 3.** Farmers' awareness of *Alectra* as a parasitic weed per gender and locality.

Variable	Awareness				Total	p-value
	Observed	Expected	Observed	Expected		
Gender						
Male	53 (61.6) [†]	51.45	33 (38.4) [†]	34.55	86	
Female	14 (53.8) [†]	15.55	12 (46.2) [†]	10.45	26	0.34 ^{ns}
Locality						
Koupela	11 (16.4) [†]	19.74	22 (48.9) [†]	13.26	33	
Tenkodogo	14 (20.9) [†]	19.14	18 (40) [†]	12.86	32	
Toussiana	42 (62.7) [†]	28.12	5 (11.1) [†]	18.88	47	<0.001**

[†]Percentage in brackets, ^{ns}not significant, **highly significant.

by farmers in the three sites as presented in Table 5. At Koupela, *S. gesnerioides* was ranked as the most

important constraint to cowpea production followed by insect pests, foliar diseases and stem diseases. *A. vogelii*

Table 4. Farmers' perception of host crops of *A. vogelii*.

Potential <i>Alectra</i> host corps identified by farmers	Number
No response	31 (27.7) †
Cowpea	73 (65.2) †
Groundnut	54 (48.2) †
Soybean	1 (0.9) †
Bambara groundnut	3 (2.7) †
Pearl millet	11 (9.8) †
Sorghum	5 (4.5) †
Rice	2 (1.8) †
Total observation	112

†Percentage of respondents in brackets

Table 5. Farmers' ranking of the importance of biotic constraints of cowpea production in the study areas.

Constraint	District						Total	Rank
	Koupela	Rank	Tenkodogo	Rank	Toussiana	Rank		
Foliar diseases	5	3	0		9	4	14	4
Stem diseases	3	4	0		2	5	5	5
Insects	19	2	23	2	27	2	69	1
<i>S. gesnerioides</i>	25	1	26	1	9	3	60	2
<i>Alectra vogelii</i>	1	5	4	3	34	1	39	3
Total	53		53		81		187	

Table 6. Farmers' perception of cowpea yield loss related to *Alectra* infestation per locality.

Locality	Yield loss				Total	p-value
	No idea	<50%	50%	>50%		
Koupela	26	2	3	2	33	
Tenkodogo	20	3	6	3	32	
Toussiana	5	6	10	26	47	<0.001**
Total	51	11	19	31		

**Highly significant.

was ranked the least important. Farmers at Tenkodogo did not recognize foliar and stem diseases as constraints to cowpea production. *S. gesnerioides*, insects and *A. vogelii* in this order were ranked as the main biotic constraints. However, at Toussiana, *Alectra* was ranked first before insects and *Striga*. Overall, the most important biotic constraints in cowpea production identified in the three sites by farmers were *S. gesnerioides*, insects and *A. vogelii* in this order. The prevalence of both *S. gesnerioides* and *A. vogelii* was linked with low soil fertility which was also identified as a production constraint. Poor soil was thought to be a channel for *A. vogelii*. Farmers at Koupela mentioned that damages caused by *Striga* are more severe when drought occurs at the reproductive stage. Besides, post-harvest problems were mentioned at Toussiana principally the

high cost of the Purdue Improved Cowpea Storage (PICS) bags for safe storage. Inaccessibility to seeds of improved varieties and other inputs (fertilizers, pesticides) also came out as constraints in cowpea production.

Farmers' perception on the effects of *A. vogelii*

Yield loss due to *A. vogelii* was differently appreciated by farmers. The chi-square test was highly significant for the estimate of yield loss between farmers from the three locations (Table 6). The yield loss estimates were grouped into three classes like mentioned in materials and methods. The proportions of farmers for their perception of cowpea yield reduction related to *Alectra* are presented in Tables 6 and 7. Many farmers (45.5%)

Table 7. Farmers' perception on cowpea yield losses due to *A. vogelii*.

Yield loss	Number of respondent
No idea	51 (45.5) [†]
Less than 50%	11 (10.5) [†]
50%	19 (17) [†]
More than 50%	31 (27) [†]

[†]Percentage in brackets.

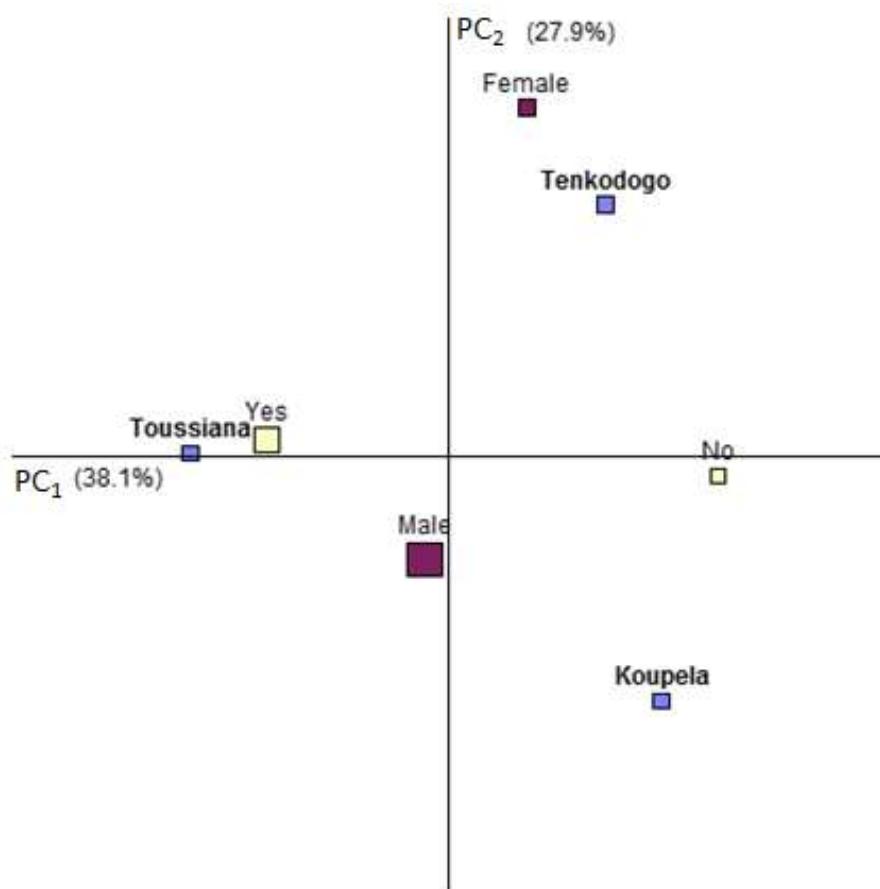


Figure 2. Factorial analysis of farmers' awareness on *A. vogelii* according to their locality and gender.

did not have an idea on the damages caused by *Alectra* on cowpea. Farmers' perceived yield losses ranged from 5 to 100% yield loss (Table 7). The study also showed that 44% of the farmers attributed at least 50% yield reduction due to *A. vogelii* (Table 7). At Toussiana 76.6% of the respondent were in favor for this opinion (Table 6). The principal component analysis graph showed that Koupela and Tenkodogo were grouped at the opposite sides of Toussiana from the origin of the graph (Figure 3). The first axe accounted for the total weight of estimated yield loss.

Production system and control measures

Two main cropping systems were used by farmers: Crop rotation (61%) and intercropping (36%) (Figure 4). The two systems were simultaneously used in some cases. Cereal-legumes or cereal-cereal (sorghum, pearl millet) rotations were used. A few of them were practicing monocropping. These practices are often used for restoring soil fertility and weed management. Cultural practices have been farmers' main method for controlling *Striga* sp. and *A. vogelii*. Manual uprooting has been the widest

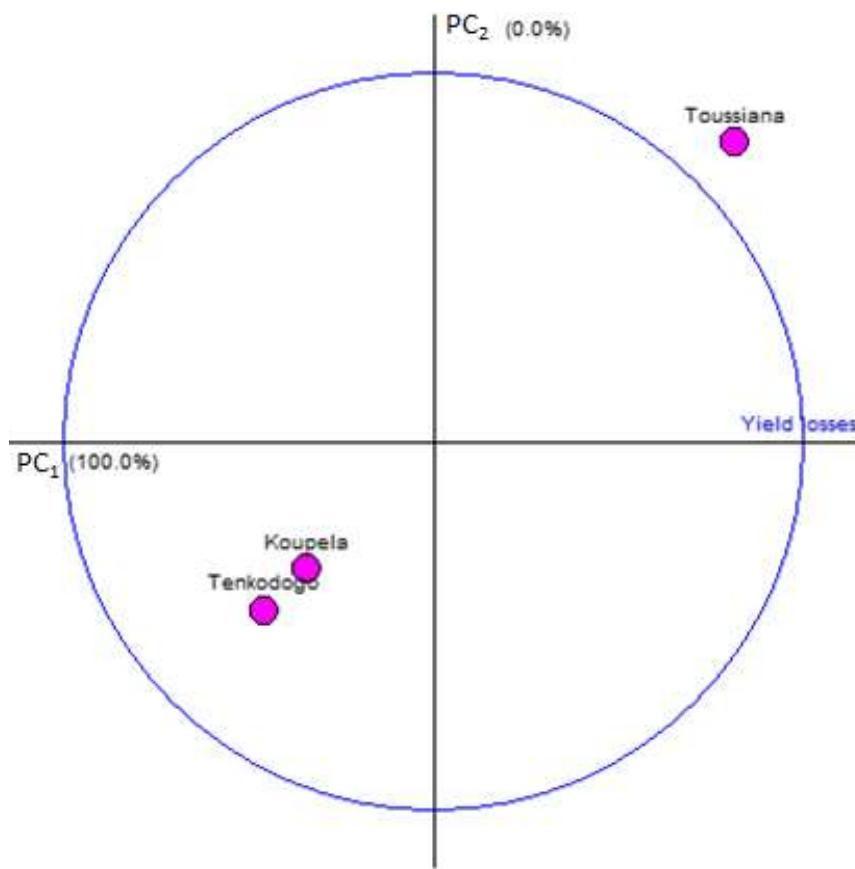


Figure 3. Principal component analysis showing the importance of estimated yield losses due to *Alectra vogelii*.

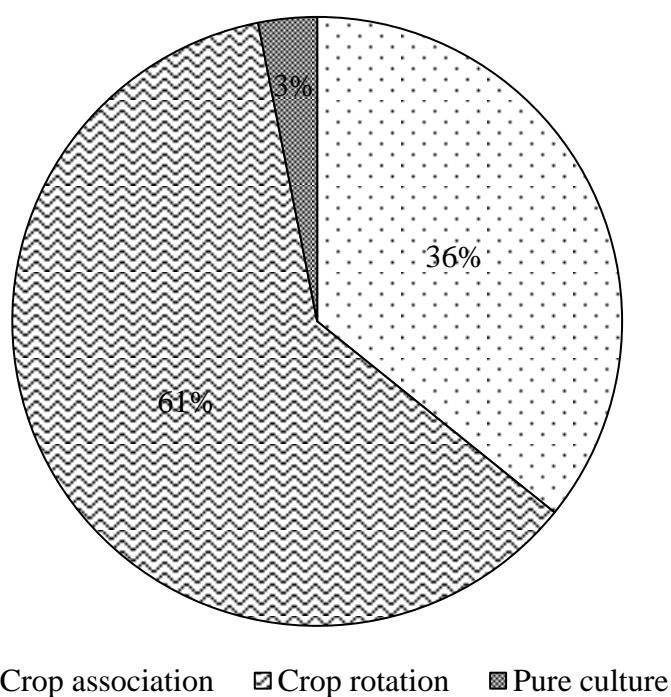


Figure 4. Cropping systems used by farmers in the studied areas.

Table 8. Farmers' preferred varietal characteristics and chi-square p-value.

Trait	Varietal characteristic	Koupela	Tenkodogo	Toussiana	Total	p-value
Growth habit	Prostrate	9	6	35	50 (44.6) [†]	0.012*
	Semi-erect	9	9	5	23 (20.5) [†]	
	Erect	18	20	12	50 (44.6) [†]	
Cycle	Short	30	27	34	91 (81.3) [†]	0.042*
	Medium	5	7	10	22 (19.6) [†]	
	Long	0	0	6	6 (5.4) [†]	
Grain color	White	33	31	45	109 (97.3) [†]	0.508 ^{ns}
	Red	0	1	4	5 (4.5) [†]	
	Black	0	0	0	0 (0) [†]	
Grain size	Big size	31	28	38	97 (86.6) [†]	0.313 ^{ns}
	Medium size	5	6	9	20 (17.9) [†]	
	Small size	0	0	3	3 (2.7) [†]	
Grain texture	Wrinkled	25	22	25	72 (64.3) [†]	0.134 ^{ns}
	Smooth	8	10	22	40 (35.7) [†]	

[†]Percentages in brackets; *Significant, ^{ns}, not significant.

practiced control measure. The application of organic matter was mentioned as a control method as well. However, farmers recognized the limitations of these methods and were therefore willing to experiment with new technologies such as the use of resistant varieties.

Farmers' varietal preferences

Farmers' preferred characteristics of cowpea varieties are presented in Table 8. Results indicated that varieties with wrinkled grain texture (64.3%) large grain size (86.4%) and white grain color (97.3%) were largely preferred by farmers across the three locations. For grain quality no difference was observed between farmers' preferences in the different locations. However, with regard to the growth habit and the cropping cycle slight differences were observed even though prostrate (44.6%) and erect (44.6%) short cycle varieties (81.3%) were preferred. Prostrate varieties were more preferred at Toussiana whilst erect varieties were preferred at Koupela and Tenkodogo. In summary, farmers' preferred cowpea varietal characteristics for all the locations were: Prostrate and erect sort cropping cycle varieties with rough big sized white grains.

DISCUSSION

The results of this study showed a large variability in farmers' awareness on the parasitic effects of *A. vogelii*. Whilst the weed was well known by farmers from

Toussiana in western Burkina Faso, its parasitic status was not known by the large majority of farmers from the districts of Koupela and Tenkodogo in the central-eastern part of the country. In contrast to its related species, *S. gesnerioides* was very familiar to the farmers from this region where it has been considered as first biotic constraint. Because of the appearance of *A. vogelii* to ordinary weeds, farmers do not pay particular attention to it until it becomes a serious problem. Actually farmers generally recognize a threat as such from the importance of its damages. For example in western Burkina Faso where *Alectra* was first observed (Subrahmanyam et al., 1989) (probably from where it was introduced into the country), it had enough time to disseminate and increase the seed bank in the soil freely without any control measure. In addition, little research and communication was done to help farmers recognize it as a constraint, because the economic importance of *Alectra* was neglected by researchers. Thus the ignorance of farmers about the parasitic weed status of *Alectra* could have contributed to its dissemination. Therefore, it is undoubtedly the gradual effects of the weed that led most farmers to get familiar with it in the district of Toussiana. The ignorance by most farmers at Koupela and Tenkodogo of the parasitic effects of *A. vogelii* could be related to its relatively low infestation density in these areas.

Though farmers identified very well the main host crops of *A. vogelii*, cowpea (65.2%) and groundnut (48.2%) were recognized to be associated with the occurrence of *Alectra* in the field. These crops are the largest grain legume crops produced in Burkina Faso. It was realized

that some farmers thought that *S. gesnerioides* changed into *A. vogelii* or *S. hermonthica*. They did not know that these weeds are different species of parasitic weeds. This opinion could be explained from their cultural practices. Legume-cereal rotation in *Alectra* infested field will make farmers think that the legume crops spread *Alectra* because the weed only emerges when its hosts (cowpea and groundnut) are grown. Farmers' knowledge on the link between a parasite and its host derives from their skill in determining host-parasite interaction in the field. This skill allows them to identify the various types of threats hampering their production. However, this study revealed that some farmers failed to recognize *A. vogelii* as a parasitic weed even though they have seen it before and identify its host crops. Collaborative research and other scientific training should be intensified to strengthen farmers' knowledge about production constraints and the new technologies as well.

The extent of *A. vogelii* damage on cowpea depends on the degree of infestation of the field. The current study revealed that farmers from western Burkina Faso, regardless of gender, knew better about *A. vogelii* compared to those from the other studied localities because the weed is more widely spread in the former region. Similarly, the highest yield loss was reported by farmers from this locality. The prevalence of *A. vogelii* in this locality was reported 30 years ago. However, rigorous measures were not implemented to control it and its highly invasive potential allowed an exponential increase of the seed bank in the soil. Consequently, in heavily infested field, 100% yield losses were registered. Elsewhere in Africa, yield losses ranged from 30 to 100% (Mbwaga et al., 2009; Omoigui et al., 2012; Karanja et al., 2013; Mbega et al., 2016). The economic importance of *A. vogelii* revealed by this study calls for urgent measures to control it. However, it is important to notice that this weed like the other related parasitic weeds is very difficult to manage. In addition, the occurrence and severity of *A. vogelii* is tightly related to poor soils. Therefore, only an appropriate integrated management strategy with genetic resistance as the principal component could allow addressing this threat.

A successful use of genetic resistance in the control of *A. vogelii* will depend on farmers' acceptance of the resistant varieties proposed. So the study also included farmers' varietal preferences as selection criteria. In all PRA sites, farmers' preferred varietal characteristics were large-sized, rough texture and white seed-coat grains. These criteria refer to productivity and market demand. Yield performance coupled with market demand are by far the most important criteria of farmers' choice (Tignegre, 2010; Gbaguidi et al., 2013; Batieno, 2014). Besides these characteristics, farmers also selected varieties for culinary characteristics. In the study areas brown and red colored seed were entirely rejected by farmers. However, Tignegre (2010) found that farmers in the Sahel (Oudalan province) preferred brown colored

seeds. Therefore, a small variation is observed in farmers' choice for this trait within the country. Similar report was done by Langyintuo et al. (2003), though white grain testa remains largely preferred by farmers in West and Central Africa according to these authors. Cowpea growth habit and reproduction cycle were also important criteria of choice in the areas covered in this study. Unanimously, farmers chose short cycle varieties in all three localities but prostrate and erect varieties were equally preferred by farmers from the western region and central-east regions respectively. On the one hand, low and irregular rainfall within and across years has heavily influenced farmers' decision about choosing short cycle varieties. Drought was mentioned by farmers as one of the most important constraints to cowpea production (Tignegre, 2010; Batieno, 2014) by putting emphasis on terminal drought (Batieno, 2014). As such, short cycle varieties will be ideal for them to escape terminal drought. On the other hand, cultural practices (pure culture or intercropping) guided farmers' choice. Whilst farmers from Western Burkina Faso (Toussiana) favored prostrate varieties, erect ones were chosen in central-east (Koupela, Tenkodogo). In the latter region, farmers have progressively moved from intercropping to cowpea mono-cropping with the release of new varieties. In contrast at Toussiana local varieties are still widely produced. Farmers mentioned the lack of improved cowpea varieties and technical packages as production constraints. From the above analysis, it can be undoubtedly inferred that to meet farmers' preferred varietal characteristics, breeding objectives in Burkina Faso should be focused on selecting varieties possessing large-sized and white-colored grain with adaptation for both the local food consumption and market demand for all the covered zones. These selection criteria corroborate those suggested by Tignegre (2010) and Batieno (2014). Such varieties should be able to withstand most biotic constraints (*S. gesnerioides*, *A. vogelii* and insects) and abiotic constraints (drought, heat).

CONCLUSION

This study showed that *S. gesnerioides*, insects and *A. vogelii* were the most important biotic constraints to cowpea production. *A. vogelii* was ranked third biotic constraint. Farmers from western Burkina Faso were more aware about its damage on the host crop. Both female and male farmers' in the three study areas preferred short cycle varieties with large-sized, rough texture and white grain for both consumption and market demand. Consequently, selecting new improved varieties for Burkina Faso should take into account the aforementioned characteristics coupled with resistance to the main biotic and abiotic constraints identified. The development of such varieties will speed up their

adoption by the stakeholders.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to disclose

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Full Length Research Paper

Impact of tsetse and trypanosomiasis control on poverty: A case of Pate Island of Lamu County, Kenya

Seth Onyango^{1*}, Sabina Mukoya-Wangia², Josiah Kinama³ and Pamela Olet¹

¹Kenya Tsetse and Trypanosomiasis Eradication Council (KENTTEC), P. O. Box 66290-00808, Wetlands, Kenya.

²Department of Agricultural Economics, Faculty of Agriculture, University of Nairobi, P. O. Box 29053, Nairobi, Kenya.

³Department of Plant Science and Crop Protection, Faculty of Agriculture, University of Nairobi, P. O. Box 29053-0625, Nairobi, Kenya.

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The livestock rearing households of Pate Island in Lamu County of the Northern Coast of Kenya had been participating in the control of tsetse flies and trypanosomiasis. The objective of this study was to estimate the change in household wealth index resulting from tsetse and trypanosomiasis control. The study was conducted on 254 project households and 282 non-project households in the County. Using structured questionnaires, the study collected data on household characteristics, number of livestock in the household by type, household durable assets and living conditions. Principal Components Analysis (PCA) was used to construct the household wealth index as an outcome to measure the well-being of households. The results indicated that the proportion of very wealthy households was higher (16%) in the project areas than in non-project areas (3.7%). Propensity Score Matching (PSM) results showed that the mean wealth index for a project participating household was 0.699 (Std. Err. = 0.048 while -0.745 (Std. Err. = 0.077 if the household had not participated in the project denoting an increase of 1.444 in the household wealth index. In conclusion, the study shows that the tsetse and trypanosomiasis control project had brought an improvement in the wealth index of the project participated households. Therefore, it is recommended that governments mobilize resources to control tsetse flies and the disease in infested areas of Africa to improve the well-being of rural households.

Key words: Impact, tsetse, trypanosomiasis, wealth.

