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African Journal of Agricultural Research

Full Length Research Paper

Responses of biofortified common bean varieties to di-ammonium phosphate fertilizer under climate variability conditions in South-Kivu, DR Congo

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Two trials with the combined effects of seeding density and application of DAP fertilizer on the yield of four biofortified bean varieties were carried out in Kashusha on the experimental site of the Université Evangélique en Afrique during the 2013-2014 agricultural long rains season (September-January) and short rains season (March-May). The overall objective was to investigate the response of biofortified common bean and its response to seeding density, mineral fertilizer application, and good agricultural practices. Four bean varieties were used (RWK-10, HM 21-7, CODMLB001 and RWR2245) and the experimental design was split-split plot whose main plot was occupied by varieties and sub-plots had DAP fertilizer crossed with two seeding spacing. Two seeding densities were considered, 250,000 plants ha^{-1} and 500,000 plants ha^{-1} respectively corresponding to the spacing between plants 40 cm x 20 cm (commonly used) and 20 cm x 20 cm. The plot was divided into 3 blocks (or repetitions), distant of 2 m and each comprising two sub-blocks separated by 1 m. A sub-block was treated with fertilizer (DAP) and the other was kept as control. The yield of bean seeds has varied from one season to another independently of varieties, which had no effect. It was better in long rains season (1725 kg ha^{-1}) than in short rains season (1087.4 kg ha^{-1}). The effect of fertilizer was also dependent on the season, no effect was observed in short rains season, with huge poorly distributed rains. However, in long rains season, the application of DAP has produced a high number of pods per plant and large grains leading to a higher yield of 1368.6 kg ha^{-1} when the fertilizer was applied compared to control which produced only 806.1 kg ha^{-1}. During the same season, doubling the density, a yield of 1842.9 kg ha^{-1} was obtained compared to the density of 250,000 plants ha^{-1} that produced 1607.1 kg ha^{-1}. The agronomic efficiency of fertilizer was low, ranging from 1.53 for the CODMLB001 variety to 5.64 for HM21-7 variety. Multi-local trials, in contrasting environments are needed to better understand the influence of the season on the bean behaviour in dissemination in South Kivu. It is also interesting to test other seeding densities, fertilizer types and doses for adequate recommendation of fertilization of this crop in Kabare territory. Due to low soil pH, it should be important for example to apply lime before applying the fertilizer.

Key words: Bean, seedling density, Di-ammonium phosphate (DAP), variety, yield

INTRODUCTION

Given their high protein content in the dry seed and their symbiotic fixation of atmospheric nitrogen capacity, food legumes are an essential component of cropping systems in the tropics. Unfortunately, these plants are often characterized by low and unstable yields, particularly because of their sensitivity to diseases, pests and low fertility of tropical soils on which they are grown in most of the cases (Baudouin, 2001). Bean, main legume encountered in the Great Lakes Region, is a plant requiring a minimum of nitrogen and optimum phosphorus for both good symbiotic atmospheric nitrogen fixation and to provide a good yield. In South-Kivu, bean is often sown at 40 cm x 20 cm spacings, or 12 plants per m^2, while densities of 15-40 plants per m^2 are possible for bush beans (Carburet and Hekimian, 2009). The low density used in South-Kivu is particularly due to soil poverty in nitrogen and phosphorus that limits the development of the plant.

For over five years, the CIAT HarvestPlus project disseminates bio fortified bean varieties with iron and zinc. The project recommends to seed-multipliers the use of 40 x 20 cm spacing and the yields range from 500 to 800 kg/ha depending on the cropping seasons without fertilizer. Carburet and Hekimian (2009) evoke yields of 1000-3000 kg/ha for growing beans in the US and Europe in intensive cropping system when fertilizers, pests control and improved varieties are used.

In traditional systems where crop association remains the rule, planting densities are relatively low. When the bean is sown with maize, it is commonly practiced at densities of 57,142 to 95,238 plants ha^{-1} against 25,000 – 40,000 ha^{-1} for maize plants (Niringiye et al*.,* 2005). Several authors have found that, in many crops, significant increases in yields are obtained by increasing seeding densities (De Bon et al., 1990; Pasquet and Baudoin, 1997; Nyabyenda, 2005), provided of course that soil fertility allows meeting the nutrient demand of plants. A study by Taffouo et al. (2008), in Cameroon, shows that seeding densities significantly affect stem collar diameter, number of leaves, leaf surface and dry matter produced in different varieties of cowpea.

Niringiye et al. (2005), on their part, have shown that increasing densities of planting climbing beans does not affect, in most varieties, the number of pods per plant, but it reduces the average weight of grains and the total quantities of grains produced per plant.

In Africa, low seeding densities are often justified by the low soil fertility, the non-use of fertilizers and the use of low-yield varieties. This poor soil fertility limits the availability of agricultural resources and led to low agricultural productivity (McCan, 2005). In this region, farmlands are lost due to their poor management, causing average annual losses of topsoil in N, P and K, respectively 22, 2.5 and 15 kg ha^{-1} (Steiner, 1996).

Thus, for better management of land resources, it is imperative to measure the initial soil fertility for its ability to provide essential nutrients for crops development (Bado et al., 2003). Mineral fertilizers play in this respect, an important role in restoring soil fertility strategy.

This study consists at identifying the combined effects of seeding density and application of DAP on 4 biofortified bean varieties performance in Kashusha during two contrasting cultural seasons.

The main research question being whether it would be possible to increase biofortified bean yields by applying DAP fertilizers and doubling the seeding density. The second question is to test whether the effects of DAP and seed density would change from one variety to another and depending on the growing season. The main purpose of this study was to evaluate the interaction between fertilizers, plants densities and varieties in order to improve biofortified common bean yield in a contrasting climate environment. It is hypothesized that increasing seeding density with DAP application will significantly increase biofortified bean yields.

METHODOLOGY

Study area

A first experiment was conducted in Kashusha on the site of the Université Evangélique en Afrique (UEA) in the short rains season (SRS), from March to May 2013. The second experiment was conducted in the long rains season (LRS), from September 12, 2013 to January 3, 2014. Kashusha is located in Kabare territory, 28 km from Bukavu city. The experimental plot was installed at an altitude of 1712 m, 028˚47'72'' latitude East and 02˚190 '05'' longitude South. Climate data during the trial period are those of the INERA-MULUNGU meteorological station, the closest station to the site. During the LRS, 163.1 mm was recorded in September for 14 days; 136.5 mm in October for 13 days, 148.6 mm for 15 days in November and finally huge amounts of rainfall were recorded in December with 214.1mm in 20 days (Table 1). The optimum rainfall of bean crop varies between 300 and 600 mm of rain for a suitable production (De Bon et al*.,* 1990). In addition to a good amount of rain, bean crop requires a good distribution of the latter. For optimal development, beans require 80-120 mm monthly precipitation from sowing to maturation (Baudouin, 2001). Thus, referring to the optimum rainfall for bean cultivation, the amount of rainfall recorded during the LRS (662.3 mm) are favourable for good bean productivity, while those recorded during the SRS shifted from the optimum (827.5 mm).

The soil of the experimental site is ferrallitique type, clay and humus on basaltic rocks. It had a pH of 4.4, a carbon percentage of 1.76%, a nitrogen content of 0.26%, a C/N ratio of 11 and a cation

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Table 1. Mulungu climate data, 2013 and 2014.

Month	Rainfall (mm)	Number of rain days	Observation
SRS 2013			
Feb	173.1	02	Rainy
March	362.6	18	Rainier
April	227.4	15	Very rainy
May	63.5	08	Rainy
Total	**827.5**	**44**	
LRS 2014			
September	163.1	14	Rainy
October	136.5	13	Rainy
November	148.6	15	Rainy
December	214.1	20	Very rainy
January 2014	220.8	20	Very rainy
Total	**662.3**	**62**	

exchange capacity of 24.31 cmol/kg. Soil pH was too acidic, away from bean requirements, which normally prefers a pH ranging from 6.0 to 7.5 with critical thresholds of 5.0 to 8.1; then, it probably would have been necessary to make a liming (Baudouin, 2001). The carbon content is acceptable; being above the recommended threshold of 0.8 by Baudouin (2001) while the amount of phosphorus is less than the optimum, estimated at 15 ppm (Godderis, 1995). This low proportion of phosphorus can inhibit root development, flower induction and pods development.

The nitrogen content (0.26%) is below the 0.3% threshold recommended in bean cultivation, though bean cultivation is not too demanding in nitrogen because of its ability to fix atmospheric nitrogen (Baudoin et al*.,* 2001) a minimum of nitrogen is required in the soil for proper development of the plant. The Kashusha soil has good organic carbon content, which is 11, the acceptable optimum being between 9.0 and 12.0 (Godderis. 1995). The C/N ratio is good; it indicates the level of mineralization of organic matter but also the accumulation of ammonium ion. The cation exchange capacity (24.31 cmol/kg) is between the recommended limits, between 10 and 25.

Materials

In this study, four biofortified common bean varieties were used namely: CODMLB-001, HM21-7, RWR2245 and RWK-10) provided by CIAT-HarvestPlus. The four varieties used have equivalent yield potential ranging from 800 - 1500 kg per hectare when suitable environmental conditions are met. Multiplication tests made previously by CIAT/ HarvestPlus and the Université Evangélique en Afrique on the same site gave yields between 500 and 800 kg/ha without fertilizer application. As fertilizers, DAP (Di Ammonium Phosphate) was used dosing 18% nitrogen, 46% P_2O_5 (18-46-0). A dose of 100 kg/ha was applied.

Experiment running

A device in "split-split-plot" has been used with the main plot being the variety (4 different varieties) and sub-plots had DAP fertilizer crossed with two spacing, 40 x 20 cm (commonly used) and 20 cm x 20 cm, giving densities of 250,000 plants ha^{-1} and 500,000 ha^{-1} plants respectively. The spacing of 40 cm x 20 cm is considered as reference and in application in the rural environment.

The plot was divided into 3 blocks (or repetitions), distant from each other of 2 m, each including two sub-blocks distant of 1 m each other; a sub-block has been treated by fertilizer (DAP) and the other was kept as a control to fertilizer response. Each block contained 16 plots (treatments) with 8 plots per sub-block; the plot area was 17.5 m^2, a length of 5 m and a width of 3.5 m. The number of rows per plot planted with spacing of 40 cm x 20 cm was 12 and that planted with spacing of 20 cm x 20 cm was 24. The trial covered a total area of 47 m x 28 m or 1316 m^2.

The DAP was applied to half of the experimental plot as dressing manure at a dose of 0.2 kg per plot corresponding to 100 kg of DAP per hectare or 0.036 kg of nitrogen and 0.092 kg of P_2O_5 per plot, 20.6 kg of nitrogen and 52.6 kg of P_2O_5 per hectare.

The maintenance consisted of two manual weeding for each trial. The first weeding occurred 26 days after planting when the crop was at the stage of pre-flowering and the second took place 57 days after sowing at the stage of pods development. During weeding, we determined the time taken to weed each plot according to the seeding density.

During the experimental period, the parameters related to vegetative development and production were measured. For vegetative parameters we estimated the germination rate (in percentage), which was obtained by a ratio of the number of germinated plants in each plot and the number of seeds sown. The emergence rate was evaluated on Tuesday, October 8, 2013; 13 days after sowing when over 50% of seeds had already sprouted.

The chlorophyll content was measured using a Konica Minolta chlorophylometer, SPAD 502, when the bean was at the pre-flowering stage. The measurements were taken from 10 plants of two diagonals of each plot. On each plant, we performed measurements on 3 different leaves per plant and the average was determined.

At the final harvest, which occurred 100 days after sowing, respectively, we determined the number of pods per plant, number of grains per pod and the weight of 100 grains. The number of pods per plant was estimated on ten plants taken at random per plot to determine the mean of each treatment. The number of grains per pod was obtained by counting the grains in each of 10 pods randomly taken from the lot of pods harvested from each plot.

To determine the weight of grains, 100 grains were taken randomly from the harvest on each plot, they were then weighed with a precision scale, Scout Pro 2000 g. Finally, the harvest obtained in each useful plot of 17.5 m² was extrapolated to the hectare.

The harvest index was calculated in % for each treatment by

Table 2. Analysis of the combined variance of the yield components of four bean varieties subjected to two seeding densities and DAP fertilization during two growing seasons.

Source of variation	df	Chlorophyll		Pods		Grains/pod		W100G		Yield	
		CM	**Prob**	CM	**Prob**	CM	**Prob**	CM	**Prob**	CM	**Prob**
Season (S)	1	155.2	0.077	222.7	**0.001**	11.5	**0.027**	12.76	0.542	9.75	**0.025**
Varieties (V)	3	88.22	**< 0.001**	14.41	**0.032**	1.3	0.057	39.52	0.29	0.52	0.605
Sx V	3	23.77	**0.01**	12.94	**0.043**	2.52	**0.008**	70.34	0.11	0.7	0.491
Fertilizer Dose (D)	1	58.68	**0.02**	36.5	**< 0.001**	0.89	**0.033**	131.7	**< 0.001**	2.14	**< 0.001**
S x D	1	19.31	0.162	3.68	0.036	0.41	0.133	32.14	**0.001**	1.66	**0.001**
V x D	3	4.72	0.671	2.44	**0.041**	0.06	0.756	4.69	0.118	0.07	0.595
S x V x D	3	7.61	0.489	1.34	0.17	0.21	0.316	20.95	**< 0.001**	0.08	0.54
Spacing ('E)	1	4.56	0.318	5.51	0.098	0.01	0.798	8.74	0.278	1.35	**0.02**
S x E	1	4.79	0.307	6.51	0.073	0	0.844	11.32	0.218	0.002	0.985
V x E	3	1.75	0.757	0.85	0.72	0.12	0.342	6.15	0.473	0.03	0.938
D x E	1	0.27	0.805	2.53	0.257	0.48	**0.046**	2.31	0.574	0.002	0.975
S x V x E	3	8.13	0.161	1.75	0.442	0.09	0.501	18.97	0.066	0.067	0.828
S x D x E	1	0	0.998	3.22	0.202	0	0.954	0.49	0.794	0.052	0.633
V x D x E	3	0.78	0.911	0.01	0.999	0.12	0.342	1.07	0.929	0.009	0.989
S x V x D x E	3	9.59	0.112	0.38	0.894	0.33	**0.046**	1.2	0.917	0.023	0.956
Residuals	32	4.44	-	1.9	-	0.11	-	7.18	-	0.22	-
CV (%)		5.9		24.3		8		7		33.9	

df: degree of freedom; CM : Mean Square; Proba: Probability of significance; NGP: Number of pods per plant; NGG: Number of seeds per pod; W100G: Weight of 100 seeds. Bold probabilities are significant.

dividing the plot grain yield by the total biomass (Donald, 1962). The agronomic efficiency (AE) was calculated using the following equation: AE = (Y1-Y2) / f. Y1, being the yield obtained by applying fertilizer; Y2, yield obtained without fertilizer and f the amount of fertilizer applied per hectare.

Statistix 8.0., Excel and Genstat softwares were used to analyze data. The analysis of variance was used to reveal differences between treatments and interactions, while the LSD test was used for comparison of mean pairwise at 5% significance level.