INTRODUCTION

Tsetse flies (*Glossina species*) transmit a fatal zoonotic disease called trypanosomiasis. The disease is known as sleeping sickness in humans and nagana in livestock. Tsetse flies infest 37 sub-Saharan African countries covering approximately 9 million square kilometres (km^2) and threaten about 60 million people and 48 million cattle

(WHO, 2001). It is one of the greatest problems hindering agricultural development in the sub-humid and humid zones of Africa. Sleeping sickness was under control in Africa during the 1960s and 1970s. However in the last two decades a spread of the disease to epidemic proportions has been observed due to the breakdown of

*Corresponding author. E-mail: sethooko@yahoo.com, Tel: +254722281123.

the control programmes causing a public health crisis in many affected areas (Smith et al., 1998). If the goal of poverty reduction and food security has to be achieved, this major hindrance to rural development needs to be removed.

To address the problem, African Heads of State and Government collectively launched the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) project in 2000 with a view of guiding the process of eradicating tsetse flies and trypanosomiasis (T&T). As part of this initiative, the African Union PATTEC (AU-PATTEC), the African Development Bank (AfDB) and the governments of affected countries prepared a proposal for a Pan-African programme, the Eradication of Tsetse and Trypanosomiasis in Sub-Saharan Africa (ETTSSA), as well as proposal for the first phase of the eradication campaign (AfDB, 2004). Six countries participated in the Phase I Project, three countries in West Africa (Burkina Faso, Mali and Ghana) and three others in East Africa (Ethiopia, Kenya and Uganda).

In Kenya, the area infested by tsetse is estimated to be 138,000 km² covering 38 out of 47 counties (KENTTEC, 2011). The disease impoverishes livestock farmers and threatens food security and livelihoods. The risk of human sleeping sickness outbreak is high in the Lakes Victoria and Bogoria basins and the Mara Serengeti tsetse belt all with a total human population of about 11 million people at risk of infection (KENTTEC, 2011; KNBS, 2010). The first phase of PATTEC-Kenya project was implemented from 2005 to 2011 covering a total area of 24,000 km² in three tsetse belts named Lake Victoria basin, Lake Bogoria and Meru/Mwea. The control of tsetse and trypanosomiasis in Pate Island served as a pilot project in the Coastal tsetse belt in an area of approximately 62 km² with the vector and disease control starting in 2010. The direct achievements of tsetse and trypanosomiasis eradication interventions under the PATTEC-Kenya programme include reduction of tsetse fly populations and reduced disease prevalence in livestock and in humans (KENTTEC, 2009, 2013, 2014, 2015, 2016; AfDB, 2011). The impact of these changes on income and wellbeing of households had not been quantified. It was therefore important to understand the impact of the project on household income for African governments to roll out tsetse and trypanosomiasis eradication campaign to other tsetse infested areas which had not been covered by such a programme. The objective of the study therefore was to estimate the change in the wealth index of the project households in Pate Island of Lamu County.

Review of theoretical literature

Theory of change

A theory of change (TOC) describes the causal

assumptions behind the links in the results chain; what has to happen for the causal linkages to be realized (Weiss, 1995; Blamey and Mackenzie, 2007; Leeuw, 2012; Rogers, 2008). Theories of change lead to the understanding of how and why the activities of the intervention are expected to lead to the desired results. The use of TOC in development evaluations has been reviewed by James (2011); Stein and Valters (2012) and Vogel (2012) and all point out that TOC uses intuitive notions of reaching some target group, changing their motivation and behaviour.

Measurement of impacts presents a large variety of econometric complications. The ultimate objective of the analysis of a treatment or intervention would be the effect of treatment on the treated individuals. In the literature it is documented that measuring this effect econometrically encounters at least two compelling computations namely endogeneity and missing counterfactuals (Greene, 2012; Roy, 1951; Rubin, 1974). In the endogeneity of the treatment problem, the analyst risks attributing to the treatment causal effects that should be attributed to factors that motivates both the treatment and the outcome. Drawing an example from tsetse control, an individual farmer who participates in tsetse control might have well succeeded more in life than their counterpart who did not participate even if they themselves did not participate in tsetse control.

On the other hand, with the missing counterfactual problem, in order to measure the impact of tsetse control on individual farmer's income, we would have to run an individual's lifetime twice, once with participation in tsetse control and once without. But, any individual is observed in only one of the two states, so the pure measurement is impossible leading to the missing counterfactual problem. Greene (2012) points out that accommodating these two problems, the endogeneity of treatment and the missing counterfactual, forms the focal point of this enormous and still growing literature on program evaluation and Rubin's causal model (1974, 1978) provides a useful framework for the analysis. This study was anchored on the theory of change to underpin the impacts of tsetse and trypanosomiasis control interventions in Pate Island. Figure 1 is a conceptual framework showing the results chain for the control of tsetse and trypanosomiasis in Pate Island in the Coastal region of Kenya.

Conceptual framework

The study conceptualized that at the inception of the tsetse and trypanosomiasis eradication project, livestock farmers were sensitized and trained on tsetse and trypanosomiasis control using different control methods. The project staff were also given technical training on tsetse and trypanosomiasis control. The trainings and the support given in terms of initial insecticides issued to farmers, tsetse target screens, tsetse traps, and

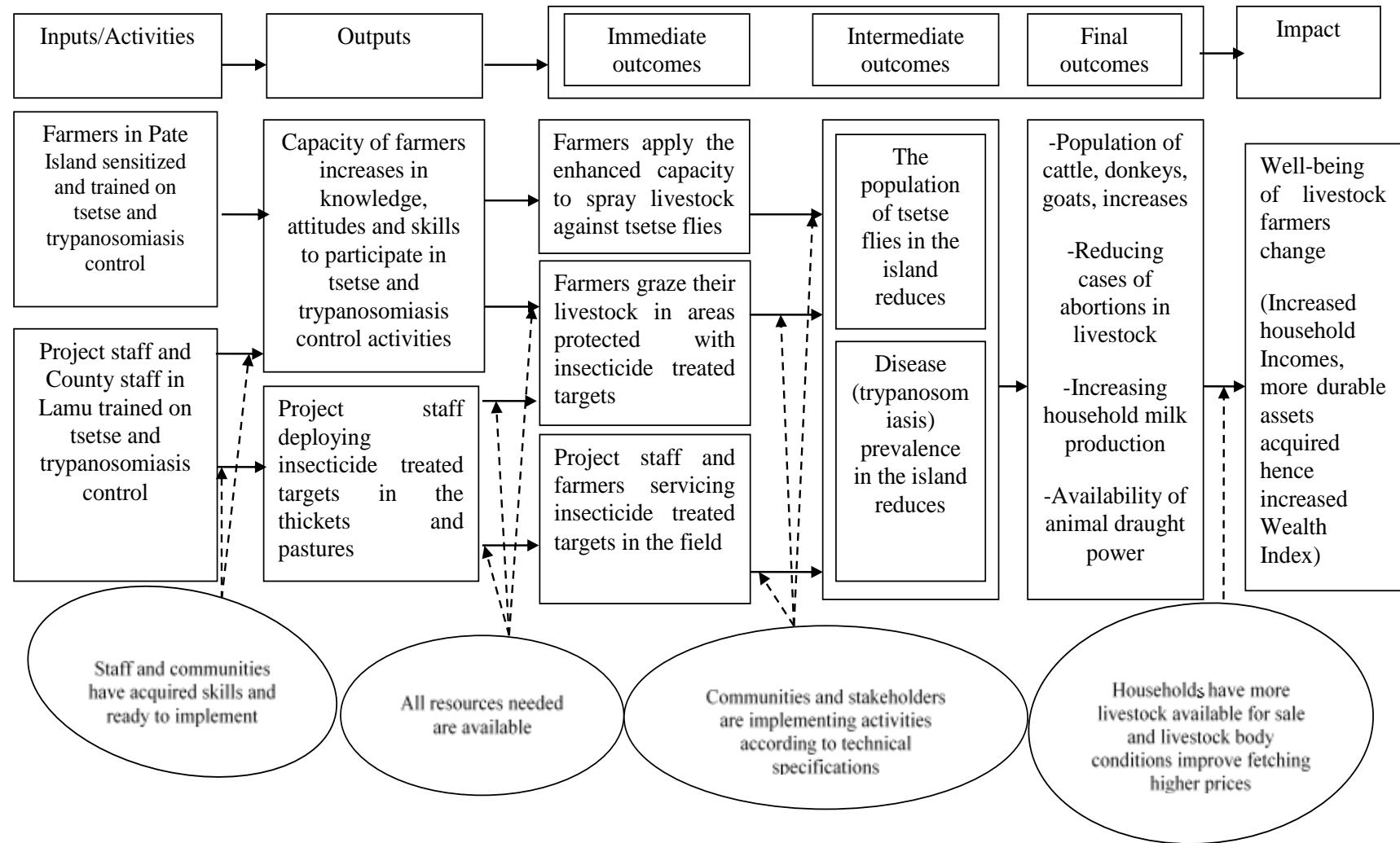


Figure 1. A conceptual framework showing the results chain for the control of Tsetse and Trypanosomiasis in Pate Island in the Coastal region of Kenya.

provision of other project operational costs served as project inputs which in the project results chain culminated into project outputs.

The outputs conceptualized in this study include the increase in farmers' capacity in terms of knowledge, attitudes and skills to control tsetse

and trypanosomiasis, project officers able to deploy the insecticide treated targets in the tsetse infested thickets. The immediate and intermediate

outcomes include farmers applying the enhanced capacity to spray livestock against tsetse flies, farmers graze their livestock in areas protected with insecticide treated target screens, project staff and farmers carrying out the servicing and replacement of worn out insecticide treated targets in the field.

The population of tsetse flies reduces in turn and disease (trypanosomiasis) prevalence in the intervention areas reduces. The final outcome of this is that the population of cattle, donkeys, goats and sheep is expected to increase, the cases of abortions in livestock expected to reduce and household milk production increases. The farmers in turn get more income from the sale of milk, cattle, sheep, goats and donkeys. The conceptual framework is presented in Figure 1. In the conceptual framework, the solid arrows represent the causal links between results; what has to happen for the causal linkages to happen. The doted arrows represent the underlying assumptions in the causal linkages for the planned change to occur.

METHODOLOGY

Study area

Pate Island is located in the Indian Ocean along the Northern Coast of Kenya in Lamu County lying between latitude 1°40, 20° 30 S and longitude 40° 15 and 40° 35 East (GoK, 2013). The Pate Island is the largest Island in the Lamu Archipelago, which lie between the towns of Lamu and Kiunga, close to the border of Kenya and Somalia. There are two administrative divisions in the Island namely Faza and Kizingitini (GoK, 2013).

The Island has a total area of 92.9 km² of which the agricultural farm land area is about 60 km². The Island has a livestock population of 8,150 heads of cattle, 6,250 goats and 3,200 donkeys. Lamu East Sub-county of Lamu County enjoys two rainy seasons and temperatures ranging between 23 and 32°C throughout the year. The long rains come in April and May while the short rains come in November and December. The main economic activities in the island include agriculture, livestock keeping and marine activities with the residents being predominantly Muslim. The study site was selected because of being an isolated area. The sea acted as a natural barrier separating the island from other islands and the main land where the project had not been implemented. This minimised the interaction between the project households and non-project households hence restricting the project spill-over effect. For example, if livestock from non-project households had access to the project area grazing lands where insecticide treated target screens were deployed, the benefits would spill over to the non-project households leading to confoundedness. Figure 2 shows a map of the study area.

Sampling

Sample size determination

The population of farm households practicing livestock rearing in the study area was obtained from the extension reports of the County Government of Lamu. According to Kothari and Gaurav (2014) the formula applicable in the case of a finite population is given as:

$$n = \frac{z_{\alpha/2}^2 N \sigma^2}{(N-1)e^2 + z_{\alpha/2}^2 \sigma^2} \quad (1)$$

where

n is the size of the sample

N is the size of population

e is the acceptable estimation error given by $e = z_{\alpha/2} \frac{\sigma}{\sqrt{n}}$

σ is the standard deviation of the population

$z_{\alpha/2}$ is the critical value using the $N(0,1)$ distribution for confidence level α

In this study, the sample size was computed using the above stated formula at a Confidence level of 95% and a margin of error of 5%. The population of project households was 734 and that of

non-project households 959. With the critical value $z_{\alpha/2} = 1.96$ and $\sigma = 0.5$, the sample size for the project households was determined as:

$$n = \frac{1.96^2 * 734 * 0.5^2}{(734-1)0.05^2 + 1.96^2 * 0.5^2} = 252.4 \approx 253$$

and the sample size for the non-project households was:

$$n = \frac{1.96^2 * 959 * 0.5^2}{(959-1)0.05^2 + 1.96^2 * 0.5^2} = 274.5.9 \approx 275$$

Sample selection

The sample of project households was drawn from the tsetse and trypanosomiasis controlled area of Pate Island. The area covers eight administrative sub-locations namely Kwatini, Kwatongani, Pate, Siyu, Shanga, Tchundwa, Kizingitini and Myabogi all with different number of livestock rearing households. The village roads were used as transects along which proportional samples were systematically drawn from each village until a total of 254 households was obtained for project households. The first household along each transect was randomly selected. There after every 5th household with livestock was selected for interviewing. The sample of non-project households was drawn from Amu and Hindi divisions of Lamu County where the Kenya Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) had not commenced tsetse and trypanosomiasis control interventions. The sub-locations covered in Hindi division were Hindi, Bargoni, Mokowe and Kilimani while those covered in Amu division were Matondoni, Kipungani, and Manda. Households with livestock in the non-project area were sampled following the same sampling protocol adopted for the households in the project area. A total of 282 non-project households were selected and interviewed.

Data collection

Using structured questionnaires administered through household

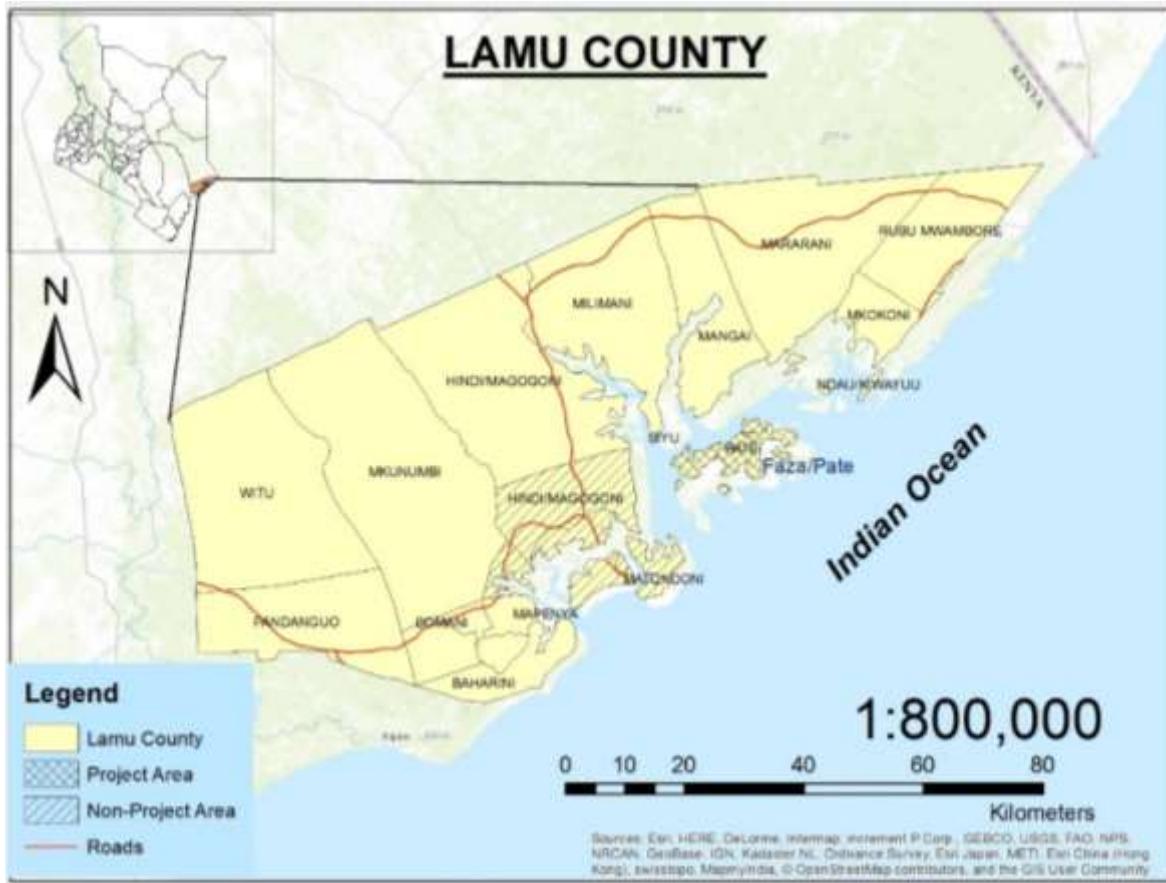


Figure 2. A Map showing the study areas of Pate Island, Amu Island and Hindi division of Lamu County, Kenya.
Source: Esri, HERE, DeLorme, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordinance Survey, Esri Japan, METI, Esri China (Hong Kong), Swisstopo, MapmyIndia, OpenStreetMap contributors, and the GIS user community.

interviews, the study collected socio-economic data including household characteristics, livestock (cattle, sheep, goats, donkeys and poultry) production, ownership of durable assets and living conditions in the household.

Indicators of household welfare

There are various indicators of welfare that may be used as outcomes to gauge impacts of a program. Baker (1960) considers poverty measures including head count index, poverty gap index, squared poverty gap and Watts's index. The head count index measures the proportion of the population living in households with income per person below the poverty line while the poverty gap for each household is the difference between the poverty line and the household income (Ravallion, 1994). The two methods are however not distribution sensitive. Some distribution sensitive measures include squared poverty gap where individual poverty gaps as a proportion of poverty line are squared before taking the mean and Watts's index which is the mean of log of the ratio of the poverty line to income (Atkinson, 1987).

According to Deaton (1997), expenditure-based economic status indicators have been found to be more reliable than indices that are income based. The main reason is the relatively high non-response rate for income based measures as well as under or over reporting

typically found in income items utilized in standard of living household surveys.

Filmer and Pritchett (2001) popularized the use of Principal Component Analysis (PCA) for estimating wealth levels using asset indicators to replace income or consumption data and concluded that PCA provides plausible and defensible weights for an index of assets to serve as proxy for wealth. Asset-based measures depict an individual or a household's long-run economic status and therefore do not necessarily account for short-term fluctuations in economic well-being or economic shocks. This study estimated the wealth index as an outcome for the assessment of impact of tsetse and trypanosomiasis control. Cordova (2008) points out that the indicator together may tap a long term dimension of economic well-being of the households.

Constructing the household wealth index

The wealth index (WI) is a composite index composed of key asset ownership variables. The WI is normally used as a proxy indicator of household level wealth. This was calculated using the PCA method performed on variables which are indicators of wealth among the farm households in Lamu County, Kenya. Table 1 shows the indicators of wealth that were used in the study and the dichotomised household responses where Yes = 1 when a

Table 1. Indicators of wealth among households and dichotomised responses.

Asset	Rich household	Poor household
Land	1= Yes	0= No.
TV Set	1= Yes	0= No.
Radio	1= Yes	0= No.
Mobile phone	1= Yes	0= No.
Bicycle	1= Yes	0= No.
Car or Boat	1= Yes	0= No.
Motorcycle	1= Yes	0= No.
Cattle	1= Yes	0= No
Improved cattle	1= Yes	0= No
Donkeys	1= Yes	0= No.
Goats	1= Yes	0= No.
Dairy goats	1= Yes	0= No
Sheep	1= Yes	0= No.
Poultry	1= Yes	0= No.
Type of dwelling unit		
Roofing material	1= Iron sheet or Tiles	0= Makuti or Grass thatch
Walling material	1= Bricks or Stone	0= Mud, lime or Timber
Floor material	1= cement	0= dust

household owns asset and No = 0 when household does not own the asset in question. The dichotomised responses were used to obtain the first principal component loadings which assigned weights for assets and housing conditions giving rise to household wealth index.

The estimation of relative wealth using PCA is based on the first principal component concept. Following the example of Cordova (2008), the study expressed the wealth index (WI) for farm household in Lamu County as:

$$y_i = \alpha_1 \left(\frac{x_1 - \bar{x}_1}{s_1} \right) + \alpha_2 \left(\frac{x_2 - \bar{x}_2}{s_2} \right) + \dots + \alpha_k \left(\frac{x_k - \bar{x}_k}{s_k} \right) \quad (2)$$

where:

y_i : is the wealth index for Lamu county farm household i .

x_1, x_2, \dots, x_k : is a vector of asset variables which include ownership of land, television set, radio set, mobile phone, bicycle, a car or boat, motorcycle, cattle, donkeys, goats, sheep, poultry; whether roofing material is made of thatch or iron sheets, whether walling material is made of stones or mud, whether floor material is made of cement or earth).

$\bar{x}_1, \bar{x}_2, \dots, \bar{x}_k$ are means of assets x_1, x_2, \dots, x_k respectively

s_1, s_2, \dots, s_k are the standard deviations of assets x_1, x_2, \dots, x_k respectively

$\alpha_1, \alpha_2, \dots, \alpha_k$ are weights for each variable x_k for the first principal component.

The procedure yielded a wealth index for every sampled household in the project area and the non-project area. Using the calculated wealth index, households were categorized into quintiles of wealth where quintile 1 = Very poor household, 2 = Somehow

poor, 3 = Middle, 4 = Wealthy and 5 = Very wealthy household.

Estimation of the PSM estimator

This study used the propensity score, $P(D = 1 | X) = P(X)$, that is, the probability for an individual to participate in a treatment given his observed covariates X . Hence, if the Conditional Independence Assumption¹ (CIA) holds, all biases due to observable components can be removed by conditioning on the propensity score (Imbens, 2004). Assuming that CIA holds and that there is overlap or common support between the participating and non-participating household groups, the PSM estimator for ATT can be written as presented in Equation 2.

$$WI_{ATT} = E[WI_i(1) | PPART = 1, P(X_i)] - E[WI_i(0) | PPART = 1, P(X_i)] \quad (3)$$

where:

WI_{ATT} = The average treatment effect on the treated (ATT) denoting the change in Wealth Index for the households participating in tsetse control project in Pate Island

WI_i = Wealth Index for the i^{th} household

$PPART_i$ = Participation of the i^{th} household in tsetse and trypanosomiasis control project ($PPART=1$ if household participated and 0 if not)

$P(X_i)$ = Propensity score defining the region of common support for project participating households and non-participating households

This is the simplest form of propensity score matching as noted by Baker (1960). This procedure involved calculating the mean value of the wealth index for the households. The difference between the calculated mean and the actual value for the project

¹ This assumption implies that systematic differences in outcomes between treated and comparison individuals with the same values of covariates are attributable to treatment

household is the estimate of the gain due to the program.

RESULTS AND DISCUSSION

The household wealth indicator variables included for the study were whether household owned assets such as bicycle, boat or car, cattle, dairy goats, donkeys, goats, improved cattle, land, mobile phone, motorcycle, poultry, a radio set, sheep and a television set. The materials used on the floor, roof and walls of the household dwelling were also included as indicators of wealth in the study area. All the variables were first dichotomized as 1=Yes and 0=No as suggested by Vyass and Kumaranayake (2006) to indicate the ownership of each household asset. The findings on general asset ownership in the study area are presented in subsequently.

Livestock ownership

It was found that 64% of the households owned indigenous cattle while the remaining percent did not. Of all the households interviewed, only 3% of had improved cattle compared to 97% who did not have. When asked about ownership of donkeys, 24.8% of the households owned donkeys while the remaining 75.2% did not have any. On the ownership of indigenous goats, the study found that 49.6% owned indigenous goats while the remaining 50.4% did not have. It was found that 2.6% of the households had dairy goats as opposed to the remaining 97.6% who did not have. Households were asked whether they owned poultry or not; in response 47.8% said they owned poultry while 52.2% did not own poultry. Finally the study found that only 8.6% owned sheep while 91.4% did not. These results are presented in Table 2.

Ownership of other assets

The study's investigation on the ownership of other assets revealed that 17% of the households owned bicycles while 83% did not have; 6.9% owned either boat or car yet the remaining 93.1% did not; 78.9% owned agricultural land while 21.1% did not. Asked whether they owned mobile phones or not, 84.1% of the households said they had phones while 15.9% said they did not. The study further found that 69.4% of the households owned radio sets while 30.6% did not; 30.6% owned television sets while 69.4% did not and finally, only 13.8% owned motorcycles while 86.2% did not. The findings are presented in Table 3.

Materials used for household dwelling

The study found that 39% of the households had the

floors of their houses made of dust (earth floors) while the remaining 61% had floors made of cement; 52.1% of the households had roofs of their dwelling units made of naturally available materials such as *makuti* or grass thatch while 47.9% had their roofs made of iron sheets. Of all the households, 59% had the walling material for their dwelling units made of naturally available materials including mud, rough stones, lime or timber while 41% had the walls made of either bricks, quarry stones or cement. The results are presented in Table 4.

Wealth index for Lamu households

The response of households on the dichotomized wealth indicator variables was captured in the Statistical Package for Social Scientists (SPSS) and Microsoft Excel spread sheets. Principal Components Analysis (PCA) procedure was used to calculate the factor scores. The weights were obtained from the first principal components loadings across the households. The first principal components assigned larger weights for assets and housing conditions that varied the most across households. In contrast, the first principal component assigned smaller weights to assets and housing conditions with minimal variations across households. The orthogonalized first principal component loadings are shown in Appendix 1.

Quintiles of wealth in Pate Island, Amu and Hindi divisions of Lamu County

Using the household wealth indices obtained from the PCA procedure, households were categorized into quintiles of wealth where quintile 1 = Very poor household, 2 = Somehow poor, 3 = Middle, 4 = Wealthy and 5 = Very wealthy household. The household quintiles of wealth were cross tabulated by whether household was in project area or not. The results of the cross tabulation revealed that 16% of households in Lamu were categorized as very wealthy and found in the project area compared to 3.7% who were very wealthy and found in non-project area. The results of household quintiles of wealth are presented in Table 5.

Propensity score matching (PSM) estimator

The project households and non-project households were matched on their propensity scores calculated using pre-project underlying covariates which included age of the household head, sex of the household head, ownership of land, other major source of livelihood for the household, and years of education of the household head. The steps of calculating the PSM estimator were followed as suggested in literature (Stuart and Rubin, 2007; Caliendo and Kopeinig, 2008; Rosenbaum and

Table 2. Livestock ownership in Pate, Amu and Hindi divisions of Lamu County.

Type of livestock	Number of households (n=536)	
	Owning livestock type	Not owning livestock type
Indigenous cattle	343(64)	193(36)
Improved cattle	16(3)	520(97)
Donkeys	133(24.8)	403(75.2)
Indigenous goats	266(49.6)	270(50.4)
Dairy goats	14(2.6)	522(97.4)
Poultry	256(47.76)	280(52.2)
Sheep	46(8.6)	490(91.4)

Note: Proportion of households (%) in parentheses.

Table 3. Household ownership of other assets in Pate, Amu and Hindi divisions of Lamu County.

Other assets	Number of households (n=536)	
	Owning asset	Not owning asset
Bicycle	91(17)	445(83)
Boat or car	37(6.9)	499(93.1)
Land	423(78.9)	113(21.1)
Mobile phone	451(84.1)	85(15.9)
Radio set	372(69.4)	164(30.6)
Television set	164(30.6)	372(69.4)
Motorcycle	74(13.8)	462(86.2)

NB: Proportion of households (%) in parentheses.

Table 4. Materials used on household dwellings in Pate, Amu and Hindi divisions of Lamu County.

Part of dwelling unit	Number of households with dwelling unit type (n=536)	
	Improved	Non-improved
Floor	327(61.0)	209(39.0)
Roofing	257(47.9)	279(52.1)
Walling	316(59)	220(41)

Note: Floor (Improved floor=tiles/cement) (Non-improved= Mud, rough stones, lime or timber)

Roof (Improved roof = Iron sheets or tiles) (Non-improved = Makuti or grass thatch)

Wall (Improved walling = Brick, quarry stones, cement((Non-improved = Mud, rough stones, lime or timber)

NB: Proportion of households (%) in parentheses.

Table 5. Quintiles of Wealth among farm households in Lamu County, Kenya.

Household category	Percentile Group of the HH wealth index					Total
	Very poor	Somehow poor	Middle	Wealthy	Very wealthy	
Non-project households	103(19.2)	85(15.9)	54(10.1)	20(3.7)	20(3.7)	282(100.0)
Project households	4(0.7)	21(3.9)	55(10.3)	88(16.4)	86(16.0)	254(100.0)
Total	107(20.0)	106(19.8)	109(20.3)	108(20.1)	106(19.8)	536(100.0)
Mean Wealth index	-1.5103	-0.5992	0.1368	0.73014	1.23921	0

Rubin, 1985; Lechner, 2001). A one to one matching algorithm that was applied resulted in 136 project

households and 136 non-project households.

The PSM results indicated that the mean wealth index

Table 6. Mean Household wealth index in project and non-project areas

Household category	Number of matched households	Mean Household Wealth Index	Std. Error Mean
Project households	136	0.699	±0.048
Non-project households	136	-0.745	±0.077
PSM estimator		1.444	

for a project household was 0.610 (Std. Err. = 0.048) compared to -0.745 (Std. Err. = 0.077) if the same household had not participated in the project denoting an increase of 1.444 in the wealth index. These results are presented in Table 6.

DISCUSSION

The 1.444 points rise in Pate Island livestock farmers' wealth index suggests that the tsetse and trypanosomiasis control activities that were carried out in the Island led to an increase in household income and wealth. On the other hand, the higher proportion of wealthy households found in the project area in this study (16%) compared to that in non-project area (3%) implies that livestock farmers' well-being improved as a result of tsetse control interventions.

The finding is consistent with that of Shaw et al. (2014) who compared farmers' livestock incomes for the 'with trypanosomosis' and 'without trypanosomosis' situations in Eastern Africa and found that the difference between the two income streams had potential benefits from the disease's absence. It is also consistent with the results of economic surveys conducted after the completion of the tsetse and trypanosomiasis eradication operations in Zanzibar which found that the average monthly income of farming households increased by 30% and the proportion of households with a monthly income of over 25 USD increased from 69 to 86% (Feldmann et al., 2005).

The findings of this study suggest that the tsetse and trypanosomiasis control activities that were carried out in Pate Island led to an increase in household wealth. The durable assets such as bicycle, boat or car, land, mobile phone, radio set, television set, motorcycle was a proxy for the medium to long term stream of income in the households during the project period. The control of tsetse flies and the elimination of disease transmission in Pate Island may have resulted to increased livestock numbers and quality.

Conclusion

The proportion of very wealthy households of Lamu County was higher in the project areas than in non-project areas resulting in a rise in Wealth Index among Pate Island households implying that the households had

higher incomes conserved over time in durable assets such as livestock, bicycles, boats or cars, land, mobile phones, radio sets, television sets, motorcycles and in improved living conditions. In conclusion, the study shows that the tsetse and trypanosomiasis control project had brought an improvement in the wealth index of the project participated households.

Recommendations

Mobilize resources from National Government, County governments and development partners to eradicate tsetse and trypanosomiasis in vast land areas of Kenya which are still heavily infested by tsetse flies. This study has demonstrated that investments in tsetse and trypanosomiasis control pays off and enhances the well-being of rural households.

Design studies to assess impact of tsetse and trypanosomiasis control in areas which are not geographically isolated. The Pate Island study was a case of an intervention area surrounded by water serving as a natural barrier. This was an ideal situation separating the treatment group from the control group of households' hence minimal infiltrations of project output into the non-project area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix 1. The orthogonalized first principal component loadings.

Coefficient matrix	Component score					
	1	2	3	4	5	6
Whether household owns land or not	-0.051	0.145	0.109	-0.238	0.039	0.509
Household ownership of radio set	-0.059	0.128	-0.240	0.033	-0.430	0.019
Household ownership of mobile phone	-0.048	0.190	-0.097	0.173	0.463	0.133
Household ownership of bicycle	-0.179	0.243	-0.087	-0.080	-0.080	0.207
Household ownership of boat or car	0.043	0.209	0.139	0.095	-0.492	-0.112
Household ownership of a motorcycle	-0.100	0.280	-0.167	-0.147	0.028	0.158
Whether household owns cattle	0.288	-0.016	-0.073	0.119	0.116	0.091
Household has improved cattle or not	0.054	0.071	-0.138	0.538	0.140	0.380
Whether household owns donkeys	0.156	0.087	0.452	0.098	-0.066	0.133
Whether household has Goats or not	-0.263	0.074	0.214	0.155	-0.093	-0.230
Whether household has dairy goats or not	-0.056	0.094	-0.143	0.489	-0.243	0.031
Whether household has sheep or not	-0.060	0.115	0.330	0.325	0.241	-0.338
Whether household owns Poultry or not	-0.121	0.212	0.355	-0.151	0.138	0.102
House roofing material is iron sheets/tiles or not	-0.105	0.179	-0.318	-0.070	0.264	-0.442
House walling material is bricks/stones or not	0.265	0.284	-0.047	-0.084	-0.043	-0.150
House floor material is cement or not	0.256	0.291	-0.013	-0.113	0.039	-0.193

Extraction Method: Principal Component Analysis.

Component Scores.

Full Length Research Paper

Chemical composition of *Urochloa brizantha* depending on the seasons and cutting frequencies

José Augusto Velazquez Duarte^{1*}, Marco Antonio Previdelli Orrico Junior², Nivaldo Passos de Azevedo Junior², Fernando Miranda de Vargas Junior², Denise Baptaglin Montagner², Poliana Campos Burin², Gustavo Daniel Vega Britez¹ Marcos Arturo Ferreira Aguero¹, Nelson David Lesmo Duarte¹ and Wilfrido Meza Giménez¹

¹Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, Pedro Juan Caballero, Paraguay.

²Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Dourados MS, Brasil.

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The aim of this research was to assess the chemical composition of *Brachiaria brizantha* cv. Piatã variety and to determine which cutting frequency is the most suitable for autumn, winter and spring. The experimental design adopted was the completely randomized in factorial scheme with plots subdivided in time. Each plot consisted of four cutting frequencies (21, 28, 35 and 42 days between cuts) and sub plots were the three seasons (autumn, winter and spring) with 12 replicates per treatment (experimental units). The assessed parameters were; crude protein content, neutral detergent fiber, lignin and *in vitro* organic matter digestibility. No significant differences were observed in crude protein content between the cutting frequencies of 21, 28 and 35 days for autumn and winter; only the cutting frequency of 42 days showed the lowest values. The cutting frequency of 35 days showed higher crude protein and digestible organic matter for autumn and winter. As for the spring season, the cutting frequency that showed higher production of crude protein and digestible organic matter was 28 days.

Key words: Animal nutrition, pastures, *Brachiaria brizantha*.

INTRODUCTION

Brazil has the largest commercial herd in the world, around 213 million head distributed virtually throughout the country. Pasture is the main source of food, and 90% of beef cattle are raised in extensive production systems (Jank et al., 2014). Production of ruminants under grazing conditions is the way to produce meat at a low cost and high quality (Guarda and Guarda, 2014).

The growing challenge of providing food for an increasing population especially animal protein, makes agricultural scientists search for better pasture species. *Brachiaria brizantha* cv. Piatã emerges as a valid option in tropical and subtropical climate of the Central-West region of Brazil. According to Jank et al. (2014), the Piata grass was developed by the breeding program of Embrapa.

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

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One of the advantages of Piata is its early flowering (January and February), which results in the production of good quality forage in late autumn and early winter (critical period), unlike the late flowering species.

The effect of vegetation height of tropical forage on elongation rate and leaf senescence is linear; however, for stem elongation, there is a quadratic effect where vegetation height management can control this process. Leaf area index is favored with the increase in vegetation height, in addition to the production or accumulation of raw forage according to Santos et al. (2017).

According to Orrico Junior et al. (2013), plants Piatã that feature long periods between cuts provide lower forage quality; however, according to Difante et al. (2011), (Marandu) pastures managed with cutting interval corresponding to three leaves emerged per tiller regardless of cutting height, accumulate large amount of leaves, a highly desirable component for animal feed. Even so, Dim et al. (2015) highlighted that the increase in height of the Piata pasture does not provide change in CP content and digestibility and increase in content of NDF (neutral detergent fiber), it only changed the NDF at 60 cm of cutting height due to the greater lignin concentration. Euclides et al. (2009) highlight the importance of Piatã variety in the Cerrado biome as a new alternative because of its nutritional values consistency in both the pre and post grazing, which due to its great flexibility of management under continuous grazing, and can be managed between 15 and 45 cm height (Nantes et al. 2013).

Due to the lack of information regarding the behavior of the chemical composition of Piatã in different times of the year or cutting heights, this study was performed with the aim to assess the effect of the seasons and cutting heights on the chemical composition Piatã.

MATERIALS AND METHODS

The experiment was performed in the municipality of Dourados-MS, Brazil ($22^{\circ} 13' 18.54''$ S, longitude $54^{\circ} 48' 23.09''$, 452 m). The study began on March 4, 2010 and lasted until December 17, 2010 so that autumn, winter and spring seasons were assessed. The climate of the region is Humid Mesothermal of the Cwa type, with average annual temperatures and precipitations varying from 20 to 24°C and 1250 to 1500 mm, respectively (Köppen classification).

The experimental design was completely randomized in factorial scheme 4×3 , four cutting frequencies (21, 28, 35 and 42 days between cuts) and three seasons (autumn, winter and spring), with 12 replicates per treatment (experimental units). *B. brizantha* cv. Piatã variety were collected with the aid of a square of 0.25 m², cut randomly four times within each bed (production per area).

The soil of the experimental area was prepared with disc harrow and leveled. The sizes of the experimental plots were 3 m x 7 m, totaling 21 m². Prior to sowing, soil samples were collected from the experimental area at the 0-20 cm layer to analyze the chemical characteristics. The results were: pH (CaCl₂) = 5.0; P (mg / dm³) = 18; K (cmolc / dm³) = 2. Ca (cmolc / dm³) = 51; Mg (cmolc / dm³) = 26; AI (cmolc / dm³) = 1.8; H + AI (cmolc / dm³) = 55; base weight (cmolc / dm³) = 79. Based on the results of the soil analysis, 500 kg/ha of gypsum were applied, and 2 kg/ha of commercial

fertilizer diluted in water and sprayed on all plots with 5% of zinc, 5% boron, 4% manganese, 2% copper, 0.5% iron, 0.5% molybdenum, 1% magnesium, 10% sulfur, and 10% potassium. Phosphorus fertilization was not carried out because the result of the soil analysis performed showed 18 mg/dm³ of phosphorus in the soil, considered a high index for forage production.

The sowing of the pasture was carried out in March 2009, with 10 kg/ha of commercial seeds with a cultural value of 50%. After 45 days, the invasive plants were controlled and nitrogen fertilization (urea) was carried out in two applications of 100 kg/ha of N, totaling 200 kg/ha of N on 12/15/2009 and 03/15/2010. After each application of nitrogen the whole surface was irrigated for better use of the nutrient. On March 4, the grass was cut for standardization to start the experiment.

The forage samples were randomly collected with a frame of 0.25 m² four times in each plot. The cutting was performed at 20 cm from the soil, simulating a 20 cm residue management (Lima, 2009). After sampling, the pasture of the plots were cut and homogenized with a costal brush cutter at 20 cm and the cut material was removed with a scraper.

The material collected was weighed and placed in plastic bags, identified and immediately transported to the laboratory where the dry matter was determined. The sample was weighed and placed in forced ventilation oven at 65°C for 72 h, for determination of dry matter content. After drying, the material was ground using a Willey type mill, with a 1 mm mesh sieve. The contents of crude protein (CP), neutral detergent fiber (NDF), lignin (Lig) and *in vitro* organic matter digestibility (IVOMD) were determined by near infrared reflectance spectroscopy (NIRS), according to Marten et al. (1985). The data that have attended the normality and homogeneity assumptions were subjected to analysis of variance and means were compared by Tukey test at 5% probability. Statistical analysis was performed using the SAS 9.1 software (SAS, 2002).

RESULTS AND DISCUSSION

Piatã showed higher crude protein content during the autumn in comparison to the other seasons of the year as indicated in Table 1.

In the autumn season, the mean content of crude protein was 11.62% in the DM, about 55% more protein than plants harvested in the winter (7.47%). Euclides et al. (2014) found values of 12.3, 11.0 and 11.1 of CP at heights of 15, 30 and 45 cm, in the region of Campo Grande, MS in autumn season, similar to the present study. However, Orrico Junior et al. (2013) found mean of 11.51% crude protein in the DM of Piatã during the autumn, with cutting intervals that ranged from 26 to 35 days, very similar to those achieved in this study. There were no significant differences in crude protein content among the cutting frequencies of 21, 28 and 35 days, for the seasons of autumn and winter, only the cutting frequency of 42 days showed lower values ($p = < 0.05$) in these seasons. The mean coefficients of organic matter digestibility were 63.40, 55.77 and 58.42% for autumn, winter and spring, respectively, in the winter period. Agulhon et al. (2004) did not find variation of Marandu organic matter digestibility, 48.69% on average, considered a low value, which was found due to the high amount of fiber. With the maturity of the plant, the concentration of digestible components such as soluble

Table 1. Mean content (% of DM) of crude protein, neutral detergent fiber, lignin, *in vitro* organic matter digestibility and productions (kg/m²) of dry matter, crude protein and digestible organic matter of *Urochloa brizantha* grass Piatã variety at different cutting frequencies (days) and seasons.

Mean content	Cutting height (cm)	Autumn	Winter	Spring
Crude protein (%)	21	12.5 ^{aAa}	7.3 ^{bAa}	7.9 ^{bAa}
	28	11.9 ^{aAa}	7.8 ^{bAa}	7.3 ^{bAa}
	35	12.8 ^{aAa}	8.1 ^{bAa}	7.5 ^{bAa}
	42	9.3 ^{aBa}	6.7 ^{cBb}	7.2 ^{bcAa}
<i>In vitro</i> organic matter digestibility (%)	21	63.3 ^{aB}	56.6 ^{cAB}	60.3 ^{bA}
	28	61.5 ^{aBC}	56.8 ^{bA}	57.5 ^{bB}
	35	68.7 ^{aA}	55.8 ^{bAB}	57.5 ^{bB}
	42	60.1 ^{aC}	53.9 ^{bB}	58.4 ^{aAB}
Neutral detergent fiber (%)	21	65.6 ^{bB}	65.4 ^{bB}	68.3 ^{aA}
	28	68.3 ^{aA}	66.1 ^{bB}	68.9 ^{aA}
	35	65.5 ^{bB}	66.8 ^{bAB}	69.14 ^{aA}
	42	67.1 ^{aAB}	68.4 ^{aA}	68.1 ^{aA}
Lignin (%)	21	2.3 ^{abA}	2.1 ^{bC}	2.4 ^{aAB}
	28	2.4 ^{aA}	2.3 ^{aB}	2.4 ^{aAB}
	35	1.8 ^{bB}	2.4 ^{aAB}	2.5 ^{aA}
	42	2.2 ^{bA}	2.6 ^{aA}	2.2 ^{bB}
Production of crude protein (kg/m ²)	21	0.06 ^{cB}	0.05 ^{cB}	0.10 ^{bA}
	28	0.10 ^{bB}	0.08 ^{aC}	0.12 ^{aA}
	35	0.86 ^{aA}	0.50 ^{abB}	0.75 ^{bcA}
	42	0.85 ^{aA}	0.50 ^{abB}	0.70 ^{bcA}

Means followed by different uppercase letters compare the seasons by Tukey test ($p = < 0.05$). Means followed by different lowercase letters compare the cutting frequencies by Tukey test ($p = < 0.05$).

carbohydrates, proteins, minerals and other cell components tend to decrease, and the proportion of lignin, cellulose, hemicellulose and other indigestible fractions. Alencar et al. (2010) stated that the autumn/winter period provides higher contents of crude protein and *in vitro* dry matter digestibility and lower content of neutral detergent fiber. According to Medeiros et al. (2007), high temperatures and extended photoperiods contribute to increased rate of photosynthesis, allowing an increase in the synthesis of new tissue. However, as the cuts approached the winter there was a decrease in the rate of photosynthesis (caused by the decrease in temperature and photoperiod), reducing the contents of CP and increasing fiber content. Autumn showed grass with better nutritional value as indicated in Table 1. Thus, the nutrient content of forages by itself does not define the best cutting interval to be adopted in a pasture.