RESULTS

Effect of season on yield parameters

The analysis of variation, showed a significant effect of the season ($p = 0.025$) on the observed parameters (Table 2). The yield varied significantly from one season to another, the best production was obtained in LRS compared to the SRS (Table 3).

In LRS the number of pods per plant and number of grains per pod was high compared to the SRS, which has seen uneven rainfall, which were abundant compared to the needs of the crop. The effects of fertilizer dose varied very significantly from one season to another ($p \leq 0.001$) as shown in Figure 1.

With or without fertilizer application, the yield was high in LRS compared to SRS (Table 3). Under the control plots, LRS the yield was 1707.2 kg/ha against 806.1 kg/ha in SRS. With the application of fertilizer, the yield in LRS was 1742.9 6 kg/ha against 1368.6 kg/ha in SRS (table 4).

The spacing, in the other hand, has had a significant effect on yield ($p \leq 0.02$) regardless of season because no interaction is observed for both seasons ($p \leq 0.985$). The spacing of 20x20 cm gave the best yield. Contrary to our initial hypothesis, fertilizer did not interact (Annex 1, $p \leq 0.975$) with bean seeding density, 100 kg dose of DAP. Ha^{-1} is probably not at optimum when applying a 20x20cm planting density.

Effect of variety

In LRS, the HM21-7, RWR2245 varieties gave the best chlorophyll rate (39.5 and 38.6 mg/kg) and higher yields that were respectively 1952.4 and 1895.2 kg/ha. Both varieties also had higher harvest indices 57 and 59% respectively for HM21-7 and RWR2245. The variety CODMLB001 produced the lowest yield (1319.2 kg/ha), which was statistically comparable to the variety RWK10 (1733.3 kg/ha). Both varieties also had the weakest harvest index (HI) but comparable. They were respectively 36.3% for RWK10 and 46.4% for CODMLB001. The low HI for RWK10 is explained by a greater production of foliage biomass than the other three varieties. This variety also gave a lot of grains per pod but low weight.

In SRS, however, no varietal effects were observed; all four varieties have produced comparable grain yields

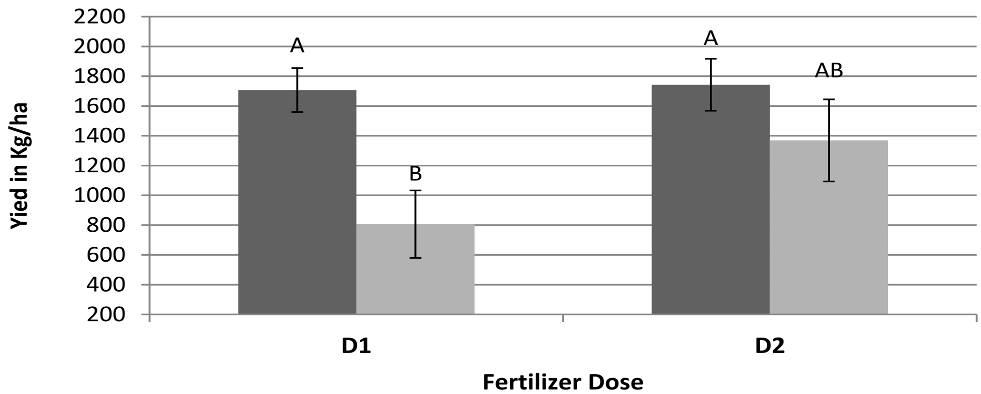

Figure 1. Effect of fertilizer dose interaction with the cropping season on bean yield (LSD = 440), error bars represent standard deviations of the mean. D1=0 $kg.ha^{-1}$ while D2 =100 $kg.h^{-1}$ of DAP fertilizer For each dose, left chat represents Long Rains Season (LRS) and right chat is Short Rains Season (SRS).

Table 4. Average effect of the studied factors (fertilizers. seeding density and varieties) on bean yield parameters in two different growing seasons.

Principal factors		Chlorophyll content (mg/g)	Pods	Grains $pods^{-1}$	W100G (g)	Yield (kg/ha)	Biomass (kg)	IR (%)
				Long rains season (LRS)				
	D1	36.6^a	6.8^b	4.5^a	37.2^b	1707.2^a	6.2^a	50.1^a
Fertilizer dose	D2	37.3^a	7.6^a	4.6^a	38.4^a	1742.9^a	6.4^a	49.3^a
	LSD	1.83	0.57	0.26	0.74	80	0.66	4.52
	E1	37.4^a	7.7^a	4.5^a	38.5^a	1607.1^b	5.8^b	50.6^a
Spacing	E2	36.5^a	6.7^b	4.5^a	37.2^b	1842.9^a	6.9^a	48.9^a
	LSD	*1.45*	*0.98*	*0.24*	*1.16*	*220*	*0.63*	*3.84*
	CODMLB	36.5^b	5.8c	4.3^b	38.8^a	1319.2^b	5.0^b	46.4^b
	HM21	39.5^a	7.3^b	4.2^b	38.7^{ab}	1952.4^a	6.1^b	57.0^a
Varieties	RWK10	33.1c	8.6^a	5.3^a	36.0^b	1733.3^{ab}	8.5^a	36.3^b
	RWR2245	38.6^a	7.2^b	4.3^b	37.7^{ab}	1895.2^a	5.6^b	59.1^a
	LSD	1.72	1.19	0.45	2.71	470	1.84	10.26
				Short rains season (SRS)				
	D1	33.2^b	3.3^b	3.7^b	36.8^b	806.1^b		
	D2	35.6^a	5.0^a	4.0^a	40.3^a	1368.6^a		
Fertilizer dose	*LSD*	2.14	0.54	0.27	1.12	290		
	E1	34.4^a	4.1^a	3.8^a	38.5^a	967.7^a		
	E2	34.4^a	4.2^a	3.8^a	38.6^a	1207.0^a		
Spacing	*LSD*	1.09	0.67	0.15	2.00	340		
	CODMLB	33.3^a	3.4^a	3.8^a	36.3^a	1134.7^a		
	HM21	35.5^a	5.5^a	4.0^a	41.0^a	1223.3^a		
	RWK10	33.5^a	3.4^a	3.7^a	40.8^a	1103.5^a		
	RWR2245	35.2^a	4.3^a	3.8^a	36.0^a	888.0^a		
Varieties	*LSD*	2.31	2.36	0.76	6.99	1180		

NGG = Number of seeds per pod; NGP: Number of pods per plant; W100G: Weight of 100 grains; IR: harvest index; D1: Without fertilizers; D2: 100 KG Contribution of DAP per hectare; CV: Coefficient of variation; a. b. c: The average of the same column and the same factor followed by the same letters are not statistically different at a probability level of 5% according to LSD test (Least Significant Difference).