During the autumn, the highest content of neutral detergent fiber of leaf blades were observed in pastures cut at 28 and 42 days. The other frequencies did not differ. During the winter, the highest content of neutral detergent fiber of leaf blades were observed in pastures

cut at 35 and 42 days and the lowest at 21 and 28 days, but without significant difference from 35 days, which shows that as the plant gets older the cellular content decreases thereby increasing the fiber, a fact also recorded by Pinho et al. (2014), according to these authors, the seasonality affects the nutritional value of Brachiaria forages in Brazilian Cerrado. In the spring there was no difference between cutting frequencies regarding the content of neutral detergent fiber of leaf blades, the season caused a rapid growth by increasing the amount of cell wall. Lignin of leaf blades was lower in the autumn at the cutting height of 35 cm; in the summer, Pinho et al. (2014) found 3.55% lignin in leaf blades at a height of 30 cm, in this season plants have higher concentration of soluble carbohydrates and less structural carbohydrates.

The cutting frequency of 35 days provided greater ($p = < 0.05$) production of crude protein and digestible organic matter for autumn and winter seasons, similar to the results reported by Pinho et al. (2014), who observed 13.38% CP at the cutting height of 30 cm, higher in spring and summer. For spring, the cutting period of 28 days

showed higher production of crude protein and digestible organic matter. An increase in height of the pasture Piatã usually causes variations in NDF and CP, and this response is affected by the season of evaluation, the NDF does not vary in relation to growing seasons according to Dim et al. (2013). Change of growing seasons affect the nutritional components of plants as the height increases, due to the fact that as the physiological age of the plant increases, so does the percentage of cellulose, hemicellulose and lignin, reducing the proportion of digestible nutrients, yet, can significantly reduce digestibility.

Conclusions

The chemical compositions of *B. brizantha* cv. Piatã variety have higher crude protein content and neutral detergent fiber of leaf during the autumn.

The cutting frequency recommended in the autumn and spring were 35 and 28 days, respectively. In winter, the lowest concentration and yield of crude protein in the cut made at 21 cm.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Quantifying the response of different maturity groups of maize (*Zea mays L.*) supplementary irrigation in the Sudan Savannah of Nigeria

Ismail Ibrahim Garba^{1*}, Adnan Aminu Adnan² and Abdulwahab Salisu Shaibu²

¹Centre for Dryland Agriculture, Bayero University Kano, PMB 3011, BUK Kano, Nigeria.

²Department of Agronomy, Faculty of Agriculture, Bayero University, PMB 3011, BUK Kano, Nigeria.

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Field trials were conducted during the 2014 rainy season under rainfed and supplementary irrigation conditions to study the response of different maturity groups of maize to supplementary irrigation in the Sudan savannah. The treatments consisted of three early (2009 EVDT, 1999 EVDT and TZECOMP5W), one late (TZL COMP1SYN), one intermediate (IWDC₂SYN) and one extra early (2009 TZEE) maturing maize varieties which were laid out in Randomized Complete Block Design and replicated thrice. Results revealed a significant difference ($p<0.05$) in terms of growth, phenology and yield among all the varieties. The yield characteristics as measured by number of kernels cob⁻¹, cob yield, and grain yield all increased with the application of supplementary irrigation except for the early varieties. Late maturing varieties performed better under supplementary irrigation with TZL COMP1SYN producing the highest grain yield (6299 kg ha^{-1}). Application of supplementary irrigation increases grain yield (kg ha^{-1}) of the late maturing varieties (TZL COMP1SYN and IWDC₂SYN) by 146 and 116%, respectively. However, there was reduction in yield for the early maturing varieties with the application of supplementary irrigation (22 and 4%), respectively. It is therefore recommended that where supplementary irrigation facilities are available, late maturing varieties should be planted as they are more high yielding.

Key words: Early maturing, late maturing, rainfed, supplementary irrigation, yield.

INTRODUCTION

Maize is considered a staple food of socio-economic importance, not only on the basis of the number of farmers that engaged in its cultivation but also because of its economic value (Olaniyi and Adewale, 2012). In Nigeria, it has almost replaced traditionally grown cereals

such as millet and sorghum (Badu-Apraku, et al. 2009). Maize has now risen to a commercial crop on which many agro-based industries depend as raw material (Iken and Amusa, 2004). In the last two decades, maize production has increased greatly in Nigeria, including the

*Corresponding author. E-mail: iigarba9@gmail.com.

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semi-arid Sudan savannah (Manyong et al. 1999) due to the development of high yielding varieties and increasing demand for maize products mainly for food and animal feeds. This led to an increase in total annual production from 658, 000 tons in 1978 to about 7,305,530 tons in 2013 (FAOSTAT, 2013). The Sudan savannah agro-ecological zone has a unimodal rainfall pattern typical of semi-arid climate where rainfall hardly exceeds 800 mm per annum (Kamara, 1986). Although average world maize yield per hectare was estimated at 4.3t/ha, they are far less in Nigeria amounting as low as 1.8t/ha in Nigeria (FAOSTAT, 2014). This is in spite of the socio-economic importance of maize in this region, where the effects of climate change including rising temperatures, evapotranspiration losses and eventually, decreasing rainfall are expected to be particularly negative (World Bank, 2007).

The entire production in this zone is mainly under rainfed condition with unreliable erratic rainfall characterized by frequent dry spell and short term droughts. However, a number of improved varieties are also grown under supplementary condition in different parts of the savannah. Maize is an efficient user of water in terms of dry matter production such that among cereals, it is potentially the highest yielding (Norman et al., 1984). Water requirements of a long season variety (150-day) range from 600 to 1000 mm of well distributed rainfall for the growing period. A medium maturity grain crop (100- to 120-day) requires from 500 to 800 mm depending on climate (Sithole 2003). Water deficits at different stages of growth have different effects on maize yields.

Because higher yielding maize varieties take longer time to mature, farmers trade-off yield and risk in selecting which varieties to grow under either rainfed or supplementary conditions (Laurense and Ninomiya, 2001). Dry land maize production varies considerably from year to year, depending on the amount and distribution of rainfall. A mixture of dry spells and erratic rainfall with annual variation, that cannot be predicted accurately, consistently has negative impact on growth and yield of Maize (Benhin 2006). Generally, yield reduction in most dry land maize growing areas occurs because seasonal rainfall distribution is erratic. Undoubtedly, water availability, specifically the lack thereof, is the most pressing and significant factor limiting the production of dry land maize.

Successful maize production requires an understanding of various management practices as well as environmental conditions that affect crop performance (Eskert, 1995). Selection of appropriate varieties, sowing dates and planting densities are cultural practices that have been shown to affect maize yield potential and stability (Norwood, 2001). Therefore, this study aimed to evaluate the response of different maturity group of

maize to supplementary irrigation and to determine the yield gap between the two growing conditions.

MATERIALS AND METHODS

The experiments were conducted at the Teaching and Research Farm of the Faculty of Agriculture (for the rainfed trial), Bayero University Kano as well as at the Orchard of the department of agronomy (for the water non-limited trial). The two farms are located at $11^{\circ} 59'49''$ N $8^{\circ}25'79''$ E; 466m a.s.l, and $11^{\circ}48'N$. $8^{\circ}25'79''$ E; 465 m a.s.l in the Sudan Savanna Zone of Nigeria. The area receives rainfall of between 600-1000 mm, annually with temperatures of 33 - 15°C (KNARDA, 2006). The treatments consisted of six improved maize varieties; 2009 EVDT, 1999 EVDT, TZECOMP5W, TZL COMP1SYN, IWDC₂SYN and 2009 TZEE, which were replicated three times and laid out in a Randomized Completely Block Design (RCBD). The land was prepared by harrowing and ridging at a spacing of 0.75 m between rows; thereafter it was marked into plots. The plantings were designed with gross plot size of 3 m x 4 m = 12m², net plot size of 1.5 m x 2 m = 3 m² and discards of 0.5 m between plots and 1.0 m between replications. Sowing was at a spacing of 0.75 m x 0.25 m. Two seeds were sown per hole, which were later thinned to one plant per stand at 2 WAS to give a plant population of 53,333 plants ha⁻¹. Hoe weeding was done manually at 3 and 6 weeks after sowing. Fertilizer was applied at the rate of 120 kg N, 60 kg P and 60 kg K using a NPK of 20:10:10 and urea (46% N). The N was applied in two split doses at planting and 6 weeks after sowing (WAS) while the P and K were applied basally at planting. Harvesting was done manually when the plants attained physiological maturity; that is, when the attachment of the grain to the cob was observed to be black, leaves turned yellow and brown, and kernels were dried in the field. The cobs were plucked, dehusked and further sun-dried. The cobs were then weighed, threshed and winnowed to obtain clean grain. Data were collected at pre-harvest [days to 50% tasselling, days to 50% silking, Photosynthetically Active Radiation (PAR), Leaf Area Index (LAI), number of days to maturity, number of leaves at flowering], and post-harvest (number of kernel/row, number of rows/ear, cob yield/hectare, grain yield/hectare and 100 seed weight).

A Time Domain Reflectometer (TDR, FieldScout TDR300, by Spectrum Technologies, Inc.) was used to measure soil moisture content throughout the period of experiment; and supplementary irrigation was given when readily available water (RAW) was fully depleted in order to ensure optimal moisture availability.

Data were analysed using GENSTAT Discovery Version 4 (VSNI, 2011). Data was subjected to Analysis of variance (ANOVA) as suggested by Snedecor and Cochran (1967). Significant means were separated using Tukey's HSD procedure (Tukey, 1953).

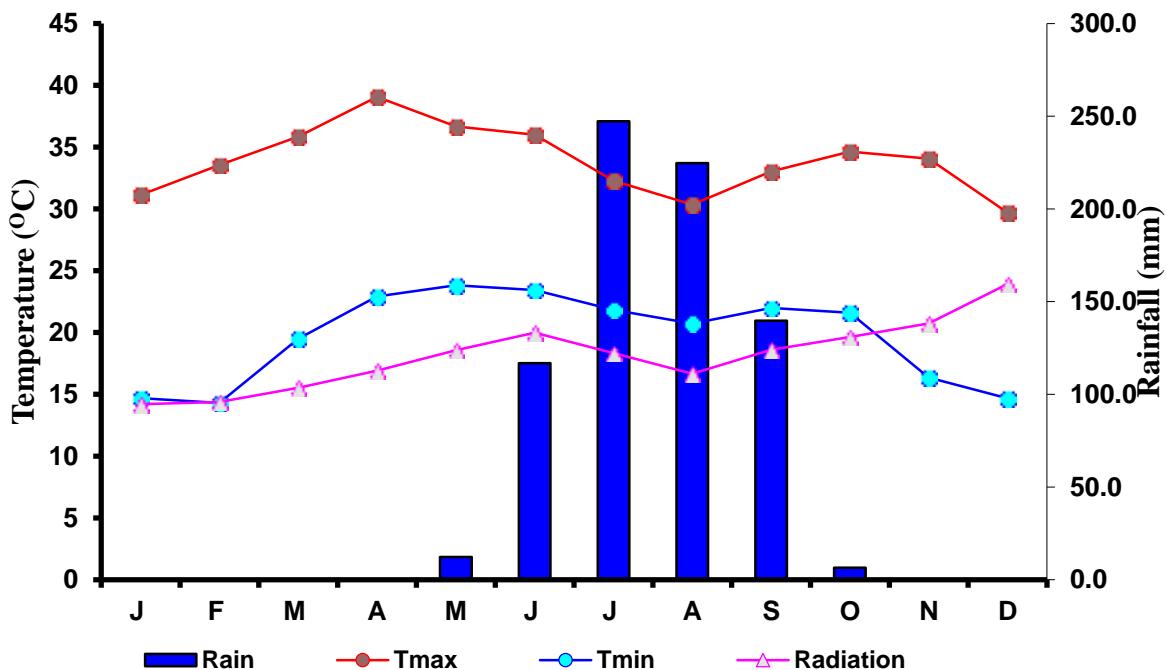
RESULTS AND DISCUSSION

Soil and weather

Table 1 show some physico-chemical properties from soil profiles dug at the two locations. The field for the rainfed trial was moderately drained with loamy texture. The Ap horizon has high bulk density (1.62 g cm^{-3}) with moderately high total Nitrogen (0.26%), acidic pH, low organic carbon (0.958) and high base saturation (75%).

Table 1. Soil physical and chemical properties of Pedons of the experimental sites.

Horizon	Depth (cm)	% Sand	% Clay	% Silt	Bd g cm ⁻³	O.C (%)	pH (H ₂ O)	pH (KCl)	% BS	EC (μs/cm)	CEC (meq /100 g)	Av P (ppm)	T N %
Rainfed site													
Ap	0-9	72.96	11.04	16	1.62	0.958	5.27	4.91	75	315	3.6	16.7	0.26
Bt(B)	9-32	53.68	27.04	19.28	1.69	0.638	5.37	4.63	85.7	330	7.3	20.3	0.61
Cv	32-78+	49.68	37.76	12.56		0.160	4.97	4.77	66.6	505	8.7	11.5	0.33
Supplementary irrigation site													
Ap	0-6	69.68	15.04	15.28	1.68	0.678	4.99	4.56	54.5	225	4.2	8.8	0.14
B	6-35	61.12	23.04	15.84	1.67	0.517	5.73	4.65	85.7	330	6	19.9	0.61
Cv	35-57+	70.96	19.76	9.28		0.238	6.38	4.9	66.7	320	6.7	18.2	0.46

**Figure 1.** Meteorological data during the 2014 rainy season at the experimental sites.

At orchard (supplementary irrigation trial), the soil has moderately low total Nitrogen (0.14%) than at the Research Farm (rainfed site) with the plough layer (Ap horizon) having low organic carbon (0.678), low base saturation (54.5%) and it is strongly acidic (4.99). Figure 1 shows the rainfall, minimum and maximum temperature and solar radiation at the experimental sites. Rainfall was unevenly distributed throughout the experimental periods and it was highest in July (250 mm) and lowest in October. Planting was done at July when the rainfall was fully established. Highest maximum temperature was recorded at April and at July (planting period), the

temperature was 32°C. Minimum temperature and solar radiation were unevenly distributed throughout the growing season with the highest solar radiation of 24 MJ/dm²/day.

Effect of supplementary irrigation on maize growth and phenology

The varietal responses of maize influence the phenology of the crop as shown in Table 2. The varieties: 1999 EVDT and 2009 TZEE, flowered and silked earlier than

Table 2. Effect of supplementary irrigation on number of days to flowering, Silking and days to physiological maturity of Maize Varieties at BUK, 2014.

Treatment	Days to tasselling		Days to silking		Days to maturity	
	Rainfed	Supp. irrig.	Rainfed	Supp. irrig.	Rainfed	Supp. irrig.
IWDC2SYN	57.67 ^a	58.67 ^a	62.67 ^a	62.00 ^a	110.33 ^a	114.00 ^b
TZL COMP1SYN	57.67 ^a	57.67 ^a	60.33 ^{ab}	61.67 ^a	111.00 ^a	120.00 ^a
TZECOMP5W	53.00 ^b	53.00 ^b	57.67 ^{bc}	56.00 ^b	93.00 ^b	90.67 ^c
1999 EVDT	52.33 ^b	50.67 ^c	55.67 ^{cd}	54.00 ^c	91.00 ^b	89.00 ^c
2009 EVDT	53.33 ^b	53.00 ^b	55.67 ^{cd}	56.33 ^b	94.00 ^b	87.67 ^c
2009 TZEE	52.33 ^b	48.67 ^d	54.33 ^d	51.33 ^d	83.33 ^c	80.33 ^d
SE ±	0.755	0.439	0.770	0.439	1.644	1.554

Means within a column having similar letter(s) are not significantly different at 5% level of significance using Tukey HSD Test.
Supp. irrig. = supplementary irrigation.

all other varieties under both rainfed and supplementary irrigation conditions; and this could be attributed to genetic factors because the two varieties are genetically early and extra early varieties, respectively. IWDC₂SYN and TZL COMP1SYN took longer time to tassel, silk and mature; probably because they are moderate to late maturing varieties. Maximum days to tasselling, silking and maturity were recorded for IWDC₂SYN under both rainfed and supplementary irrigation. Days to harvest maturity were significantly affected by maize varieties. Minimum days to harvest maturity were observed in 2009 TZEE. Extra early maturing varieties had a better chance to overcome the water stress problem, because they can mature quickly before the midsummer drought begins (Cross, 1990; Franzky, 1996; Lewis, 1998). This is of utmost importance, because excessive heat during flowering can inhibit pollination, which results in yield reduction. Results for days to silking, tasselling and maturity are similar to the results of previous studies that reported shorter days to

silking and tasselling for 2009 EVDT and 2009 TZEE due to varietal effect under rainfed conditions (Afifa, 2014).

Effect of supplementary irrigation on maize yield and yield attributes

Grain yield was significantly different among the varieties. There was no significant difference for cob yield for all varieties under rainfed conditions. However for supplementary irrigation, significant differences among the varieties were observed. TZECOMP5W produces the highest cob yield and grain yield under rainfed conditions even though it does not produce the highest number of kernels per cob; this may be attributed to its larger kernel size as compared to other varieties. Similar trend was observed for IWDC₂SYN which produced the highest cob and grain yield under supplementary irrigation. However, 2009 TZEE did not perform well under supplementary irrigation as it produces the lowest number of kernels per cob and lowest

grain yield per hectare. Highest number of kernels per cob was produced by 2009 EVDT under rainfed conditions even though it produces the lowest grain yield under rainfed conditions whereas IWDC₂SYN produced the highest number of kernels per cob under supplementary irrigation (Table 3). Muhammad et al. (2004) identified number of rows per cob and grain weight per cob as important traits contributing to maize grain yield.

Performance of varieties under rainfed and supplementary irrigation in terms of cob yield, number of kernel per cobs and grain yield was generally better under supplementary irrigation except for 2009 EVDT and 2009 TZEE, which produce higher cob yield and high grain yield under rainfed condition. The result agrees with findings of Aziz et al. (1992) who reported that to increase maize yield per unit area, it is imperative for the plant breeders to develop maize varieties that are high yielding, early maturing, disease resistant, responsive to improved production practices and adjustable in the existing cropping

Table 3. Effect of supplementary irrigation on cob yield (kg ha^{-1}), number of kernels per cob and grain yield (kg ha^{-1}) of maize varieties at BUK, 2014.

Treatment	Cob yield (kg ha^{-1})		% Change in yield	No. of kernels/cob		Grain yield (kg ha^{-1})		% Change in yield
	Rainfed	Supp. irrig.		Rainfed	Supp. irrig.	Rainfed	Supp. irrig.	
IWDC2SYN	3069	6644 ^b	116	408.2	495.0 ^a	1947 ^b	6039 ^a	210
TZL COMP1SYN	3044	7504 ^a	146	447.4	488.2 ^a	2328 ^b	6299 ^a	171
TZECOMP5W	5326	4103 ^c	-22	436.0	472.7 ^{ab}	3704 ^a	3819 ^b	3
1999 EVDT	3333	3843 ^c	15	395.3	433.7 ^c	2841 ^{ab}	3391 ^c	19
2009 EVDT	4213	4040 ^c	-4	458.8	454.1 ^{bc}	2578 ^{ab}	3770 ^b	46
2009 TZEE	2899	3080 ^d	6	411.1	403.0 ^d	3546 ^a	2949 ^d	-17
SE ±	790.7	108.6		23.17	7.52	324.50	99.7	

Means within a column having similar letter(s) are not significantly different at 5% level of significance using Tukey HSD Test.
Supp. irrig. = supplementary irrigation.

pattern. Moreover, late maturing varieties performed better under supplementary irrigation producing higher cob and grain yield. While 2009 EVDT performed best under rainfed condition. This could be because it is an early maturing variety and drought tolerant; therefore, it was able to withstand the drought stress during the rainfed period. The variation in yield between rainfed and supplementary irrigation conditions could be attributed to differences in soil types and the amount of water in the root zones, which ensured higher transpiration leading to absorption of more nutrients from the soil and the translocation of photosynthates within the plant systems, resulting in a significantly higher yield. There was a dry spell during tasselling in the rainfed conditions (Figure 1), this may have led to reduction of yield. Babalola et al. (2007) reported that in agricultural fields, yield variability is partly caused by soil variability and varying topography features of the fields; and this may be the basis for difference in grain yield and cob yield in the two experimental

sites. Application of supplementary irrigation increases the yield of the intermediate and late maturing varieties (IWDC₂SYN and TZL COMP1SYN) by 1.5 fold (46%) and 116%, respectively. However, for TZECOMP5W and 2009 EVDT, there was a reduction in grain yield by 22 and 4%, respectively. The increase in yield as a result of the application of supplementary irrigation could be attributed to the crucial role played by water in several physiological processes in the plant system with consequent effect on yield.

Conclusion

Results of this study reports the effects of supplementary irrigation during the growing season on the growth and yields of early and late maturing maize varieties in the Sudan Savanna of Nigeria. The early and extra early maturing varieties produces higher yields than the late

maturing varieties under the rainfed condition but with the addition of supplementary irrigation, higher yields were observed with the late maturing varieties. Application of supplementary irrigation ensures that late maturing varieties attain their potential by ensuring water availability where rain ceases early or were intermittent drought is adamant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of tillage and mulching on agronomics performances of maize and soil chemical properties in Linsinlin Watershed of Centre of Benin

**Akplo Tobi Moriaque¹, Kouelo Alladassi Félix^{1*}, Azontonde Hessou Anastase²,
Hougnandan Pascal¹, Benmansour Moncef³, Rabesiranana Naivo⁴ and Mabit Lionel⁵**

¹Laboratory of Soil Microbiology and Microbial Ecology, Faculty of Agronomics Sciences, University of Abomey-Calavi,
01 BP 526 Cotonou, Benin.

²Institut National de Recherches Agricoles du Bénin, Benin.

³Centre National des Energies, Sciences et Techniques Nucléaires (CNESTEN), Rabat, Morocco.

⁴Institut National des Sciences et Techniques Nucléaires (INSTN), Antananarivo, Madagascar.

⁵SWMCNS, Joint FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture, Vienna-Seibersdorf, Austria.

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Soil conservation has become an important aspect in achieving food security. The objective of this study is to assess the effect of two agricultural practices on the agronomic performance of maize and the chemical properties of soil. A field experiment was carried out on loamy-sand soil using Fisher Block design. Tillage systems and mulching significantly affected maize growth and yield components. The interactive effect of tillage and mulching were not significant on growth and yield components of maize. While, the highest growth rate (2.38 cm/day), leaf area (65.70 cm²), collar diameter (1.39 cm), grain yield (4148.71 kg DM ha⁻¹), straw yield (5077.65 kg DM ha⁻¹) and harvested index (40%) were obtained with the treatment combining isohypse ridging and mulch. Tillage increased soil organic matter. Treatment combining isohypse ridging to mulching allowed obtaining the highest level of soil organic matter after trial. The level of nitrogen and available phosphorus did not vary under the treatment. Throughout, this experiment, isohypse ridging under mulching constitute the effective soil conservation practice to combat soil erosion and improve maize productivity on the watershed of Linsinlin in Southern Benin.

Key words: Tillage, mulching, maize yield, soil chemical properties, watershed, Djidja.

INTRODUCTION

In the current context of high population growth and increased pressure on resources, tropical soils are

particularly threatened (Kouelo, 2016). Traditional production systems are no longer able to maintain soil

*Corresponding author. E-mail: fkouelo@yahoo.fr.

fertility and production capacity (Séguy, 2006). Chemical and physical degradation affects most of the present agricultural land in Africa (Henao and Baanante, 1999). However, soil productivity is essential for agriculture sustainability, food security and the living conditions of the poor people (OECD, 2009). Continuous land use, near total exports of crop residues through burning, deforestation, and low mineral input are the main causes of declining soil fertility in Africa (Saïdou et al., 2012a).

In Benin, traditional agriculture is characterized by a reduction of the fallow periods without any other measure aimed at restoring nutrients used by previous crops (Saïdou et al., 2009). This form of agriculture is the only one of its kind. The majority of cropping systems used lead to soil degradation (Baco et al., 2011). Thus, food production and the sustainability of production systems are compromised (Egah et al., 2014). In the central agro-ecological zone of Benin, the dynamics of agro-systems and agrarian structures have led to a negative evolution of soil (Agossou and Igué, 2002). Under the effect of population pressure (3.5% as growth rate), fallow practice is greatly reduced or even suppressed in favor of continuous cropping systems, overexploitation of soils without organic or mineral fertilizers. In order to grow, agriculture must learn to save (FAO, 2011). New cropping systems have become necessary to ensure sustainable agricultural production. Conservation tillage (CT) practices (e.g., no-till) have become increasingly common in recent years (Corbell et al., 2006; Ziadi et al., 2014). Considered from the point of view of its function on agricultural production in tropical environments, tillage aims to ensure the establishment of crops and the functioning of the roots, to improve the circulation of water and air in the soil, promote its warming and limit weed infestations and a number of soil-borne bio-aggressors (Roger-Estrade et al., 2011). The literature is unanimous concerning the positive effects of mulch-based systems. Vegetation cover protects the soil surface against the effect of raindrops, increases structural stability, maintains soil moisture, and maintains high soil biological activity (Douzet et al., 2010; Mazarei and Ahangar, 2013). However, opinions remain divergent concerning the effect of tillage. Some authors consider that tillage ensures crop establishment and root functioning, improves the flow of water and air into the soil, and limits infestation (Kurothe et al., 2014). Others consider that tillage limits erosion if it disturbs the least surface residues (Roger-Estrade et al., 2010). Labreuche et al. (2007) consider that plowing is generally considered to be an unfavorable factor for carbon storage and therefore unfavorable for soil organic matter. This multiple role of tillage is often reinforced by the permanent presence of vegetation cover as mulch (Roger-Estrade et al., 2010). The objectives of this study were to determine how the various types of tillage and mulching affect the chemical properties of soil in this region and to use the results to identify sustainable land

management practices that would increase production (that is, maize).

MATERIALS AND METHODS

Study area

The experiment was carried out at Linsinlin watershed (latitude 7° 20' 46" North; longitude 1° 56' 8" East and altitude 190 m) at Djidja district in Benin from May 29, 2016 to August 31, 2016 (Figure 1). The study area is situated on the Precambrian basement of the Penepaline Cristalline base rocks as embrechites and granites (Igué, 2000). The soil is locally known as 'Depleted, Little Ferruginized Tropical Ferruginous Soils'. The soil texture of Linsinlin watershed is a loamy-sand according USDA textural triangle. These chemical characteristics are 0.11% for total nitrogen, 0.53% for total organic carbon, 72.62 ppm for available phosphorus, 25.94 meq.100 g⁻¹ for Exchangeable Cation Capacity, and 5.75 to 5.78 for acidity in average. Linsinlin has a bimodal rainfall distribution with a long rainy season from March to July and a short rainy season from September to November. The annual rainfall of the site is 1200 mm. The average temperature is around 28°C. The average slope is 5% (Kouelo et al., 2015).

Experimental design

The effects of tillage and mulching on growth and yield components of maize were evaluated. The experiment was a four replicated experiment arranged to Fisher block design. The experiment comprised 24 plots, each measuring 6 m × 3.5 m. Tillage had three levels: no-tillage (SL), minimum tillage (ML) and isohypse ridging (B). Mulching had two levels: without mulch (0% soil cover rate) and with mulch (50% coverage rate). The treatment was constituted by the combination of the modalities of factors (Table 1). In order to obtain the 50% soil cover rate, 3 t/ha of crop residue was applied two weeks after sowing (Kouelo, 2016). The modalities of these factors are combined to form the installed treatments. A total of six (6) treatments were installed. Maize variety DMR was sown at 50 × 40 cm² (for good soil cover). The fertilizer dose recommended was applied: 200 kg of complete NPK (15-15-15). Weeds management was done manually. The harvest of maize was done after 90 days after sowing.

Data collected and treatment

Growth parameters

The growth parameters taken were plant height, dimension of leaves and collar diameter. The measurement of all parameters was performed on 8 plants on four lines selected randomly by experimental unit: height of the maize plant (from 15 to 75th DAS at intervals of 15 days, that is, 15, 30, 45, 60 and 75 Days After Sowing); dimension of leaves (on 75th Days After Sowing); collar diameter (on 60th Days After Sowing). Maize growth speed was estimated fitting linearly maize plant height. Growth speed rate was represented by coefficient *a* of regression equation. Leaf area was calculated using the method of Ruget et al. (1996). This method uses the number of growing leaves as well as the number and size of full-grown leaves.

Yield components

Maize was harvested from square performance within each plot.

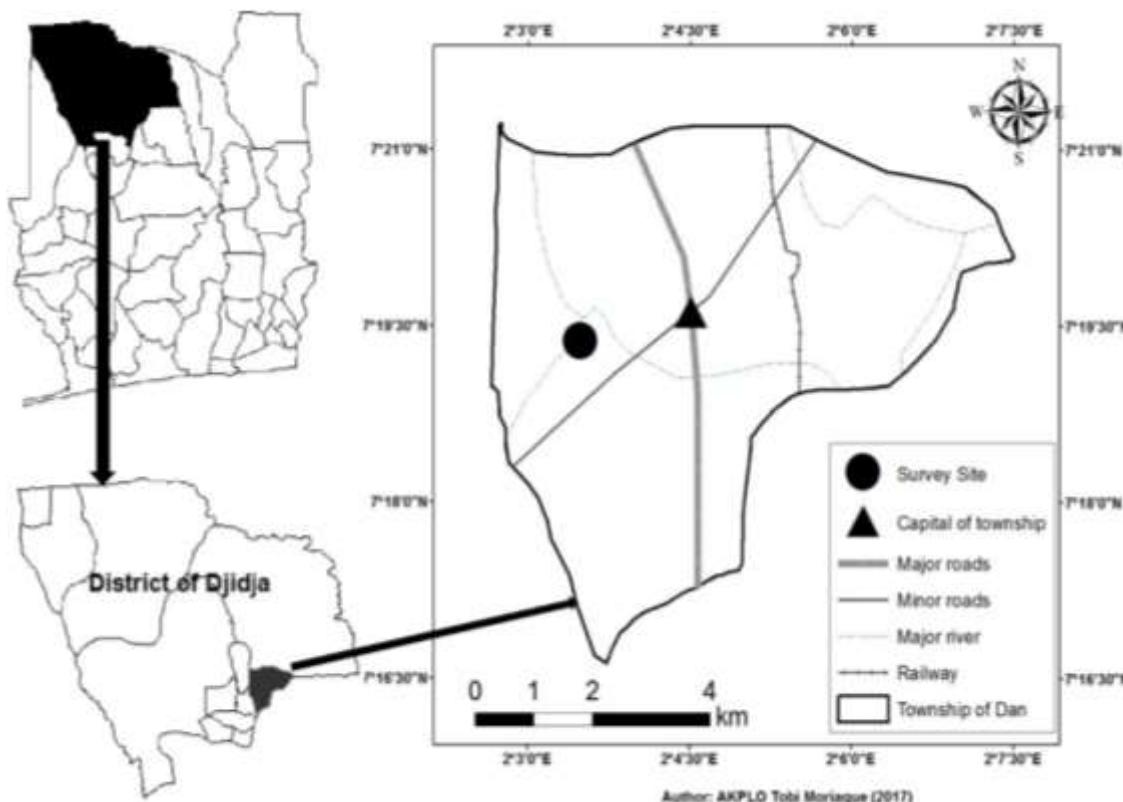


Figure 1. Map showing the study area.

Table 1. Treatment details.

Treatment code	Description
BM50	Isohypse ridging + with mulch
BM0	Isohypse ridging + without mulch
MLM50	Minimum tillage + with mulch
MLM0	Minimum tillage + without mulch
SLM50	No tillage + with mulch
SLM0	No tillage + without mulch

Thus, the harvested effective area per plot was 4 m² (2 m × 2 m). The straw and grain were weighed and sub-samples were taken. These samples were dried in an oven at 75°C during 72 h. These dry weights were recorded. Yield calculations were done using the following expressions (Saïdou et al., 2012b):

Dry matter factor (MS) = sample dry weight / sample fresh weight;

Shelling factor (n) = dry weight of grain/ dry weight of cob

Economic yield (kg DM ha⁻¹):

$$EY = \frac{10000 \times P \times MS \times n}{Ea}$$

Biological yield (kg DM ha⁻¹):

$$BY = \frac{10000 \times P \times MS}{Ea}$$

The Harvest Index, HI (Beadle, 1985):

$$HI = \frac{EY}{EY+BY}$$

where economic yield (EY)= weight of seeds; biological yield (BY)= above ground biomass; P= total fresh weight; MS= dry matter factor; n= shelling factor; Ea= effective area (4 m²).

Chemical properties of soil

Sampling was carried out in the first two soil horizons (0-20 and 20-

40 cm) on each plot at the beginning and at the end of the trial. The samples were air dried and sieved through a 2 mm mesh. Total soil nitrogen content was determined by the Kjeldahl method (Jones et al., 1991). Available phosphorus was estimated using of Bray I method (Bray and Kanz, 1945), soil organic carbon using the method of Walkley and Black (1934).

Statistical analysis

The data collected and the calculated parameters were subjected to a two-way analysis of variance (ANOVA) according to the General Linear Model procedure with SAS 9.2 software. The effects tested are those of tillage, mulching as well as those of their combinations. Means separation was done using the Student-Newman-Keuls test. The threshold of significance used is 5%.

RESULTS

Maize growth

Growth speed

Analysis of the results of the analysis table (Table 2) shows that tillage significantly ($p < 0.05$) influenced growth speed of maize. Indeed, isohypse ridging and minimum tillage had increased maize growth speed by 30 and 14%, respectively compared to no-tillage (Table 3). The effect of mulching was not significant on growth speed. However, mulch practice had increased growth by 6.13% compared to no-mulch. The interactive effect of tillage and mulching were not significant on the growth speed growth. However, on the basis of the values, the treatments can be classified in descending order as follows: SLM0 < SLM50 < MLM0 < MLM50 < BM0 < BM50 (Figure 2).

Leaf area

Tillage and mulching significantly ($p < 0.05$) influenced the leaf area. For tillage system, isohypse ridging had increased the leaf area from 110.43 to 74.18 cm² (Table 3). Considering the indeed, mulch practice had allowed an increase of 16% compared to no-mulch. The effect of the combination of tillage system and mulching was not significant on leaf area ($p < 0.05$). Nevertheless, arithmetic differences exist between treatments. Treatment combining isohypse ridging and mulch (BM50) had allowed the highest maize leaf area (120.96 cm²) followed successively by BM0 (99.90 cm²); SLM50 (82.66 cm²); MLM50 (76.10 cm²); MLM0 (74.88 cm²); and SLM0 (65.70 cm²) (Figure 3).

Collar diameter

Isohypse ridging and minimum tillage have significantly increased collar diameter of maize, respectively by 14

and 11% compared to no-tillage (Table 3). Mulching has not significant effect on collar diameter. However, there is little difference between mulch (1.59 cm) and no-mulch (1.54 cm). The interactive effect of tillage system and mulching was not significant. Thus, the highest maize collar diameter was obtained under the treatment combining isohypse ridging with mulch (BM50) (Figure 4).

Maize yield

Grain yield

The results on maize grain yield under different tillage system, mulching levels and these combinations are shown in Table 2. The results show that tillage system and mulching significantly increased the grain yield of maize. Isohypse ridging gave the highest grain yield (3893.11 kg DM ha⁻¹) followed by minimum tillage which increased grain yield by 16% compared to direct tillage. For the mulching factor, the mulch practice allowed an increase of grain yield by 28% compared to the no-mulch-level (Table 3). The interactive effect of tillage system and mulching were not significantly on grain yield ($p > 0.05$). But, an arithmetic difference exists between treatments. Indeed, isohypse ridge combined with the mulch provided the highest grain yield (4148.71 kg DM ha⁻¹) and the lowest grain yield was obtained with the minimum tillage without mulch (Figure 5).

Straw yield

Tillage significantly improved straw yields of maize from 3938.42 to 4972.58 kg DM ha⁻¹ (Table 3). Despite of a non-statistical significance of the effect of mulching, the mulch practice increased maize straw yield by 6% compared to no-mulch modality. The maize straw yield was not influenced by the treatments combining tillage system and mulching ($p > 0.05$). Nevertheless, the treatment combining isohypse ridging with mulch practice (BM50) provided the highest straw yields (5077.65 kg DM ha⁻¹) (Figure 5).

Harvest index

The results (Table 2) showed that tillage system significantly affected the harvest index of maize. Tillage improved the harvest index by 30% (for no-till) to 40% (for ridging), an increase of 10%. Although the effect of mulching was not significant on harvest index, mulch practice provided an increase of 7% compared to no-mulch. The interactive effect of tillage system and mulching were no-significant on the maize harvest index (Table 2). From analyzing of Figure 6, however, the treatment combining isohypse ridging and minimum tillage with mulch (BM50 and MLM50) and isohypse ridging without mulch (BM0) generated the highest

Table 2. Summary of analysis of variance (ANOVA) for various parameters studied in the experiment.

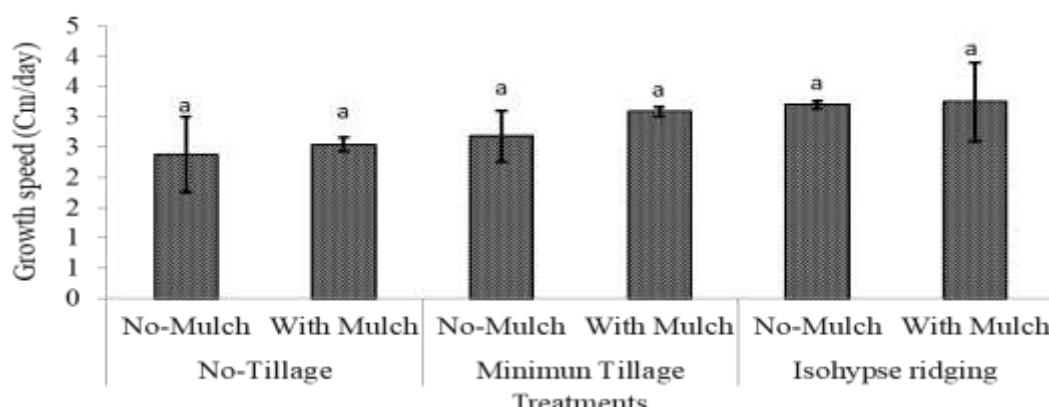
Parameters studied	Growth parameters			Yield components			Chemical properties of soil		
	Growth speed	Leaf area	Collar diameter	Grain	Straw	Harvest index	Available phosphorus	Total nitrogen	Organic carbon
Tillage	0.0056**	<.0001***	0.0104*	0.0158*	0.04362*	0.0729 ^{ns}	0.329 ^{ns}	0.31 ^{ns}	0.01*
Mulching	0.3067 ^{ns}	0.0013**	0.4127 ^{ns}	0.0410*	0.6889 ^{ns}	0.0475*	0.371 ^{ns}	0.21 ^{ns}	0.55 ^{ns}
Tillage vs. Mulching	0.5425 ^{ns}	0.0713 ^{ns}	0.6570 ^{ns}	0.6319 ^{ns}	0.8814 ^{ns}	0.2408 ^{ns}	0.119 ^{ns}	0.69 ^{ns}	0.15 ^{ns}

*Stands for significant at $p \leq 0.05$; **Stands for significant at $p \leq 0.01$; ***Stands for significant at $p \leq 0.001$; ^{ns}No significant.

Table 3. Effect of tillage and mulching on growth parameters and yield component of maize and chemical properties of soil (mean \pm standard error).

Factor	Levels	Growth parameters			Yield components			Chemical properties of soil		
		Growth speed (cm/day)	Leaf area (cm ²)	Collar diameter (cm)	Grain (kg DM.ha ⁻¹)	Straws (kg DM.ha ⁻¹)	Harvest index	Available Phosphorus (ppm)	Total N (%)	Organic carbon (%)
Tillage	No-tillage	2.46 \pm 0.15 ^b	74.18 \pm 5.28 ^b	1.43 \pm 0.06 ^b	1994.69 \pm 251.71 ^b	3938.42 \pm 614.58 ^b	0.30 \pm 0.04 ^b	17.02 \pm 2.23 ^a	0.12 \pm 0.003 ^a	0.52 \pm 0.03 ^b
	Minimum tillage	2.88 \pm 0.13 ^{ab}	75.49 \pm 1.50 ^b	1.60 \pm 0.06 ^a	2383.48 \pm 402.38 ^b	4434.58 \pm 366.02 ^b	0.33 \pm 0.03 ^b	14.58 \pm 0.98 ^a	0.11 \pm 0.01 ^a	0.53 \pm 0.03 ^b
	Isohypse ridging	3.23 \pm 0.15 ^a	110.43 \pm 4.36 ^a	1.67 \pm 0.04 ^a	3893.11 \pm 572.43 ^a	4972.58 \pm 381.91 ^a	0.40 \pm 0.03 ^a	14.17 \pm 0.79 ^a	0.13 \pm 0.01 ^a	0.81 \pm 0.1 ^a
Mulching	No-Mulch	2.77 \pm 0.18 ^a	80.16 \pm 5.21 ^b	1.54 \pm 0.06 ^a	2321.24 \pm 498.70 ^b	4311.12 \pm 530.76 ^a	0.31 \pm 0.03 ^a	16.12 \pm 1.80 ^a	0.13 \pm 0.01 ^a	0.65 \pm 0.1 ^a
	With Mulch	2.94 \pm 0.09 ^a	93.24 \pm 6.09 ^a	1.59 \pm 0.04 ^a	3234.18 \pm 309.57 ^a	4575.65 \pm 344.50 ^a	0.38 \pm 0.03 ^b	14.56 \pm 0.62 ^a	0.11 \pm 0.01 ^a	0.60 \pm 0.04 ^a

For each column, the mean followed by the different letter are significantly different.

**Figure 2.** Interactive effect of tillage and mulching on maize growth speed. The error bars correspond to the standard deviation. Different letters indicate statistically significant difference.

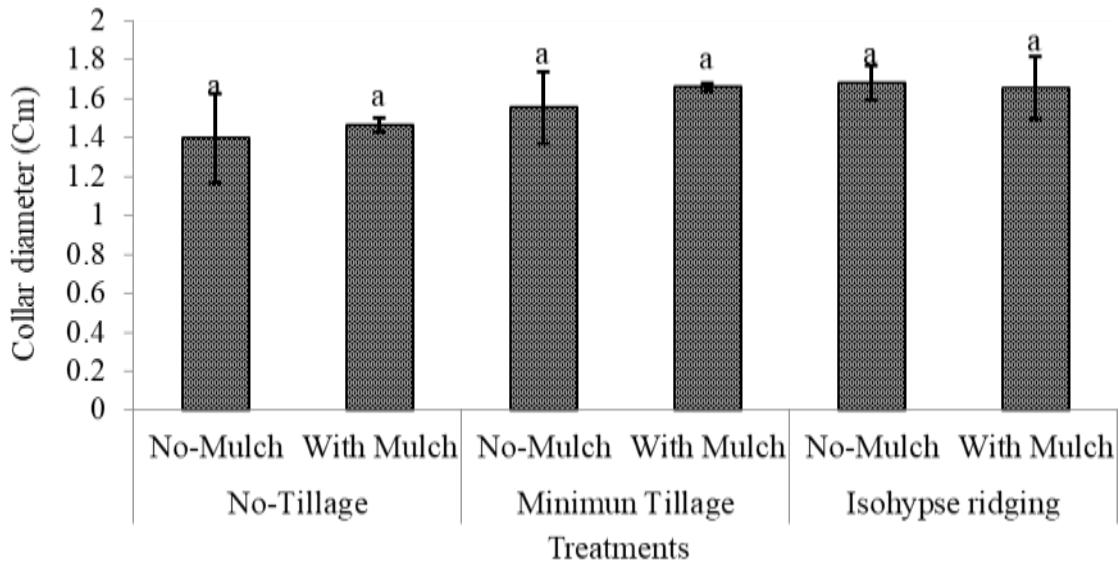


Figure 3. Interactive effect of tillage and mulching on maize collar diameter. The error bars correspond to the standard deviation. Different letters indicate statistically significant difference.