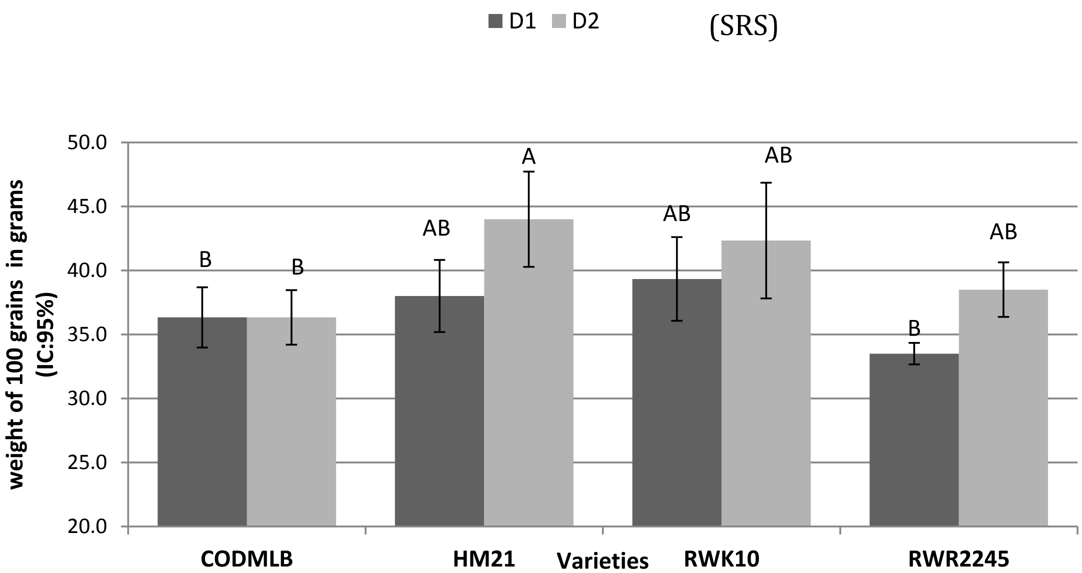

Figure 2. Effect of fertilizer dose on the weight of 100 grains of four bean varieties (SRS; Short Rains Season, LSD = 7.01). The error bars represent the standard deviations on the mean. D1=0 kg.ha^{-1} while D2 =100 kg.h^{-1} of DAP fertilizer.

varying from 888 kg/ha for RWR2245 to 1223.3 kg/ha for HM21-7. These yields were statistically low compared to those obtained in LRS, because of the abundant rainfall observed in SRS during the vegetative growth phase. This is a consequence of climate disruption, because in the Kashusha conditions, usually the rainiest season is the SRS.

Effect of seeding density

As indicated in Table 4, in LRS, sowing density influenced the number of pods per plant, grain weight, total biomass and definitely grain yield. The spacing of 20 x 20 cm was better compared to 40 x 20 cm. Its yield was 1842.9 kg/ha while the 40 x 20 cm spacing gave 1607.1 kg/ha. The spacing of 20 x 20 cm produced a reduced number of pods per plant as well as low weight of grains. The observed difference from yield point of view is primarily attributed to the number of harvested plants per unit area varying from the simple (12 plants/m^2) to twice (24 plants/m^2) as a function of the spacing.

In SRS, once more, the study factor, here the seeding density, has not affected all yield parameters.

Effect of DAP application

In LRS, though DAP application has slightly influenced the number of pods per plant and grain weight, in final, grain yield has not changed. It looks like that it was more dependent on the number of grains by pods. An interaction between factors under observation might also explain the results.

In SRS however, the application of 100 kg DAP fertilizer allowed a seed yield (1368.6 kg/ha) which was higher than the control (806.1 kg/ha). The fertilizer has positively influenced all yield parameters including grain weight (Figure 2) and the number of pods per plant (Figure 3).

Figure 2 shows that with the addition of 100 kg of DAP fertilizer, the HM21-7 and RWR2245 varieties gave the best grain weight in SRS. Figure 3 shows that with the addition of 100 kg of the DAP fertilizer, HM21-7 varieties, RWK10 and RWR2245 gave a high number of pods per plant in SRS. To get an idea on the ability of the different varieties to capitalize applied fertilizer to different densities, we computed the agronomic effectiveness of the fertilizer. The results are shown in Table 5.

At 40 cm x 20 cm spacing, HM21-7 variety produces 4 kg of grains for each application of 1 kg of DAP per hectare, and at the spacing of 20 cm x 20 cm each kg of DAP provides 5 kg of dry bean grains. The performance of the HM21-7 variety is seasonal; it is low and even negative in LRS for the density of 12 plants/m^2. It seems that when rainfall is at optimum and well distributed during the crop cycle, then it is not necessary to apply a dose of 100 kg of DAP. The low agronomic efficiency of fertilizer is observed for the CODMLB001 variety for which 1 kg of DAP applied gave on average 1.53 kg of dry grains for the density of 24 plants/m^2. Nevertheless, although the density of 24 plant/m^2 gave good results, it increases the necessary time spent on weeding. Indeed, on average 1 h 13 min were needed to weed a plot of 17.5 m^2 sown at the spacing of 20 x 20 cm against 40 min when the spacing was 40 x 20 cm. The high density

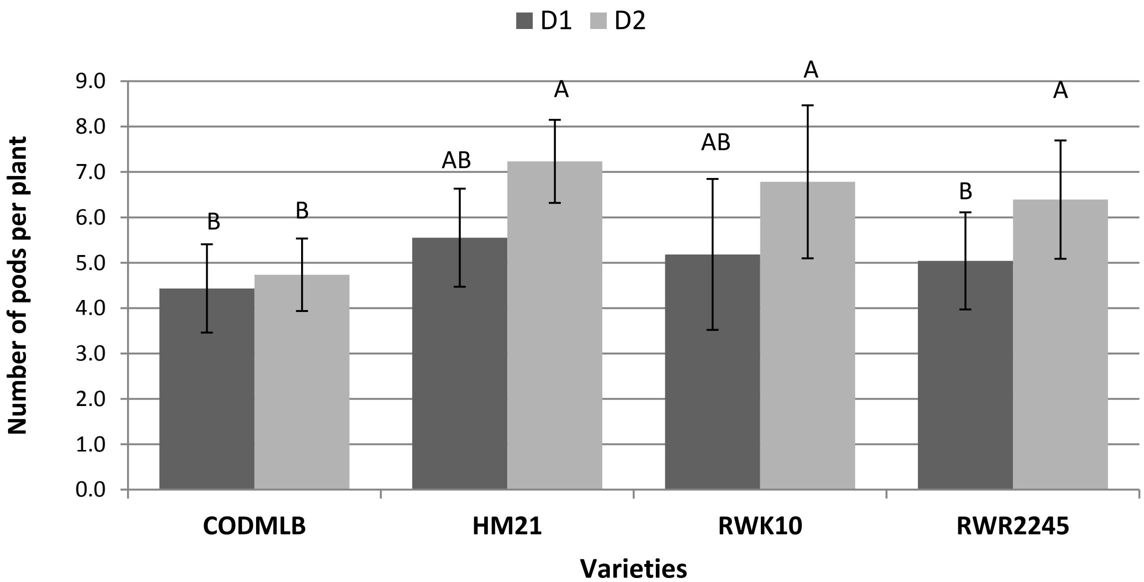

Figure 3. Effect of fertilizer dose on the number of pods per plant for four bean varieties (LSD = 1.25). D1=0 kg.ha^{-1} while D2 =100 kg.h^{-1} of DAP fertilizer. The error bars represent the standard deviations of the mean.

Table 5. Agronomic effectiveness of fertilizer.

Sowing density	Varieties	Seasons		Average
		LRS	SRS	
12 plants/m^2	HM21-7	-0.95	9.00	4.02
	RWK10	1.52	4.65	3.09
	RWR2245	-1.52	6.27	2.37
	CODMLB001	0.38	4.34	2.36
24 plants/m^2	HM21-7	3.05	8.23	5.64
	RWK10	0.38	5.25	2.81
	RWR2245	0.76	4.78	2.77
	CODMLB001	0.57	2.49	1.53

LRS: Long rains Season, SRS: Short Rains Season.

not only induces an additional cost in terms of seed and planting work but it also increases the weeding time.

DISCUSSION

Season has influenced all bean yield parameters

Regardless of the varieties, the yield of bean grains varied from one season to another. It was high in the LRS in which rainfall was well distributed and was close to the optimum required for beans cultivation. In fact, the optimum rainfall on bean crop varies between 300 and 600 mm of rain for a good production (De Bon et al*.,* 1990).