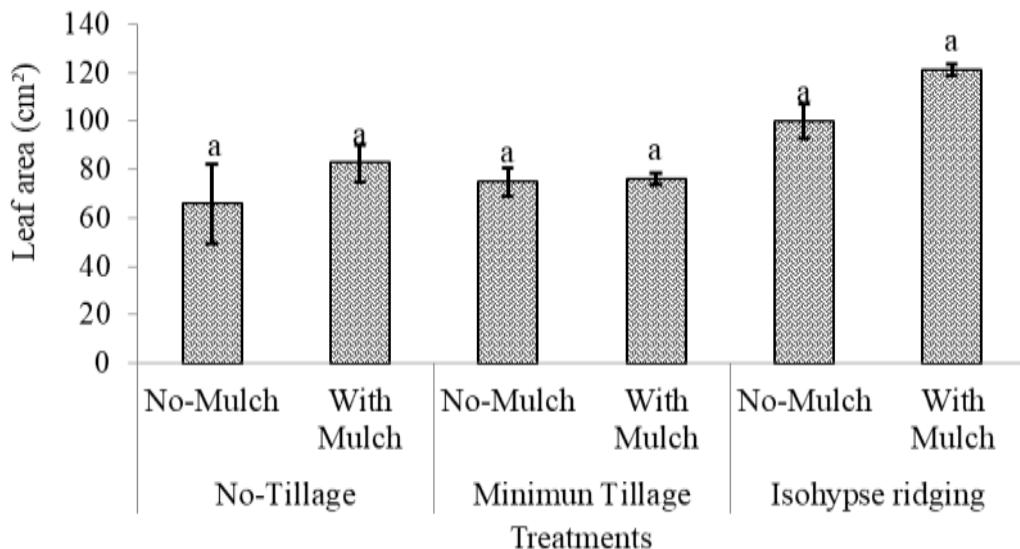


Figure 4. Interactive effect of tillage and mulching on maize leaf area. The error bars correspond to the standard deviation. Different letters indicate statistically significant difference.

harvest index. No-tillage without mulch (SLM0) generated the lowest harvest index, around 15% less than the BM50, MLM50 and BM0.

Chemical properties of soil

Tillage system significantly influenced soil organic carbon ($p<0.05$). The effects of both tillage system and mulching were not significant on total nitrogen and available phosphorus ($p=0.329$ and $p= 0.31$, respectively). In fact,

ridging has induced a 36% increase soil organic matter compared to minimum tillage and no-tillage. On the contrary of the case of organic carbon, ridging has decreased available phosphorus content of soil. The highest available phosphorus pool was recorded with direct seeding (17.02 ppm). Total nitrogen has varied little according to the modalities of tillage system. Despite of the not significant effect of the mulching on total nitrogen content, organic carbon content and available phosphorus content of soil, there are arithmetical differences. No-mulch allowed the highest values for the

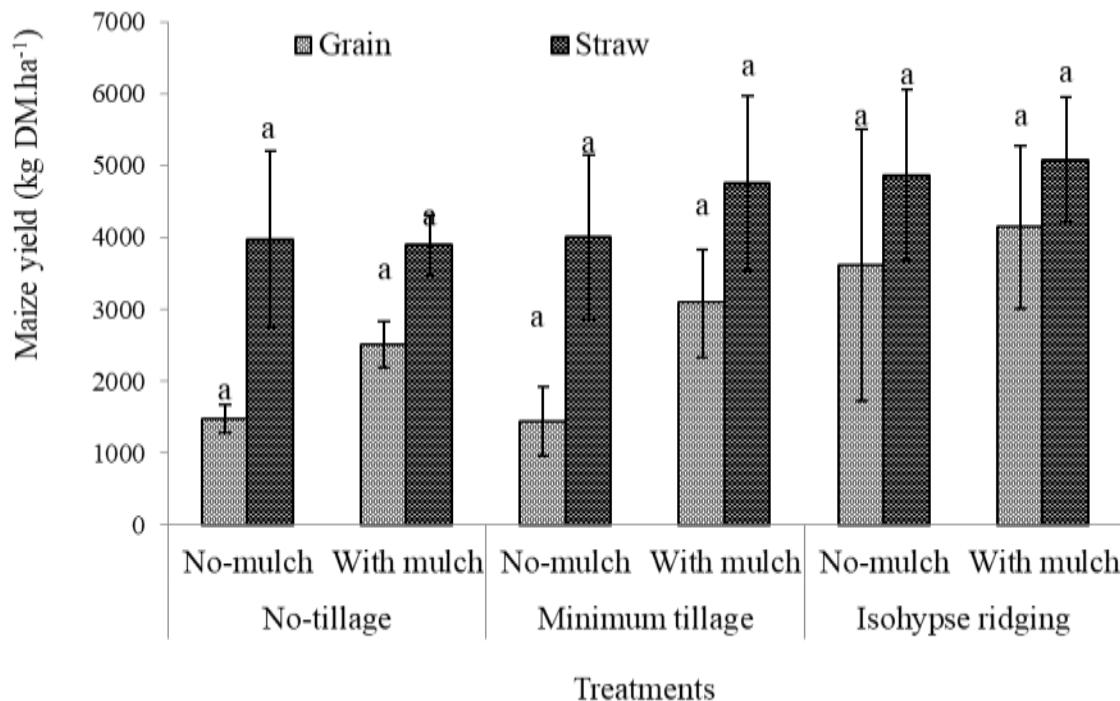


Figure 5. Interactive effect of tillage and mulching on maize yield. The error bars correspond to the standard deviation. For the same parameter, Different letters indicate statistically significant difference.

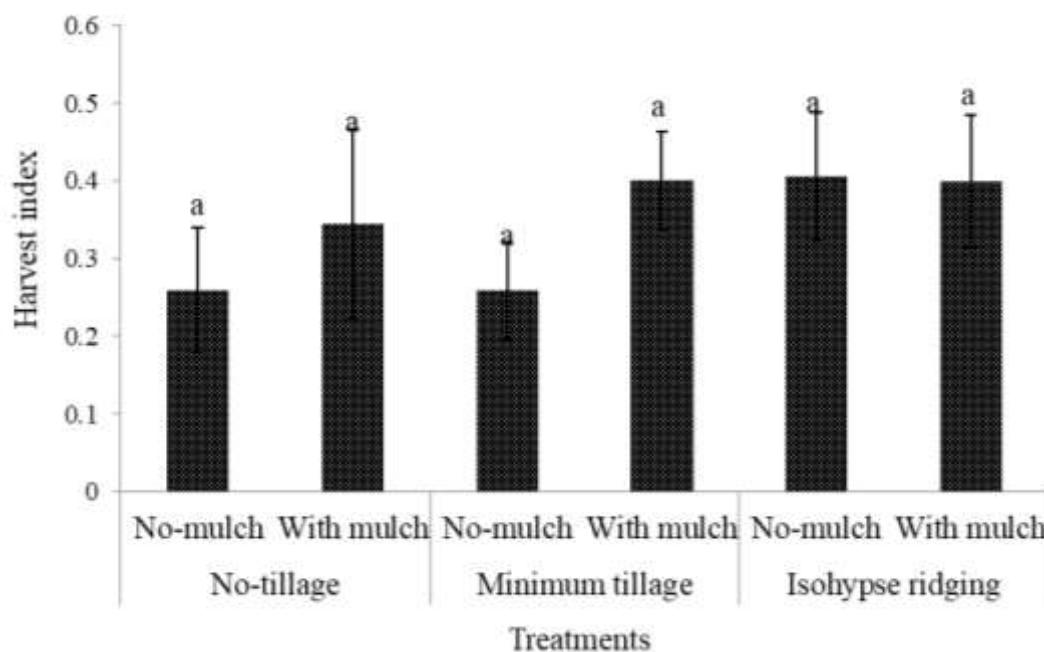


Figure 6. Interactive effect of tillage and mulching on maize harvest index. The error bars correspond to the standard deviation. Different letters indicate statistically significant difference.

three chemical parameters (Table 3). As shown, no-mulching creased by 10, 15 and 8%, respectively, the available phosphorus, total nitrogen and organic carbon content of soil after trial compared to mulch modality

(Table 3). The interactive effect of tillage system and mulching were not significant ($p > 0.05$). However, the statistical classification obtained shows that for total nitrogen and organic carbon content of soil, the BM0

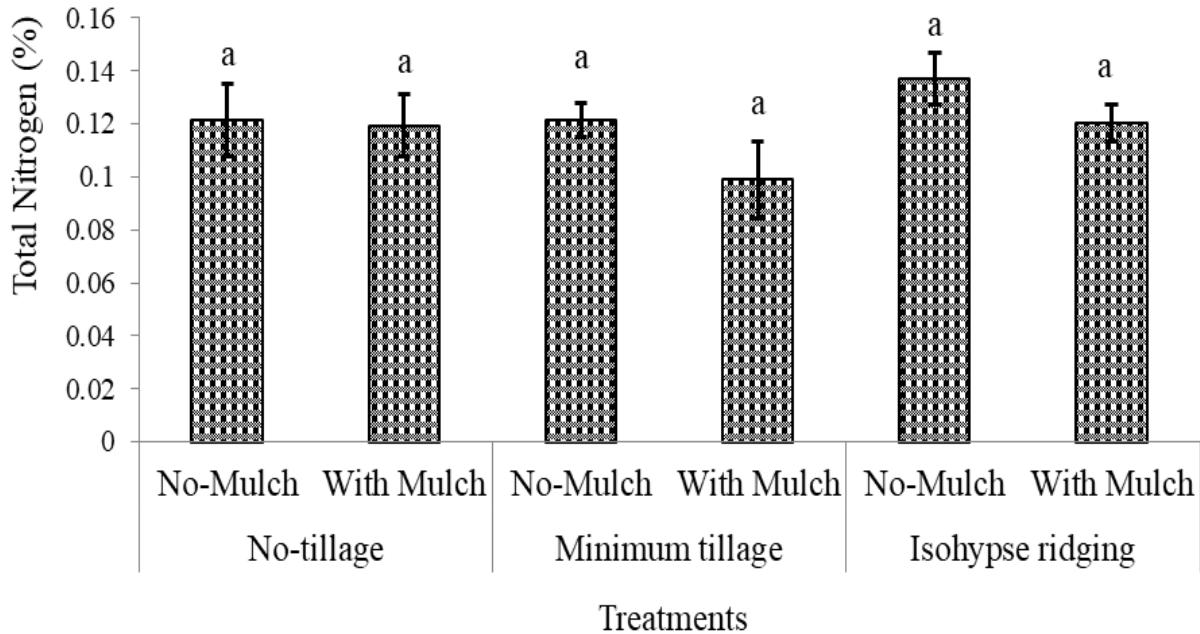


Figure 7. Interactive effect of tillage and mulching on total nitrogen content of the soil. The error bars correspond to the standard deviation. Different letters indicate statistically significant difference.

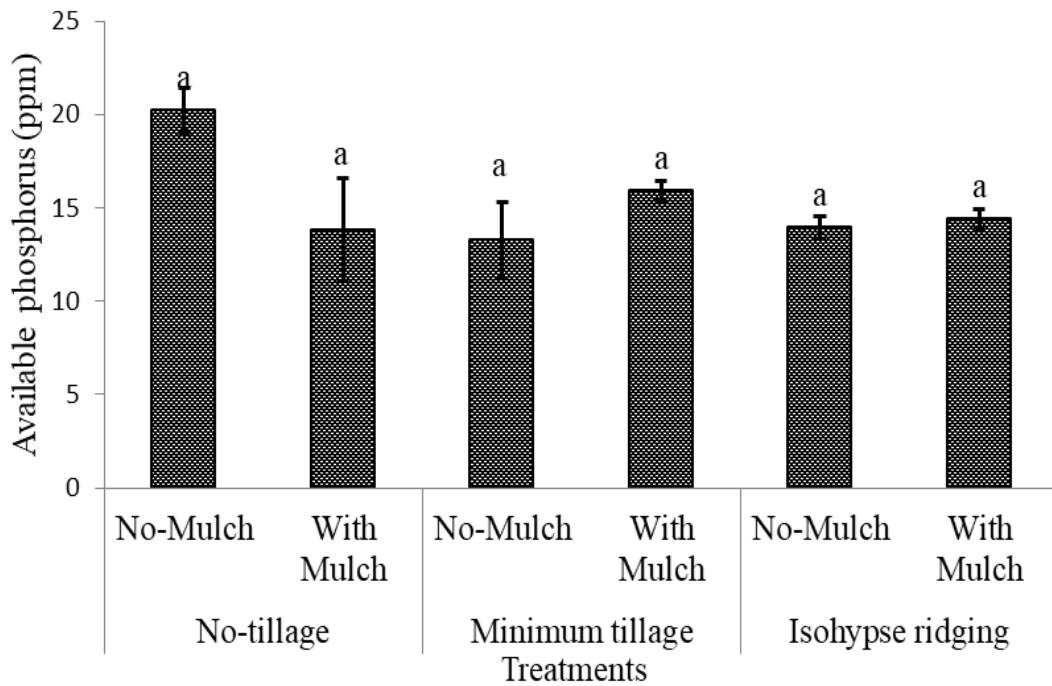


Figure 8. Interactive effect of tillage and mulching on soil available phosphorus pool (The error bars correspond to the standard deviation. Different letters indicate statistically significant difference).

allowed to obtain the high total nitrogen and organic carbon content of soil after trial (Figures 7 and 8). For the

available phosphorus, the highest pool was obtained with SLM0 (Figure 9).

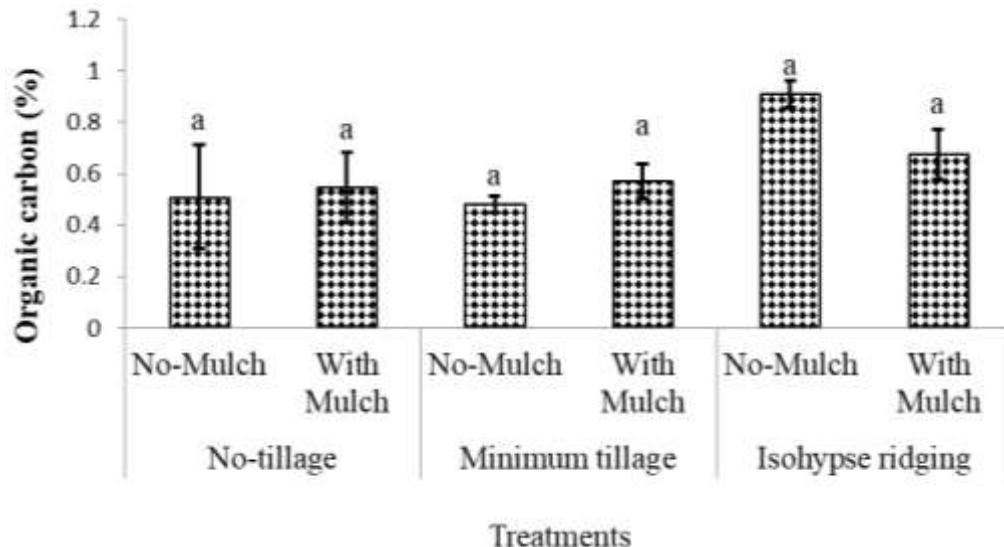


Figure 9. Interactive effect of tillage and mulching on soil carbon organic content. The error bars correspond to the standard deviation. Different letters indicate statistically significant difference.

DISCUSSION

Maize growth

In general, the results of this study have testified to this fact. Indeed, these results show that tillage system and mulching significantly influenced maize growth. Ridging allowed for faster growth, higher leaf area and higher collar diameter. No-tillage allowed slow growth. In fact, under no-tillage, the soil is compact and does not allow good water infiltration and good root development. As opposed to no-tillage, ridging allowed the soil to be aired by the loosening of the humiferous layer, thereby creating an environment favorable to the root activity of maize plants. Similar results were obtained by Abdellaoui et al. (2006), Tomavo (2014), Hountongninou (2016) and Kouelo (2016). These authors related that the growth of maize or wheat was significantly increased by tillage. Tomavo (2014) and Hountongninou (2016) in a study on the effect of tillage, mulching and nitrogen on maize growth in southern Benin showed that flat tillage increased the growth speed, of around 50% compared to no-tillage. Kouelo (2016) obtained the same results on three watersheds in southern Benin. The author concludes that maize grows slowly at no-tillage. The mulch treatments resulted in a significant improvement in maize growth in the study area. The present results confirm those obtained by the authors (Findeling et al., 2003; Diallo et al., 2006; Barthes et al., 2010; Pervaiz, 2009). Researchers agree the role of mulch on the improvement of physical, chemical and biological properties of the soil allows the growth of plants. Indeed, mulch increases soil moisture and nutrients availability to plant roots in turn, leading to higher plant growth.

Maize productivity

Maize productivity was assessed through grain and straw yields and harvest index. Isohypse ridging significantly increased grain and straw yield of maize. This reflects the strong growth favored by ridging compared to no-tillage and minimum tillage. Indeed, the suppression or minimization of the topsoil reversal has significantly reduced the maize productivity components in the watershed of Linsinlin. These results confirm those of Osunbitan et al. (2005) and Kouelo (2016) which attribute this to the formation of crusts and nutrient depletion. Indeed, no-tillage deeply modifies the physical properties (Peigne et al., 2007). The practice of mulch significantly increased maize grain yield and harvest index. The presence of a vegetation cover applied in mulching on the soil preserves the humidity of soil, promoting a microclimate favorable to microbial life, which has the direct consequence of improving the physicochemical properties of the soil. In addition, the mulch cover constitutes a source of organic matter (Pervaiz, 2009). Our results are confirm to those of Barthès et al. (2010) and Badou et al. (2013). Treatments that combine either of the working methods with the practice of mulch (BM50, MLM50 and SLM50) have increased the productivity of maize, respectively compared to the treatments without mulch (BM0, MLM0 and SLM0). Indeed, BM50 combines the beneficial effects of the turning of the soil and those due to the presence of mulching. The effects of non-turning of the soil are attenuated by the presence of mulch. In a no-till situation combined with mulching, the organic matter content of soils (MOS) increases essentially on the surface: West and Post (2002) estimate that 85% of the organic matter accumulates in

the first 7 cm. In this regard, Thévenet et al. (2002) stressed the importance of the management (return to soil or removal) of organic residues (quantity, quality and fate). Badou et al. (2013) recommended mulch practice for sustainable land use.

Chemical properties of soil

The results show that the effect of both tillage system and mulching was not significant on the available phosphorus and total nitrogen content of the soil. The effect was significant on soil organic carbon pool. Furthermore, the available phosphorus and total nitrogen pool decreased from no-tillage to ridging and from the no-mulch to with mulch. Hountongninou (2016) reported a decrease in total nitrogen and available phosphorus on flat tillage plots compared to non-tillage plots. The decrease of soil total nitrogen pool could be explained by its high use rate due to the high maize growth on the tilled plots. The reduction of available phosphorus on tilled plots compared to no-tillage plots can be explained by the fact that tillage systems, even in relation to the input of organic matter, promote nutrients depletion, especially phosphorus (Müller et al., 2008). Organic carbon has been increased by ridging and mulching. This can be explained by the fact that a large quantity of organic matter was buried during ridging. Therefore, the presence of mulching constitutes a potential source of soil organic matter. Agricultural practices adopted in agro-systems have positive or negative impacts on soil organic matter content and functions (Aholoukpè, 2013).

Conclusion

Tillage system and mulching, taken separately, significantly affect maize growth and yield. Combined, maize growth and yield is also improved, but the difference is not significant. This improved maize performance has exhausted the nutrient pool of the soil. No-tillage generated the lowest performance, although it was combined with mulching, while mulch-covered logging produced the best maize performance. The soil, poor in organic matter and nutrients, is therefore degraded and consequently exhibits poor physical and chemical properties. The soil would be compact, making it difficult to develop roots and infiltrate rainwater. Regardless of the type of tillage, mulching has improved maize performance. Isohypse ridging and mulching constitute two effective practices for the sustainable use and conservation of agricultural soils in the central agro-ecological zone of Benin.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Adaptability and stability of saccharine sorghum cultivars

Guilherme Cassicala Eculica¹, Pedro César de Oliveira Ribeiro², Aluízio Borém de Oliveira², Nádia Nardelly Lacerda Durães Parrella³, Pakizza Sherma da Silva Leite⁴ and Rafael Augusto da Costa Parrella^{5*}

¹Department of Biology, Federal University of Viçosa, Viçosa, MG, Brasil.

²Department of Fitotecnia, Federal University of Viçosa, Viçosa, MG, Brasil.

³Department of Agrarian Sciences, Federal University of São João del-Rei, UFSJ, Sete Lagoas, MG, Brasil.

⁴Department of Biology, Federal University of Lavras, UFLA, Lavras, MG, Brasil.

⁵Department of Sorghum Breeding, Brazilian Research Company, Embrapa Maize and Sorghum, Sete Lagoas, Mg, Brasil.

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The Brazilian bioenergy sector has been trying the use of saccharine sorghum in order to optimize ethanol production. However, there are few varieties fitted to this objective and little knowledge about their adaptability and stability. Then, the purpose of this work was to study the adaptability and stability of saccharine sorghum, taking into account the effects of the G x E interaction, to select superior genotypes and validate if the two selection methods for identification of genotypes with better phenotypic stability complement one another. Thus, the methodologies of Eberhart and Russell as well as Cruz et al. were used; and according to Eberhart and Russell, the BRS 511 genotype is preferred in ethanol production because it presents highly predictable and responsive behavior to changes in environments for all the traits evaluated in specific or broad conditions. The method also identified the genotypes CMSXS644, CMSXS647 and Sugargraze for green mass production; CMSXS629, CMSXS630, CMSXS646, CMSXS647, BRS 508, BRS509 and CV198 for tons of brix per hectare; and finally, CMSXS629, CMSXS630, CMSXS643, CMSXS646, BRS 506 and BRS 509 for total soluble solid content. The methods used in this study were not complementary to selection genotypes.

Key words: Bioethanol, ethanol production, genotype and environment interaction, genetic breeding, *Sorghum bicolor* (L.) Moench.

INTRODUCTION

Brazil is a country with different renewable energy alternatives and with favorable weather to production,

one of the leaders in world ethanol production (Lamers et al., 2011; Li et al., 2018). The bioethanol is a fuel that is

*Corresponding author. E-mail: rafael.parrella@embrapa.br.

produced in large quantities in the world, being motivated by the creation of flex-fuel vehicles and by the more accessible and lower price of this fuel in relation to gasoline. This biofuel can be obtained from sources saccharose rich agricultural crops, as it is traditionally done in a large scale from sugarcane in Brazil or starch-rich crops as corn in the USA (AIE, 2010).

Sugarcane is considered the main crop for biofuel production; however, other species are also viable for this purpose such as the saccharine sorghum [*Sorghum bicolor* (L.) Moench]. This crop is a C₄ plant with a high photosynthesis efficiency being the most efficient energy producer in order to produce concentrated energy from renewable sources. Thus, the saccharine sorghum is a promising alternative to complement the supply of raw material for sugar-ethanol industry, since this crop has the potential to supply the demand for raw material for bioethanol production in the sugarcane harvesting season without cessation of service in the production (Dar et al., 2018). The main purpose of breeding programs involving sorghum hybrids is the identification of genotypes with high production potential, wide adaptability and good stability. According to Cruz and Carneiro (2006), the genotype adaptability indicates the ability to respond advantageously to environmental improvements, meanwhile genotype stability refers to the ability to present expected behaviors in relation to environmental variations. In Brazil, breeding programs aiming the production of sorghum varieties work intensively evaluating trials in several environments and years. The high cost of research activities requires a rational use of resources, since there is a decrease in the number of environments for testing and proper selection of the right method to do this analysis.

Furthermore, selection can be maximized by using methodologies permitting a better interpretation of genetic and environmental effects individually, which allows selection to be carried out considering only the genetic effects. In this case, the method proposed by Eberhart and Russel (1966) and Cruz et al. (1989) are recommended to be used in data analysis as an attempt to maximize selection. These methodologies mainly try to quantify genotype behavior in relation to genotype adaptability and stability in various environments, besides quantifying environmental differences when selecting the ideal environment and, therefore, providing high genetic gains. Although both methodologies are very informative separately, their association can provide a better understanding about genotype and environment interaction.

Eberhart and Russel (1966) method evaluate only a regression coefficient, which can be estimated by a unique analysis considering all the tested environments. In this method, the ideal genotype can be discarded because it has a double slope and, then, deviations that should be evaluated in different environments could be quite high in relation to the estimated straight line. Thus,

this method was improved by Cruz et al. (1989) with the use of bissegmented linear regression, which makes possible to adjust a straight-line segment to unfavorable and poor environments and also another straight-line segment to the best and favorable environments (RAMALHO et al., 2012).

Thus, the purpose of this study was to analyze the adaptability and stability of saccharine sorghum, considering the effects of genotype and environment interactions for selecting superior genotypes for ethanol production, and compare the two methods of genotype identification with better phenotypic stability.

MATERIALS AND METHODS

Experimental design

Eight experiments (2013 -2014) were performed in order to obtain the crop values and uses as depicted in Table 1.

Sixteen sorghum saccharine genotypes obtained from a genetic breeding program of *Embrapa Milho e Sorgo* were evaluated in eight environments. Ten of these genotypes are fertility restoration lineages (BRS506, BRS508, BRS509, BRS511, CMSXS629, CMSXS630, CMSXS643, CMSXS644, CMSXS646 and CMSXS647) and the other six are hybrids (CV198, CV568, Sugargraze, V82391, V82392 and V82393).

The experiment was carried out in a randomized complete block design with 16 saccharine sorghums (treatments) and three replications. The plots (experimental units) were considered to be four rows of 5 m. Row spacing was 0.7 m and eight plants were left per linear meter. Two central rows were considered as useful plots. Weeding and pest control was performed as needed. Fertilization was performed using 400 kg ha⁻¹ of the 8N-28P-16K and 200 kg ha⁻¹ of urea in dressing. No micro nutrients were applied.

Evaluated traits

The evaluated traits were green mass production (GMP), content of total soluble solids (TSS) and tons of brix per hectare (TBH). The values for GMP were obtained by measuring the weight of all plants in each plot, with no panicles. The plants were harvested when the seed reached physiological maturity, and their weight was determined in kilograms (kg) for each plot in tons per hectare (t ha⁻¹). The values for TSS was determined from six plants per plot which were taken at random by using a juice sample from plants an automatic digital refractometer, with the reading in degrees Brix. Finally, the values for TBH was obtained by the multiplication of the values for GMP by the values for TSS (TBH = GMP x TSS).

Statistical analysis

All the statistical analysis described below was performed in the statistical genetic software GENES. The first analysis conducted before the analysis of variance (ANOVA) was performed to verify the following assumptions: If the errors follow a normal distribution, are independent and present a constant variance (homogenous variance) (RAMALHO et al., 2000), then, individual analysis of variance was performed considering each environment separately and all the evaluated traits. The statistical model adopted for the individual analysis of variance for each environment was the following:

Table 1. Municipalities where experiments with sorghum saccharine genotypes were conducted with their geographical coordinates, respectively.

Environment	Geographical coordinate		
	Latitude	Longitude	Altitude (m)
Santa Vitória-MG	18° 50' 19" S	50° 07' 17" O	498
Sete Lagoas-MG	19° 27' 57" S	44° 14' 49" O	767
Lavras-MG	21° 14' 43" S	44° 59' 59" W	919
Nova Porteirinha-MG	15° 47' 00" S	43° 18' 00" O	533
Piracicaba-SP	22° 43' 31" S	47° 38' 57" W	547
Sinop-MT	11° 51' 51" S	55° 30' 09" W	345
Planaltina-DF	17° 35' 03" S	47° 42' 30" W	1.100
Dourados-MS	22° 13' 16" S	54° 48' 20" W	430

$$Y_{ij} = \mu + g_i + b_j + e_{ij}$$

Where: Y_{ij} is the individual observation (plot) of the genotype i in the block j ; μ represents the overall mean and is a constant associated to the observations; g_i is the effect of genotype i ($i=1, 2, \dots, 16$); b_j is the random effect of block j ($j = 1, 2, \dots, 3$); and e_{ij} is the experimental error associated to Y_{ij} .

Before performing the combined analysis, the Cochran (1947) test was performed to verify the homogeneity of variances for all variables considered in this study. Since there are statistical differences between the mean squared errors, it used the method proposed by Cochran (1954) accordingly to recommendations of Pimentel-Gomes (2000), in which the residual mean squares of the combined analysis were obtained by the degrees of freedom adjusted of each variable. Then, it was possible to perform an analysis of variance combined with the decomposition of the sum of squares for environments. The following statistical model was fitted to the data when performing the analysis of variance combined between the environments:

$$Y_{ijk} = \mu + G_i + B/A_{jk} + A_j + GA_{ij} + e_{ijk}$$

Where: Y_{ijk} is the individual observation (plot) of the genotype i inside the environment j and in the block k ; μ represents the overall mean and is a constant associated to the observations; G_i is the fixed effect of the genotype i ($i=1, 2, \dots, 16$); B/A_{jk} is the effect of the block k inside the environment j ; A_j is the effect of the environment j ($j=1, 2, \dots, 8$); GE_{ij} is the effect of the interaction between the genotype i and the environment j ; and e_{ijk} is the experimental error associated to Y_{ijk} .

Analysis of variance was first conducted for each environment. After verifying the assumptions of homogeneity of residual variances, analysis of variance for all sites and the Scott-Knott test (1974) at 5% probability were performed as illustrated in supplementary Tables S1, S2 and S3. Finally, adaptability and stability analysis was conducted, after determining significant genotype by environment interaction. The analysis of phenotypic adaptability and stability for the genotypes were performed using both the methodologies of Eberhart and Russell (1966) and Cruz et al. (1989). The method proposed by Eberhart and Russel (1966) is based on linear regression analysis and uses the mean of productivity as an adaptability parameter for each genotype (β_{oi}), the linear regression coefficient (β_{1i}) as a response pattern of the genotype in different environments, and finally the genotype stability is evaluated by the mean squared deviation of regression (σ^2_d) and/or by the coefficient of determination (R^2). The following statistical model was fitted to the data when performing analysis

using the method of Eberhart and Russel (1966):

$$Y_{ij} = \beta_{0i} + \beta_{1i}I_j + \delta_{ij} + \varepsilon_{ij}$$

where: Y_{ij} is the mean of productivity for the genotype i in the environment j ; β_{0i} is the mean of the genotype i in all environments; β_{1i} is the linear regression coefficient of the genotype i ; I_j is the environmental index estimated by the difference between the environmental mean and the general mean; δ_{ij} is the regression deviation of the genotype i in the environment j ; ε_{ij} is the mean of the experimental error associated to the observation Y_{ij} , presenting normal and independent distribution, with a mean of zero and constant variance. The adaptability parameter (β_{0i}) was estimated by using the following equation:

$$\hat{\beta}_{1i} = \frac{\sum Y_{ij} I_j}{\sum I_j^2}$$

Under the null hypothesis $H_0: \beta_{0i}=1$ and the alternative hypothesis $H_1: \beta_{0i} \neq 1$, the β_{0i} estimates were evaluated by student's t -test using the following equation:

$$t = \frac{\hat{\beta}_{1i} - 1}{\sqrt{\hat{V}(\hat{\beta}_{1i})}} \sim \mathcal{N}(\hat{\beta}_{1i}) = \frac{\sigma^2}{r \sum I_j^2}$$

The stability parameter (σ^2_{di}) was estimated by the following equation:

$$\sigma^2_{di} = \frac{MSD_i - MSE}{r},$$

in which: MSD_i is the mean squared deviation of genotype i ; MSE is the mean squared error; and r is the number of repetitions.

Then, under the null hypothesis of $H_0: \sigma^2_{di}=0$ and the alternative hypothesis of $H_1: \sigma^2_{di} \neq 0$, the estimates for σ^2_{di} were analyzed by F -test using the following equation:

$$F_{\text{calculated}} = \frac{MSD_i}{MSE},$$

Where: MSD_i is the mean squared deviation and MSE is the mean

squared error.

Thus, as proposed by Eberhart and Russel (1966), the environment effect can be split in two components: a linear and nonlinear component. The regression coefficient β_{1i} is associated to the linear component and indicates the genotype adaptability, which is the genotype ability to respond to environmental improvements. On the other hand, the regression deviations σ^2_{di} are associated to the nonlinear component and indicate the phenotypic stability.

The method of Eberhart and Russel (1966) reveals that a stable genotype occurs when $\sigma^2_{di} = 0$ and a non-stable genotype is found when $\sigma^2_{di} \neq 0$. Also, a genotype has wide adaptability if $\beta_{1i} = 1$; is adapted to favorable environments, in case that $\beta_{1i} > 1$, and adapted to unfavorable environments, if $\beta_{1i} < 1$. The coefficient of determination (R^2) for each genotype was proposed by Pinthus (1973) as an additional measure for the Eberhart and Russel (1966) method and was also used in this study as a measure to define the phenotypic stability and to quantify the ratio of phenotypic variation of each genotype that is explained by linear regression.

According to Cruz et al. (2004), genotypes with higher means of productivity and presenting σ^2_{di} statistically different from zero can occur and, then, it would be necessary the selection of some genotypes from the group with low stability. In these cases, an auxiliary measure of comparing genotypes is the coefficient of determination (R^2_i), which is estimated by the following equation:

$$R^2_i = \frac{SS(\text{Linear Regression})}{SS(E/G_i)} \cdot 100,$$

Where; SS is the sum of squares.

For evaluating stability of the genotype, the method proposed by Cruz et al. (1989) was used in the analysis. This method is based on the bissegmented regression analysis and has the mean as an adaptability parameter (β_{0i}) and a linear response to both unfavorable (l_j) and favorable environments ($l_j + l_+$). Then, stability of the genotype is evaluated by the regression deviation of each genotype in relation to the environmental variations following this statistical model:

$$Y_{ij} = \beta_{0i} + \beta_{1i}l_j + \beta_{2i}T(l_j) + \delta_{ij} + e_{ij}$$

where: Y_{ij} is the mean of genotype i in the environment j ; β_{0i} is the general mean of genotype i ; β_{1i} is the linear regression coefficient associated to the variable l_j ; l_j is the environmental index; β_{2i} is the linear regression coefficient associated to the variable $T(l_j)$; if $l_j < 0$, $T(l_j)=0$; if $l_j > 0$, $T(l_j)=l_j - l_+$, in which l_+ is the mean of the positive indexes l_j ; δ_{ij} is the linear regression deviation; and e_{ij} is the mean of the experimental error; β_{0i} indicates the maximum productivity found in all the experimental essay; $\beta_{1i} = 0.5$ and $\beta_{2i} = 1$ are values established by Cruz and Carneiro (2006). $\beta_{1i} = 0.5$ indicates a low response to unfavorable environments and $\beta_{1i} + \beta_{2i} = 1.5$ reveals the responsiveness to favorable conditions.

RESULTS AND DISCUSSION

The homogeneity of variances showed that the ratio between the bigger and lower mean squared error was higher to the approximate ratio of 7:1, therefore, the maximum F was statistically significant and then the null hypothesis was rejected for all the variables evaluated in this study. Then, the combined analysis was carried out and the results showed that the source of genotype variation was non-statistically significant only for GMP,

indicating there are none significant statistical differences between the genotype means in the environments. On the other hand, for the variables TBH and TSS, it was possible to identify significant differences between genotype means in the environments. The G x E interaction was statistically significant ($P<0.01$) by F-test, indicating that the performance of sorghum cultivars evaluated in this study is statistically different in the environments. And furthermore, the coefficients of variation (C.V.) for the variables indicate good experimental precision; for GMP is 15.77%, for TSS is 12.37% and for TBH is 19.51% as illustrated in Table 2. In fact, Figueiredo et al. (2015) also studied about the adaptability and stability of saccharine sorghum and used the methods of GGEBiplot and Toler in data analysis. Their same variables GMP, TBH and TSS were also identified as statistically significant, indicating statistical differences between genotype means in the environments. For this reason, the significant differences between genotype means inside each environment reflects the need of doing a more detailed study, aiming to identify genotypes that present better adaptability and stability.

The variance analysis for the phenotypic adaptability and stability were evaluated by the linear regression method proposed by Eberhart and Russel (1966) and the results are shown in Table 3. Significant differences were observed for the effects of genotypes, environments and G x E interactions, which mean that genotypes presented different behaviors in non-similar environments, confirming the variability of evaluated genotypes and environments.

Significant effects of G x E interaction indicate that there are differences between regression coefficients of the genotypes being evaluated, and that a part of G x E interaction can be explained by a linear relationship between genotypes and environments. Furthermore, regression deviations inform about phenotypic stability and they were also identified to be statistically significant in this study. Thus, linear and nonlinear components of stability are involved in the performance of genotypes in the environments. Also, the significance of regression deviations informs that in general the evaluated genotypes showed an unstable and unpredictable behavior. However, by its magnitude, the linear environment was mainly responsible for the explanation of genotype behavior.

The mean estimates of GMP, TBH and TSS for genotypes ($\hat{\beta}_{0i}$), the regression coefficient (β_{1i}), the variance of regression coefficients (σ^2_d) and the coefficient of determination (R^2) for each genotype were obtained by Eberhart and Russel (1966) method and the results are in Table 4. The genotypes BRS 506 and CMSXS643 presented $\beta_{1i}>1$ for GMP, which indicates a higher production than the general mean reflecting a specific adaptability to favorable conditions and adaptability to environments with high productivity.

Table 2. Summary of the combined analysis of variance of sixteen saccharine sorghum genotypes that were evaluated in eight environments and the following variables were considered in the analysis: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

Variable	Source of variation	Degree of freedom	Mean square	F
GMP	Blocks/environments	-	16	172.23
	Environments (E)	-	7	6556.59
	Genotype (G)	-	15	367.84
	G x E	-	81	227.08
	Residue	-	179	77.15
	Mean	55,7	-	-
	Coefficient of variation (%)	15,77	-	-
TBH	Blocks/environments	-	16	0.8
	Environments (E)	-	7	52.33
	Genotypes (G)	-	15	6.17
	G x E	-	86	2.27
	Residue	-	191	0.68
	Mean	4,21	-	-
	Coefficient of variation (%)	19,51	-	-
TSS	Blocks/environments	-	16	3.47
	Environments (E)	-	7	143.12
	Genotypes (G)	-	15	81.14
	G x E	-	77	13.16
	Residue	-	168	3.39
	Mean	14,88	-	-
	Coefficient of variation (%)	12,37	-	-

Values with * were statistically significant at 1%; values with ns are non-significant by F-test.

Similarly, Souza et al. (2013) also studied the adaptability and stability of the saccharine sorghum and, by using the method of Annichiarico, found out that the genotype BRS 506 showed a high stability and adaptability to favorable and unfavorable environments, meanwhile the CMSXS643 genotype did not present a high index for stability and adaptability.

Also, by using Eberhart and Russel (1966) method in this study, it is possible to recommend the BRS 511 genotype as a good cultivar for ethanol production because it shows a highly predictable and responsive behavior to variations in environments with specific or wide conditions to all the evaluated traits and shows good mean in variables of agronomist as presented in supplementary Table S1, S2 and S3. Similarly, Figueiredo et al. (2015) used the GGEbiplot method and verified that the following genotypes BRS 511 and CMSXS647 presented higher adaptability and stability for the variable TBH. This fact corroborates the results of this present work, in which the BRS 511 variety presents adaptability, stability and good means, making it possible to be recommended as a cultivar for ethanol production.

When analyzing the mean squared deviation (σ^2_d), a parameter that classifies the stability of a genotype, it

was possible to identify that BRS 511, CMSXS647 and TBH genotypes presented a good foreseeability with $\sigma^2_d = 0$. Genotypes with wide adaptability in the environments are the ones with $\beta_1=1$, and thus with unpredictable behaviors ($\sigma^2_{di} \neq 0$ and $R^2 < 80\%$). In this situation, the CMSXS630, CMSXS643, BRS 506, CV 198, CV 568 and V82393 are superior genotypes for the variable GMP; CMSXS643, CV 568, Sugargraze and V82393 for the variable TBH; and finally, BRS 508, CV 198, Sugargraze, V82391 and V82393 for the variable TSS. According to Eberhart and Russel (1966), the superior genotypes in the analysis are the ones showing a higher mean than the overall mean, overall or wide adaptability ($\beta_1 = 1$), good foreseeability ($\sigma^2_d = 0$) and with good response both in favorable and unfavorable environments. The results of this analysis are in Table 4 and it can be inferred that the CMSXS644, CMSXS647, BRS 511 and Sugargraze are superior genotypes for the variable GMP. The CMSXS629, CMSXS630, CMSXS646, CMSXS647, BRS 508, BRS 509, BRS 511 and CV198 genotypes are superior for the variable TBH. And finally, the CMSXS629, CMSXS630, CMSXS643, CMSXS646, BRS 506, BRS509 and BRS 511 genotypes are superior for the variable TSS.

Table 3. Summary of analysis of variance combined with decomposition of the sum of squares of environments according to the method of Eberhart and Russell (1966).

ANOVA			
Variable	Source of variation	Degree of freedom	Mean square
GMP	Environment (E)	7	6556.592**
	Genotypes (G)	15	367.840**
	E x G interaction	105	175.176**
	Env/Gen. (E/G)	112	574.014**
	Linear Env. (LE)	1	45896.146**
	G x EL interaction	15	128.869**
	Combined deviation (E/G)	96	171.463**
	Residue	179	1
TBH	Environments (E)	7	52.332**
	Genotypes (G)	15	6.174**
	E x G interaction	105	1.861**
	Env/Gen. (E/G)	112	5.016**
	Linear Env. (LE)	1	366.324**
	G x LE interaction	15	1.265**
	Combined deviation (E/G)	96	1.838**
	Residue	191	1
TSS	Environments (E)	7	143.116**
	Genotypes (G)	15	81.135**
	E x G interaction	105	9.653**
	Env/Gen. (E/G)	112	17.995**
	Linear Env. (LE)	1	1001.811**
	G x LE interaction	15	7.167**
	Combined deviation (E/G)	96	9.439**
	Residue	168	1

Values with * were statistically significant at 1% by F-test. Sixteen saccharine sorghum genotypes were evaluated in eight environments and the following variables were considered in the analysis: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

The results of the adaptability and stability analysis for the variable GMP, TBH and TSS using the method proposed by Cruz et al (1989) are found in Table 5. The β_1 estimates genotype performances in unfavorable conditions. For both GMP and TBH, most of the genotypes did not differ from one ($\beta_1=1$), with the exception of V82392 and BRS 506 that showed to be more demanding in this same condition ($\beta_1>1$). And finally, for the variable TSS, all the genotypes did not differ significantly from one ($\beta_1=1$). On the other hand, when considering unfavorable conditions, it was not possible to identify responsive genotypes to variations in the environment.

In relation to the linear response of favorable environments ($\beta_1+\beta_2$), the genotype CMSXS630 was the most responsive in this environment to the variable GMP and the genotype CMSXS643 to the variable TBH, which showed statistically significant results (linear response) higher than one ($\beta_1+\beta_2>1$). However, it was found that

only the genotype CMSXS643 is adapted to favorable environments and responsive to environmental improvements, for having a higher mean than the overall mean. For the TSS variable, all the genotypes were non-statistically significant from one ($\beta_1+\beta_2=1$).