In addition to a good amount of rain, bean requires a good distribution of the latter. For optimal development, beans require 80-120 mm monthly precipitation from sowing to maturation (Baudouin*,* 2001). Thus, the amount of rainfall recorded during the growing LRS (662.3 mm) supported the good bean productivity; while those recorded during the SRS were shifted from the optimum (827.5 mm) and were poorly distributed. Leading to a fall of flowers and a seedling rot in some varieties like CODMLB001 in which the agronomic efficiency of fertilizer was low. These results are in agreement with Bildirici and Yilmaz (2005), who showed that the yield in bean grains varies depending on seasons and the average of all climatic factors such as temperature as well as rainfall amount and distribution.

All the four varieties were affected in the same way by heavy rainfall in SRS, yields were low and comparable, ranging from 888 kg/ha for RWR2245 to 1223.3 kg/ha for HM21-7. However, in LRS, HM21-7 and RWR2245 varieties gave the best yields, respectively 1952.4 and 1895.2 kg/ha. CODMLB001 and RWK10 varieties produced the lowest yields (1319.2 and 1733.3 kg/ha). Indeed, next to nitrogen and phosphorus deficiency, soil moisture is considered to be the main factor that affects growth, nitrogen fixation and yield in most grain legumes (Graham et al*.,* 2003). Bean is considered to be a plant that does not tolerate high humidity of soil but also water stress, and whose performance can vary greatly from one season to another (Khan et al*.,* 2007). Our results show that, under the conditions of seasonal climate disruption, it would be interesting to have on one side, the varieties that tolerate water excess and the other side, varieties resistant to waterlogging.

Effects of fertilizer varied from season to season

The effect of fertilizer varied from one season to another, no effects were observed in SRS with large poorly distributed rains. However, in LRS, the application of DAP has produced a high number of pods per plant and large grains. This is consistent with Wery et al. (1988) and Streeter (1981), who found that; nitrogen intake has a positive effect on legume growth by increasing their ability to fix atmospheric nitrogen and thus contributing to high grains production. A strip application of 200 to 400 kg of nitrogen per hectare in some soils in Latin America showed a positive response (Schwartz et al*.,* 1978). In acid and volcanic ash soils in Colombia, a negative response occurred from a strip application over 80 kg of nitrogen per hectare during the drought; in the same soil, the application of 320 to 640 kg of nitrogen per ha gave negative results due to low pH and subsequent induction of the manganese toxicity. Further, in soils where phosphorus is the limiting factor, bean does not respond to the nitrogen until the amount of phosphorus to be applied is sufficient (Howard and Schaniel*,* 1980; Lunze et al*.,* 2002), thus, the usefulness of having brought the DAP to overcome both the lack of nitrogen as well as phosphorus. Indeed, Wortmann et al. (1998) found nitrogen and phosphorus deficiency in 93 out of 95 soils from East Africa. We should have probably also provided potassium to improve root development. In Burundi, the application of NPK at a rate of 18-46-30 kg/ha gave an average yield increase of 97% compared to the average yield without NPK fertilizer; estimated at 642 kg/ha (Godderis, 1995), the yield increases were variable depending on soil conditions, changes in rainfall and parasitism from one season to another and differences among farmers regarding the application of adequate cultivation techniques (Godderis, 1995).

Agronomic efficiency of DAP has varied from season to season and depending on the common bean variety

The agronomic efficiency of fertilizer was low, ranging from 1.53 for the CODMLB001 variety to 5.64 for HM21-7 variety. According to Dos Santos and Fageria (2007) a bean variety is considered to have responded positively to fertilizer when its agronomic efficiency (AE) is greater than 12. High AE values therefore mean a good response to the supply of fertilizer but should also be calculated. The use efficiency of the fertilizer by the plant, meaning the capacity of the variety to transfer nutrient from soil to grains (Dorvincil et al*.,* 2007). In this work, we did not measure the amount of nitrogen or phosphorus extracted from the ground by the plant; therefore, it is impossible to calculate the efficiency of utilization of nitrogen and phosphorus by the plant. The low agronomic efficiency of fertilizer could be partly explained by the high soil acidity (pH = 4) observed on our test site, which would have reduced the ability of the plant to symbiotically fix nitrogen and to collect soil nutrients (Hungria and Vargas 2000). It could also be related to the intrinsic values of the varieties used. Nodulation for example, is drastically reduced at pH=4.5 (Hungria and Vargas 2000; Streeter, 1981) and the availability of certain nutrients is reduced (Tessier, 2002). A study by Fageria et al (1989) shows that the maximum development of the bean is obtained at pH=5, leaf and root growth was positively correlated with pH and soil exchangeable aluminium. In this study and in the conditions of too acidic soil, it would be interesting to apply liming, for example, to improve soil pH before applying DAP. In acidic and in most tropical soils, only 20 to 30% of brought phosphorus is used by the plant, the rest is often attached or precipitated as non-usable form by the plant (Hissinger, 2001; Vance et al*.,* 2003).

Increasing the seeding density improved the yield of dry grain of beans

Several authors have found in many crops that significant increases in yields are obtained by increasing seeding densities (De Bon et al*.,* 1990; Pasquet and Baudoin, 1997; Nyabyenda, 2005) provided of course that the fertility of the soil makes it possible to meet the nutrient demand of plants. Niringiye et al. (2005) showed that increasing densities of climbing beans does not in most varieties affect the number of pods per plant, but it reduces the average grain weight and the amount of total grains produced per plant. In this work, increasing seeding density from 12 to 24 plants/m^2, has led to increased yields from 1287 to 1525 kg/ha, regardless of the cropping season. In contrast, Hug and Winterthur (2011) working on sunflower crop found no effect of seeding density on yield, probably because of low soil fertility. When increasing plant density and the level of fertilizers in the soil does not cover the nutritional needs of the crop there is a risk of observing a decrease in yield. Contrary to our initial hypothesis, no interaction was obtained between the increase in density and application of fertilizer, except for the number of grains per pod (P = 0.046), but unfortunately not significantly affected grain yield (P = 0.975). It is possible that in this study the dose of DAP; 100 kg/ha does not fully cover the needs of beans with increasing plant density.

Conclusion

The effect of fertilizer on common bean grain yield varied from season to season independently to variety, showing that the chemical fertilizer depends on soil water. In long rains season, the application of DAP has produced a high number of pods per plant and large grains leading to a higher yield of 1368.6 kg ha^{-1} when the fertilizer was applied compared to control which produced only 806.1 kg ha^{-1}. During the same season, doubling the density, a yield of 1842.9 kg ha^{-1} was obtained compared to the density of 250,000 plants ha^{-1} that produced 1607.1 kg ha^{-1}. The agronomic efficiency of fertilizer was low, ranging from 1.53 for the CODMLB001 variety to 5.64 for HM21-7 variety. Therefore, in the context of climate change it's very difficult to have an accurate dose of chemical fertilizer, which will lead to optimum yield without taking into account unpredictable rainfall or weather disturbance. Applying 100 kg.ha^{-1} of DAP seems to not be efficient when rainfall is not sufficient or when its distribution among cultural cycle is not conform to bean's water demand. It would be interesting to test several seeding densities and different doses of DAP fertilizer in order to determine the density and the dose that achieve an economic yield in dry grains of beans. As said earlier, it would be also interesting not only to raise the pH level with a liming before applying the DAP but in addition to compare the effect of DAP and NPK in different contrasting environmental conditions. Finally, an attention should be given to soil water management when dealing with chemical fertilizer in a context of climate change.

Conflict of Interests

The author has not declared any conflict of interest.