Phenotypic stability or genotype predictability is evaluated by regression deviations in relation to the linear response to the environmental improvement. Then it was possible to identify the following genotypes CMSXS629, CMSXS630, CV198, CV568 and V82393 showing regression deviations different from zero ($\sigma^2_d \neq 0$) for the variable GMP; the genotypes CV568 and V82393 for the variable TBH, and finally the genotypes BRS508, CV 198, Sugargraze and V82391 for the variable TSS. Thus, these genotypes can be classified as unstable both on favorable and unfavorable environments.

By Cruz et al. (1989), the ideal genotype should present a high mean (high β_0), the phenotypic averages shown in supplementary Tables S1, S2 and S3 are less

Table 4. Estimates of the regression coefficients (β_0 and β_1), mean squared deviation of regression (σ^2_d) and coefficient of determination for the following variables: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

Genotype	Variable											
	GMP				TBH				TSS			
	Mean (β_0)	β_1	σ^2_d	R ² (%)	Mean (β_0)	β_1	σ^2_d	R ² (%)	Mean (β_0)	β_1	σ^2_d	R ² (%)
CMSXS629	51.93	0.83 ^{ns}	21.74 ^{ns}	70.12	4.42	0.74 ^{ns}	0.26 ^{ns}	54.22	15.91	0.79 ^{ns}	-0.92 ^{ns}	66.43
CMSXS630	53.42	1.16 ^{ns}	100.52 ^{**}	62.93	4.32	0.68 ^{ns}	0.22 ^{ns}	51.33	16.1	0.64 ^{ns}	-0.46 ^{ns}	47.59
CMSXS643	56.04	1.23 ^{ns}	55.82 ^{**}	74.98	4.53	0.89 ^{ns}	0.80 ^{**}	47.33	15.9	0.50 ^{ns}	0.87 ^{ns}	23.1
CMSXS644	57.15	0.95 ^{ns}	8.54 ^{ns}	80.95	4	0.99 ^{ns}	-0.16 ^{ns}	87.70	14.22	0.60 ^{ns}	-1.69 ^{ns}	79.43
CMSXS646	52.58	0.71 ^{ns}	-14.77 ^{ns}	88.44	4.68	0.87 ^{ns}	0.00 ^{ns}	74.49	17.28	1.33 ^{ns}	-1.53 ^{ns}	92.61
CMSXS647	58.54	0.96 ^{ns}	-1.80 ^{ns}	86.22	4.57	0.92 ^{ns}	0.04 ^{ns}	74.24	14.76	1.32 ^{ns}	1.31 ^{ns}	64.39
BRS 506	58.16	1.13 ^{ns}	32.46 [*]	77.87	4.57	1.55 ^{**}	0.15 ^{ns}	86.43	14.93	1.07 ^{ns}	-0.36 ^{ns}	70.61
BRS 508	53.06	0.85 ^{ns}	-1.99 ^{ns}	83.36	4.33	0.77 ^{ns}	0.19 ^{ns}	59.18	17.45	0.55 ^{ns}	5.46 ^{**}	12.39
BRS509	53.73	1.41 [*]	8.25 ^{ns}	90.4	4.34	1.13 ^{ns}	0.14 ^{ns}	77.52	16.41	1.40 ^{ns}	-0.99 ^{ns}	86.86
BRS 511	57.1	0.81 ^{ns}	-3.52 ^{ns}	82.80	4.84	0.78 ^{ns}	-0.01 ^{ns}	70.54	16.9	1.00 ^{ns}	-0.05 ^{ns}	63.80
CV 198	59.56	0.93 ^{ns}	71.34 ^{**}	58.92	4.34	1.10 ^{ns}	0.19 ^{ns}	74.81	15.24	0.63 ^{ns}	3.20 [*]	20.91
CV 568	65.51	0.69 ^{ns}	99.84 ^{**}	37.53	4.47	0.89 ^{ns}	1.05 ^{**}	42.1	13.12	0.96 ^{ns}	0.01 ^{ns}	61.06
Sugargraze	59.14	0.90 ^{ns}	28.90 ^{ns}	70.27	4.17	1.31 ^{ns}	0.41 [*]	74.59	13.36	1.20 ^{ns}	5.41 ^{**}	40.19
V82391	51.61	1.08 ^{ns}	21.11 ^{ns}	80.15	3.32	1.24 ^{ns}	0.05 ^{ns}	83.72	12.15	1.17 ^{ns}	3.60 [*]	46.07
V82392	52.86	1.34 ^{ns}	23.10 ^{ns}	85.53	3.32	1.18 ^{ns}	0.47 [*]	69.02	12.07	1.42 ^{ns}	1.71 ^{ns}	65.34
V82393	50.83	1.04 ^{ns}	62.45 ^{**}	66.22	3.17	0.96 ^{ns}	0.70 ^{**}	53.23	12.25	1.42 ^{ns}	2.44 [*]	61.03
Overall mean	55.7	-	-	74.79	4.21	-	-	67.53	14.88	-	-	56.36

Values for β_1 , parameters with * were statistically significant at 1%, respectively; values with ^{ns} are non-significant by Student *t*-test. Values for σ^2_d parameters with ** or *** were statistically significant at 1% or 5%, respectively; values with ^{ns} are non-significant by *F*-test. The genotypes of saccharine sorghum were analyzed following the method proposed by Eberhart and Russel (1966).

demanding to unfavorable environment (lower β_1), have the ability to respond to environmental improvements (the highest $\beta_1 + \beta_2$) and present a high stability in the evaluated environments ($\sigma^2_d=0$ or $R^2 > 80\%$). According to the data on Table 5, there was no genotype satisfying this condition, and for this reason the results obtained by this method was not coincident to the results of the method proposed by Eberhart and Russel (1966), which do not allow a better description of genotypes in relation to genotype and environment interaction. In case of coinciding the

results between these two methodologies, it would be possible to identify genotypes with better adaptability and especially its discrimination in relation to the phenotypic stability in the different environments evaluated in this study. The difficulty in identifying the ideal cultivars by the method proposed by Cruz et al (1989) can be due to the positive correlation that exists between β_{1i} and $\beta_{1i} + \beta_{2i}$ (Miranda et al., 1998).

Depending on the degree of association between the methodologies used in this study, they could be an auxiliary measure to choose the

stability parameter which results in the best adjustment (Duarte and Zimmermann, 1995). However, there is a lack of association between the methodologies proposed by Eberhart and Russel (1966) and Cruz et al. (1989), which indicates that there exists a redundancy in the information provided by methods based on regression and, for this reason, they should not be used together. The association between the methodologies of Eberhart and Russel (1966) and Cruz et al. (1989) are mainly explained, by the fact that these methodologies use the same

Table 5. Mean and parameter estimates of adaptability and stability for the following variables: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

Genotype	Variable														
	GMP				TBH				TSS						
	X (β_0)	β_1	($\beta_1+\beta_2$)	σ^2_d	R ² (%)	X (β_0)	β_1	($\beta_1+\beta_2$)	σ^2_d	R ² (%)	X (β_0)	β_1	($\beta_1+\beta_2$)	σ^2_d	R ² (%)
CMSXS629	51.93	0.86 ns	0.74 ns	30.05 ns	70.39	4.42	0.66 ns	1.21 ns	0.43 ns	57.88	15.91	0.83 ns	0.36 ns	0.12 ns	68.23
CMSXS630	53.42	0.83 ns	2.28 **	53.68 **	80.49	4.32	0.66 ns	0.77 ns	0.44 ns	51.51	16.1	0.59 ns	1.11 ns	0.66 ns	49.99
CMSXS643	56.04	1.08 ns	1.77 ns	55.21 **	79.16	4.53	0.62 ns	2.50 **	0.46 ns	73.44	15.9	0.54 ns	0.05 ns	2.26 ns	24.86
CMSXS644	57.15	0.89 ns	1.16 ns	12.30 ns	82.09	4	0.98 ns	1.00 ns	-0.02 ns	87.71	14.22	0.61 ns	0.55 ns	-0.74 ns	79.49
CMSXS646	52.58	0.74 ns	0.58 ns	-14.22 ns	89.33	4.68	0.79 ns	1.34 ns	0.11 ns	78.35	17.28	1.34 ns	1.27 ns	-0.54 ns	92.63
CMSXS647	58.54	1.00 ns	0.81 ns	1.07 ns	86.83	4.57	0.87 ns	1.16 ns	0.20 ns	75.13	14.76	1.19 ns	2.68 ns	2.15 ns	70.78
BRS 506	58.16	1.03 ns	1.45 ns	37.69 ns	79.71	4.57	1.54 *	1.59 ns	0.35 ns	86.44	14.93	1.03 ns	1.50 ns	0.79 ns	71.67
BRS 508	53.06	0.83 ns	0.92 ns	1.79 ns	83.53	4.33	0.56 ns	2.03 ns	-0.01 ns	85.83	17.45	0.52 ns	0.89 ns	7.81 **	12.81
BRS509	53.73	1.34 ns	1.63 ns	11.57 ns	91.07	4.34	1.04 ns	1.64 ns	0.27 ns	80.25	16.41	1.36 ns	1.81 ns	0.04 ns	87.54
BRS 511	57.1	0.82 ns	0.76 ns	0.12 ns	82.88	4.84	0.63 ns	1.67 ns	-0.04 ns	86.09	16.9	0.97 ns	1.31 ns	1.20 ns	64.36
CV 198	59.56	1.13 ns	0.27 ns	65.30 **	67.71	4.34	1.25 ns	0.25 ns	0.21 ns	82.5	15.24	0.57 ns	1.27 ns	4.98 **	22.93
CV 568	65.51	0.91 ns	-0.06 **	92.78 **	50.64	4.47	1.16 ns	-0.72 **	0.76 **	65.63	13.12	0.97 ns	0.82 ns	1.30 ns	61.18
Sugargraze	59.14	0.89 ns	0.90 ns	39.15 ns	70.27	4.17	1.47 ns	0.38 ns	0.44 ns	81.07	13.36	1.20 ns	1.14 ns	7.79 **	40.2
V82391	51.61	0.98 ns	1.44 ns	22.79 ns	82.66	3.32	1.35 ns	0.55 ns	0.11 ns	88.08	12.15	1.25 ns	0.34 ns	5.34 **	48.24
V82392	52.86	1.46 **	0.91 ns	21.75 ns	88.13	3.32	1.32 ns	0.37 ns	0.56 ns	74.67	12.07	1.44 ns	1.22 ns	3.33 ns	65.47
V82393	50.83	1.21 ns	0.46 ns	60.37 **	72.33	3.17	1.08 ns	0.25 ns	0.88 **	58.23	12.25	1.58 ns	-0.31 ns	3.07 ns	69.4
Overall mean	55.7	-	-	-	78.58	4.21	-	-	-	75.8	14.88	-	-	-	58.11

Under the null hypothesis $H_0: \beta_1 = 1$ and $H_0: (\beta_1+\beta_2) = 1$ and the alternative hypothesis $H_0: \beta_1 \neq 1$ and $H_0: (\beta_1+\beta_2) \neq 1$, the adaptability parameter was decomposed in favorable and unfavorable environments and then evaluated by the Student's t-test. Values with * and ** are statistically different at 1% and 5%, respectively, and ns are non-significant values. Under the null hypothesis $H_0: \hat{\sigma}_{\delta_i}^2 = 0$ and the alternative hypothesis $H_0: \hat{\sigma}_{\delta_i}^2 \neq 0$, the stability parameter was evaluated by F-test. Values with * and ** are statistically different at 1% and 5%, respectively, and ns are non-significant values. Sixteen genotypes of saccharine sorghum were analyzed in eight environments following the method proposed by Cruz et al. (1989).

stability parameters (σ^2_d and R²).

Conclusion

The method of Eberhart and Russel (1996) was efficient in identifying the genotype BR511 as a cultivar for ethanol production. Considering each trait individually, the following superior genotypes were also identified: CMSXS644, CMSXS647 and Sugargraze for the trait GMP; CMSXS629 CMSXS630, CMSXS646, CMSXS647, BRS 508, BRS 509 and CV 198 for the trait TBH; and

CMSXS629, CMSXS630, CMSXS643, CMSXS646, BRS 506 e BRS 509 for the trait TSS. The method of Cruz et al. (1989) was not appropriate to identify the superior genotypes to be recommended for ethanol production. The compared methodologies were non-complementary, then the combined use of them is not recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of

interests.

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SUPPLEMENTARY TABLES**Table S1.** Average of green mass production (GMP), *, in t ha⁻¹, of 16 cultivars of sweet sorghum grown in five environments, in 2013/2014 season in Brazil.

Genotype	Enviroment										Mean						
	S. Vitória	Sete Iagoas	Lavras	N. Porteirin	Piracicaba	Sinop	Planaltina	Dourados									
CMSXS629	52.00	B c	49.49	B b	62.12	A b	54.72	B a	44.29	B b	42.25	B b	37.27	B a	73.31	A a	51.93
CMSXS630	47.25	B c	50.99	B b	92.19	A a	57.78	B a	37.66	C b	53.31	B a	39.31	C a	48.89	B b	53.42
CMSXS643	47.57	C c	63.35	B a	88.56	A a	54.96	B a	33.56	C b	57.01	B a	41.32	C a	61.95	B b	56.04
CMSXS644	52.75	B c	59.97	B a	76.67	A b	46.67	B a	52.52	B a	57.18	B a	39.64	B a	71.81	A a	57.15
CMSXS646	55.54	A c	57.20	A a	67.34	A b	51.64	A a	41.40	B b	51.93	A a	40.09	B a	55.46	A b	52.58
CMSXS647	54.37	B c	67.89	A a	75.73	A b	54.25	B a	48.33	B a	56.69	B a	40.16	B a	70.91	A a	58.54
BRS 506	51.40	C c	67.15	B a	85.72	A a	48.57	C a	53.16	C a	43.26	C b	45.04	C a	71.00	B a	58.16
BRS 508	47.38	B c	60.50	A a	70.83	A b	49.70	B a	41.48	B b	53.13	B a	38.98	B a	62.46	A b	53.06
BRS 509	52.88	B c	59.21	B a	86.09	A a	56.80	B a	31.84	C b	44.36	C b	35.26	C a	63.36	B b	53.73
BRS 511	60.22	B c	61.64	B a	75.38	A b	56.02	B a	41.27	C b	56.62	B a	45.71	C a	59.98	B b	57.1
CV 198	76.67	A b	56.34	B a	70.34	A b	61.13	B a	59.85	B a	44.62	C b	35.11	C a	72.45	A a	59.56
CV 568	89.78	A a	61.54	C a	74.84	B b	65.14	C a	64.48	C a	59.91	C a	43.70	D a	64.68	C b	65.51
Sugargraze	62.87	A c	61.27	A a	78.30	A b	49.48	B a	64.85	A a	46.68	B b	40.94	B a	68.78	A a	59.14
V82391	49.57	B c	50.85	B b	75.61	A b	46.59	B a	54.63	B a	39.05	C b	30.93	C a	65.68	A b	51.61
V82392	63.65	A c	51.52	B b	72.23	A b	51.25	B a	35.03	C b	40.44	C b	31.71	C a	77.04	A a	52.86
V82393	71.67	A b	45.55	B b	65.81	A b	48.06	B a	30.94	C b	45.11	B b	35.06	C a	64.41	A b	50.83
Means	58.47	- -	57.78	- -	76.11	- -	53.3	- -	45.96	- -	49.47	- -	38.76	- -	65.76	- -	55.7

*Means followed by the same lowercase and same capital letters do not differ at same column and row, respectively, by Scott Knott test at 5% probability.

Table S2. Average of tons of brix per hectare (TBH)*, in t ha⁻¹, of 16 cultivars of sweet sorghum grown in five environments, in 2013/2014 season in Brazil.

Genotype	Enviroment										Mean						
	S.Vitória	Sete Iagoas	Lavras	N. Porteirin	Piracicaba	Sinop	Planaltina	Dourados									
CMSXS629	3.74	B c	5.95	A b	4.38	A a	4.61	A a	5.06	A a	2.52	B b	3.89	B a	5.22	A a	4.42
CMSXS630	4.40	B c	5.36	A b	6.22	A a	3.49	B a	4.00	B b	3.70	B a	3.96	B a	3.43	B a	4.32
CMSXS643	4.25	B c	7.13	A a	5.89	A a	2.98	B a	3.38	B b	4.30	B a	4.32	B a	3.95	B a	4.53
CMSXS644	3.84	B c	5.63	A b	4.70	A a	2.67	B a	4.79	A a	2.40	B b	3.57	B a	4.39	A a	4.00
CMSXS646	5.56	A b	6.79	A a	4.75	B a	3.93	B a	4.75	B a	3.87	B a	3.98	B a	3.81	B a	4.68
CMSXS647	4.80	B c	6.63	A a	4.74	B a	4.04	B a	5.60	A a	3.68	B a	3.37	B a	3.71	B a	4.57
BRS 506	4.44	B c	7.27	A a	5.58	A a	2.31	C a	5.95	A a	2.18	C b	4.35	B a	4.51	B a	4.57
BRS 508	3.37	B c	6.53	A a	4.89	B a	3.28	B a	4.34	B a	3.68	B a	4.03	B a	4.51	B a	4.33
BRS 509	5.25	A b	6.49	A a	5.85	A a	3.55	B a	3.40	B b	2.81	B a	3.48	B a	3.91	B a	4.34
BRS 511	5.20	B b	6.96	A a	5.15	B a	4.27	B a	4.49	B a	3.95	B a	4.67	B a	4.02	B a	4.84
CV 198	4.51	A c	5.66	A b	4.87	A a	3.64	B a	6.21	A a	2.14	B b	3.14	B a	4.58	A a	4.34
CV 568	7.23	A a	5.16	B b	4.56	C a	3.95	C a	5.30	B a	2.76	C a	3.51	C a	3.29	C a	4.47
Sugargraze	4.08	A c	5.67	A b	5.03	A a	3.03	B a	6.22	A a	1.21	C b	3.63	B a	4.46	A a	4.17
V82391	3.39	A c	4.80	A b	4.41	A a	2.39	B a	4.68	A a	0.82	B b	2.19	B b	3.87	A a	3.32
V82392	4.86	A c	4.48	A b	4.50	A a	2.36	B a	3.16	A b	0.99	B b	1.72	B b	4.50	A a	3.32
V82393	5.57	A b	4.24	B b	3.67	B a	2.74	C a	2.49	C b	1.13	C b	2.09	C b	3.47	B a	3.17
Means	4.65	- -	5.92	- -	4.95	- -	3.33	- -	4.61	- -	2.63	- -	3.49	- -	4.10	- -	4.21

* Means followed by the same lowercase and same capital letters do not differ at same column and row, respectively, by Scott Knott test at 5% probability.

Table S3. Average of total soluble solids (TSS) in °Brix, of 16 cultivars of sweet sorghum grown in five environments, in 2013/2014 season in Brazil.

Genotype	Enviroment																		Mean						
	S.Vitória		Sete Iagoas		Lavras		N. Porteirin		Piracicaba		Sinop		Planaltina		Dourados										
CMSXS629	15.07	A	b	17.37	A	a	17.07	A	a	16.97	A	a	18.22	A	a	13.87	A	a	14.45	A	a	14.25	A	a	15.91
CMSXS630	18.80	A	a	15.23	A	a	16.57	A	a	15.90	A	a	17.05	A	a	17.03	A	a	14.15	A	a	14.10	A	a	16.10
CMSXS643	17.87	A	a	16.63	A	a	16.07	A	a	14.90	A	a	16.42	A	b	18.13	A	a	14.44	A	a	12.78	A	a	15.90
CMSXS644	14.63	A	b	14.03	A	b	14.97	A	a	15.60	A	a	15.18	A	b	14.27	A	a	12.83	A	a	12.21	A	a	14.22
CMSXS646	20.03	A	a	17.67	A	a	18.03	A	a	18.37	A	a	19.94	A	a	16.33	B	a	14.11	B	a	13.74	B	a	17.28
CMSXS647	17.60	A	a	13.73	B	b	14.53	A	a	15.43	A	a	18.66	A	a	16.20	A	a	11.33	B	b	10.58	B	a	14.76
BRS 506	17.23	A	a	15.63	A	a	16.07	A	a	14.10	B	a	18.13	A	a	11.93	B	b	13.79	B	a	12.57	B	a	14.93
BRS 508	14.17	B	b	17.10	B	a	18.47	A	a	20.00	A	a	19.36	A	a	21.13	A	a	15.02	B	a	14.33	B	a	17.45
BRS 509	19.87	A	a	16.07	B	a	16.80	A	a	17.87	A	a	19.36	A	a	14.90	B	a	13.97	B	a	12.44	B	a	16.41
BRS 511	17.47	A	a	17.80	A	a	17.00	A	a	17.03	A	a	20.25	A	a	17.97	A	a	14.26	B	a	13.41	B	a	16.90
CV 198	11.87	B	b	14.97	B	b	18.13	A	a	16.23	A	a	17.68	A	a	16.97	A	a	13.39	B	a	12.70	B	a	15.24
CV 568	16.03	A	b	12.00	B	b	14.40	A	a	15.53	A	a	13.54	A	b	11.00	B	b	12.27	B	a	10.18	B	a	13.12
Sugargraze	12.97	B	b	13.47	B	b	16.87	A	a	16.13	A	a	15.87	A	b	6.57	C	c	12.02	B	a	12.95	B	a	13.36
V82391	14.10	A	b	13.77	A	b	14.43	A	a	12.90	A	a	14.24	A	b	5.73	C	c	9.97	B	b	12.03	A	a	12.15
V82392	15.20	A	b	12.73	A	b	14.90	A	a	12.07	A	a	14.65	A	b	7.63	B	c	7.62	B	b	11.72	A	a	12.07
V82393	15.30	A	b	13.67	A	b	13.23	A	a	15.93	A	a	13.25	A	b	7.10	B	c	8.58	B	b	10.91	B	a	12.25
Means	16.14	-	-	15.12	-	-	16.10	-	-	15.94	-	-	16.99	-	-	13.55	-	-	12.64	-	-	12.56	-	-	14.88

*Means followed by the same lowercase and same capital letters do not differ at same column and row, respectively, by Scott Knott test at 5% probability.

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