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Full Length Research Paper

Performance of *Peltophorum dubium* under intraspecific tree competition and cardinal directions as possibility for integrated livestock-forestry systems

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***Peltophorum dubium* can be an alternative of forestry species to be included in integrated crop-livestock-forestry systems in tropical region. The aim of this research was to evaluate the performance of *P. dubium* under intraspecific tree competition and cardinal directions to measure the possibility of introduction in integrated livestock-forestry systems. The experiment was performed in a Nelder Wheel competition, which is used in forestry research to test tree competition. The experimental design was factorial (10×2×5) with 12 replicates. The treatments were accomplished by ten tree competitions (159, 201, 255, 322, 408, 516, 653, 827, 1,046 and 1,324 trees ha^{-1}), two cardinal directions (North-South and East-West) and five times after planting (12, 18, 24, 30 and 36 months). The highest tree competition (1,342 trees ha^{-1}) in 36 months after planting, promoted increase in the cylindrical volume of the tree in both North-South and East-West cardinal directions. Nevertheless, the cardinal direction East-West was profitable to increase tree height, diameter at breast height (DBH), cylindrical volume per tree and cylindrical volume per hectare. The DBH measured in 24 months after planting was 5.0 cm, which is considered the minimum DBH to introduce livestock into the integrated system without significant damage on trees. Regarding these preliminary results in the first three years, *P. dubium* was promising as Brazilian native species to be inserted in integrated livestock-forestry system or single forestry as an option for *Eucalyptus* spp.**

Key words: Forestry, dendrometry, Nelder Wheel, integrated systems, agroforestry.

INTRODUCTION

Sustainable agricultural systems have increased through the last decade. The integrated crop-livestock-forestry systems (ICLFS) have pointed out as a quite promisor production system to be used to recover degraded pasture in Brazilian Cerrado (Almeida et al., 2013). ICLFS is defined as integrated production system that enables to have three components (crop, livestock and forestry) at the same area, with at least two components at the same time. Nevertheless, forestry is always in the area and determines the cycle of the system. ICLFS allows increase in the diversity of products at the same production area. In this ICLFS, trees species might be well defined because of its impact on crop, pasture and livestock combined in integrated systems of production.

The benefits of trees in integrated systems for animal grazing is related to better microclimate and animal thermal comfort (Karvatte et al., 2016). However, the trees can result in negative effects on grain crops yields in tree crop zone (Nasielski et al., 2015) and pasture in case of higher tree competition (Burner and Brauer, 2003). As reported by Franchini et al. (2014), decrease in soybean grain yield can be related to the age of the trees, which was not observed significant effects in the first two year of *Eucalyptus* species age; nonetheless, the decrease of soybean grain yield can achieve 27% after four years of *Eucalyptus* spp. establishment.

Nevertheless, the most common ICLF systems use *Eucalyptus* spp. as tree species because of faster growth and enables to introduce livestock sooner than native forest species; besides *Eucalyptus* spp., multiple use and high commercial value (Grossman, 2015). Even with many positive feature of *Eucalyptus* spp., the possibility to have native forestry species, as *Peltophorum dubium,* might be considers. Monocrop of *Eucalyptus* spp. is not profitable for balanced ecosystems; this way is quite relevant to have other forestry species as the alternative to maximize the diversity of the ecologic system.

As possibility of native species, *P. dubium* shows some features that have been pointed as a profitable species to introduce in ICLS with some positive features as narrow crown area (Matos et al., 2015), which increases the incidence of sunlight in crops and pasture cultivated under the trees. Soybean, rice and corn as crop components and *Brachiaria* species as pasture are profitable to be inserted in ICLFS. *P. dubium* (Spreng) is a leguminosae found in tropical seasonal semi-deciduous forest in Brazil (Lisi et al., 2008). Moreover, *P. dubium* can be found in Atlantic forest of Brazil and can reach 20 m of height and 90 cm of trunk diameter at breast height (DBH).

As reported by Lima et al. (2015), *P. dubium* is very adaptable in different regions, which may imply in different response with the environment conditions. To insert the *P. dubium* in an integrated crop-livestock-forestry system is quite important to know the growth rate, because tree height and DBH determine the moment to introduce the livestock into the integrated system of production. Faster growth shown by *Eucalyptus* spp. makes this species widely used in tropical climate in integrated systems (Grossman, 2015). However, the *P. dubium* shows higher wood basic density (0.65 g cm^{-3}) in ten-years-old (Vivian et al., 2010) in comparison to *Eucalyptus grandis* (0.52 g cm^{-3}), at the same age (Githiomi and Kariuki, 2010).

The preferable destination of wood in ICLS is for timber, as veneer wood and sawmills (Almeida et al., 2013), which turn *P. dubium* as a profitable option due to its higher wood basic density. To obtain high quality of wood from integrated system is necessary to conduct the trees with pruning and find the profitable plant density and cardinal directions to improve wood quality and growth.

The tree competition has been pointed as the variable that affects some dendrometric features. Matos et al. (2015) observed decreasing in cylindrical volume of trees in higher *P. dubium* competition (1,324 trees ha^{-1}), on the other hand, lower tree competition results in less volumetric trunk production per hectare (Folkard et al., 2012). The ideal tree competition might be found for *P. dubium* to improve the recommendation as native species to be chosen as forest component in ICLS. The aim of this research was to evaluate the performance of *P. dubium* under intraspecific tree competition and cardinal directions to be possible inference of some dendrometric parameters to assess the viability of inserting the native species in integrated livestock-forestry systems.

MATERIALS AND METHODS

Location of the experiment

The experiment was carried out from November 2010 to November 2013 on the experimental field of Brazilian Agricultural Research Corporation (Embrapa Western Agriculture), followed by geographic coordinates, 22°33'07'' S, 55°38'37'' W, and average altitude 496 m, the experimental area belongs to the municipality of Ponta Porã, state of Mato Grosso do Sul, Brazil. The weather condition is classified as Aw Köppen-Geiger (Fietz, 2008), with rainy summer and dry winter. The average rainfall and temperature in the region of the experimental is shown in Figure 1.

Soil physical and chemical properties

The experimental site topography was under 5.0% of slope. The soil of the study area was classified as dystroferric Red Latosol, according to Santos et al. (2013), the landscape originally covered

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Abbreviations: DBH, Diameter at breast height; **TH,** tree height.

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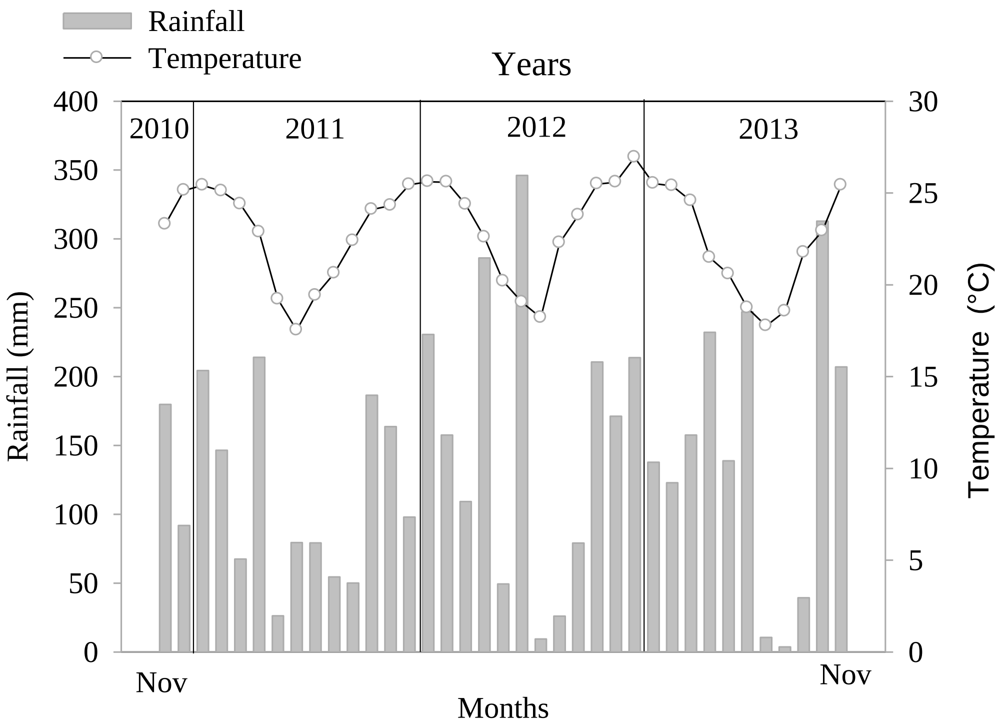

Figure 1. Monthly average rainfall and temperature in the period from November 2010 to November 2013.

Table 1. Some initial soil physical and chemical properties from the experimental area.

Soil properties	Depth (cm)	
	0-10	10-20
pH ($CaCl_2$)	4.42	4.26
SOM (g kg^{-1})	31.78	28.27
CEC ($cmol_c$ dm^{-3})	4.43	3.49
P (mg dm^{-3})	16.86	7.03
K^+ ($cmol_c$ dm^{-3})	0.37	0.21
Ca^{2+} ($cmol_c$ dm^{-3})	2.40	1.46
Mg^{2+} ($cmol_c$ dm^{-3})	0.95	0.53
H+Al ($cmol_c$ dm^{-3})	10.65	11.46
BS (%)	3.72	2.19
Clay (%)	54.9	57.0
Silt (%)	8.68	8.70
Sand (%)	36.43	34.30

SOM: Soil organic matter; CEC: cation exchange capacity; total acidity pH 7.0 (H^+ $+Al^{3+}$); Exchangeable Ca and Mg (KCl 1 mol L^{-1}); BS: Base Saturation=(∑cations/CEC)x100.

by savanna (Brazilian Cerrado biome). The values of soil physical and chemical properties were obtained at 0 to 10 and 10 to 20 cm depth, as the following results in Table 1. These analyses were accomplished at the Laboratory of Soil Fertility from Embrapa Western Agriculture.

Implementation of the experiment

The whole area of the experiment was 1.33 hectares, defining according to the Nelder Wheel design. The soil tillage was carried out with heavy harrow and leveling harrow, followed by the planting

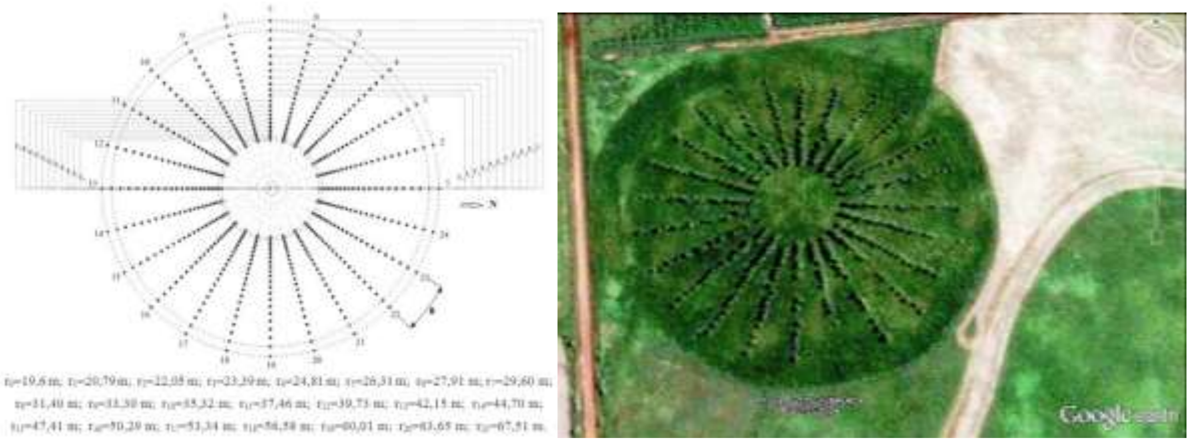

Figure 2. Scheme of Nelder Wheel to define the tree competition of *P. dubium*, and the image of the experiment on the field.

of 528 *P. dubium* seedlings with height of 20 cm in November, 2010. This seedling height is defined as the ideal one for field planting. In order to supply the nutritional requirement of *P. dubium,* each seedling was fertilized with 150 g of N-P-K (6-30-6 + 1% B + 0.5% Zn + 0.5% Cu), at the same day of planting. The application of fertilizer was established in two portions of 75 g; both of them were put in 15 cm apart from the stem of the seedling.

The topdressing fertilization was applied 30 days after the planting. In this occasion, 120 g of the fertilizer formulated as 20-0-20 (N-P-K) was used. The location of the fertilizer was in the area around the tree, being applied at the end of the crown projection. The arrangement of the trees followed the design proposed by Nelder (1962) (Figure 2). The trees distances and angles were determined according to the Equations 1, 2 and 3 (Namkoong, 1965):

$$r_n = r_0 . \propto^n \quad (1)$$

$$A_n = \tan\left(\frac{\theta}{2}\right) . \left[\frac{r_n^2}{4}\right] . f(\alpha) \quad (2)$$

$$f(\alpha) = (1+\alpha)^2 - (1+\alpha^{-1})^2 \quad (3)$$

where r_n is the radial distance to the last trees, r_0 is the radial distance to the first tree in each ray, A_n means the area of tree in each ray, θ is the angles between adjacent rays, and α is the constant that determines the rate of change in space growth.

The Nelder Wheel competition was defined by 22 concentric circles, with distance from the center ranging between 19.60 m (r_0) and 67.51 m (r_{21}). The decrease rate was 12.5% in the trees densities from (r_0) to (r_{21}), resulting in the increase of 6.066% in the distance for each new circle, represented by α=1.06066. Both inner and outer circles were considered borders. The angle θ between the Nelder Wheel rays was of 15°, resulting in 24 rays and 24 seedlings implanted in each ray (Figure 2). The ray number one was located in the direction of the north. This arrangement allowed the evaluation in the experimental circles from r_3 to r_{21} of the trees densities as shown in Table 2.

Treatments and experimental design

The experimental design was factorial (10×2×5) with 12 repetitions (12 trees per Nelder Wheel arc in each cardinal orientation evaluated). The treatments were accomplished by ten tree competitions (159, 201, 255, 322, 408, 516, 653, 827, 1046 and 1324 trees ha^{-1}), two cardinal directions (North-South and East-West) and five times after planting (12, 18, 24, 30 and 36 months).

Measurement of dendrometric variables of *P. dubium*

The dendrometric variables were measured at 12, 18, 24, 30 and 36 months after seedling planting. In all trees, the circumference at 1.3 m of height (C1.3), the tree height (TH) and cylindrical volume per tree (CVT) were measured. In order to obtain these measurements, the tape-measure and graduated scale were used. Based on these measurements earlier, the diameter of trunk ($DBH_{1.3\ m}$= $C_{1.3}$/pi), the transversal area of trunk (g= pi/($D_{1,3}^2$/4) and the cylindrical volume (CV= g × TH) were defined. The cylindrical volume per hectare (CVH) was defined with the relation of CVT and tree densities evaluated.

Statistical analysis

The variables evaluated in the experiment were submitted to the analysis of variance (ANOVA) by the *F*-test. The response surface was adjusted in case of significant interaction (p<0.01) between trees competition and time after planting. The simple Pearson's correlation matrix of dependent variable was performed to obtain the degree of relationship between them. In case of significant correlation (p<0.05), the strength was defined as Table 3, according to Hinkle et al. (2003). These statistical analyses were carried out with the software SPSS for Windows, version 11.0.0 (SPSS Inc., Chicago, IL, EUA).

RESULTS AND DISCUSSION

P. dubium tree competition impact on tree height

The dendrometric parameters of *P. dubium* changed with the treatments applied (Table 4). The tree height (TH) and diameter at breast height (DBH) were affected (p<0.01) by tree competitions, time after planting and the

Table 2. Trees competition in each circle of the Nelder Wheel competition.

Series	Circle	Radial distance (m)	Area per tree (m^2)	Trees competition (trees ha^{-1})
-	-	r_n	-	-
1	0	19.60	-	-
2	1	20.79	-	-
3	2	22.05	7.55	1324
4	3	23.39	8.50	1177
5	4	24.81	9.56	1046
6	5	26.31	10.75	930
7	6	27.91	12.10	827
8	7	29.60	13.61	735
9	8	31.40	15.31	653
10	9	33.30	17.22	581
11	10	35.32	19.37	516
12	11	37.46	21.79	459
13	12	39.73	24.51	408
14	13	42.15	27.59	362
15	14	44.70	31.03	322
16	15	47.41	34.90	286
17	16	50.29	39.27	255
18	17	53.34	44.18	226
19	18	56.58	49.71	201
20	19	60.01	55.92	179
21	20	63.65	62.91	159
22	21	67.51	-	-

Table 3. The rule for interpreting the size of Person's correlation coefficients.

Size of correlation	Interpretation
0.90 to 1.0 (-0.90 to -1.0)	Very high positive (negative) correlation
0.70 to 0.90 (-0.70 to -0.90)	High positive (negative) correlation
0.50 to 0.70 (-0.50 to -0.70)	Moderate positive (negative) correlation
0.30 to 0.50 (0.30 to -0.50)	Low positive (negative) correlation
0 to 0.30 (0 to -0.30)	Negligible correlation

Table 4. Summary of analyses of variance (ANOVA) for tree height (TH), diameter at breast height (DBH), cylindrical timber volume per tree (CTV), and cylindrical volume per hectare (CVH) of *Peltophorum dubium.*

Source of variation	*df*	Mean square			
		TH (m)	DBH (cm)	CVT (m^3)	CVH (m^3 ha^{-1})
Tree competition (TC)	9	14.22**	14.32**	0.000456**	2467.049**
Time after planting (TAP)	4	337.65**	365.38**	0.009281**	3370.711**
Cardinal directions (CD)	1	0.08^{ns}	0.28^{ns}	0.000008^{ns}	0.0113*
TC × TAP	36	0.31^{ns}	0.28^{ns}	0.000037**	226.6965**
TC × CD	9	1.14**	0.68*	0.000028*	28.1409**
TAP × CD	4	0.70^{ns}	0.49^{ns}	0.000006^{ns}	1.8094^{ns}
TC × TAP × CD	36	0.13^{ns}	0.13^{ns}	0.000006^{ns}	5.256**
Residual	814	0.41	0.31	0.000014	14.279
CV (%)	-	13.8	11.8	36.5	20.6

^{ns}, **, * no significant effects, significant at level of 1 and 5% by F-value, respectively. Df: Degree of freedom.

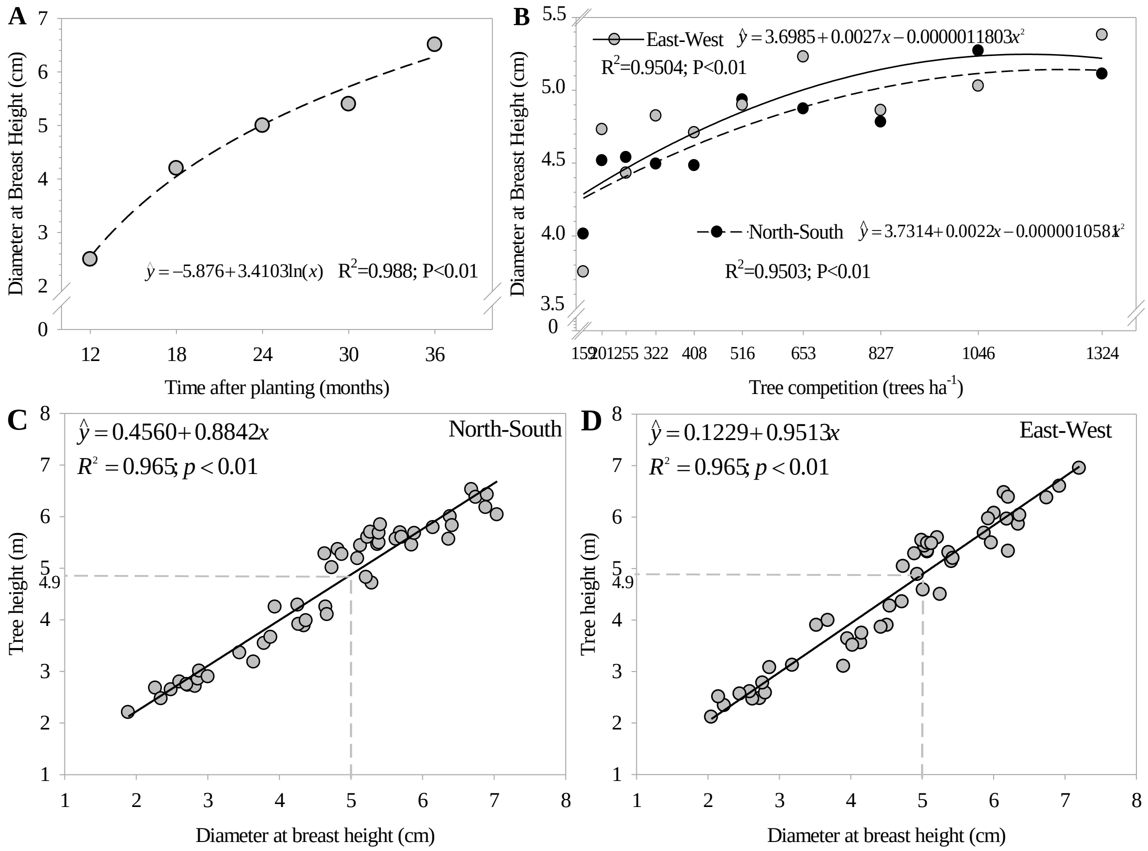

Figure 3. Diameter at breast height (DBH) of *Peltophorum dubium.* (A) Effects of time after planting on DBH; (B) Effects of tree competition on DBH; (C) correlation between tree height *vs* DBH in North-South cardinal direction; (D) correlation between tree height *vs* DBH in East-West cardinal direction.

interaction between tree competitions *vs*. cardinal directions (Table 4). The tree height of 2.62 m (12 months after planting) was compatible for what was obtained by Oliveira et al. (2009), who obtained the range of 1.5 to 3.5 m at 12 months after planting for native species including *P. dubium*. On the other hand, tree height was 29.1% higher than the average found by Matos et al. (2015) for *P. dubium* in the same region of studying in 12 months after planting. As reported by Carvalho (1994), *P. dubium* showed high adaptive plasticity, resulting in different behavior among the same location of plantation.

The height of *P. dubium* showed faster growth between 12 and 24 months after planting and slow between 24 and 36 months. The absence of growth between 24 and 30 months may be related to the freezing occurred in winter season (Figure 1), which may have compromised the growth during this period; anyway, the growth in summer season after fall-winter season was not as fast as the initial growth which comprised between 12 and 24 months.

The cardinal directions affected the tree height (Figure 3B). The cardinal direction East-West showed improvement in tree height in comparison with North-South (Figure 3B). Tree competition of 1047.6 trees ha^{-1} was necessary to achieve the highest tree height in cardinal direction North-South, occasioning in 4.88 m of tree height, and cardinal direction East-West of tree rows required 1143.78 trees ha^{-1} to achieve the highest tree height (5.24 m). The row directions of East-West promoted increase of 6.87% of *P. dubium* height, which is possibly related to improvement of sunlight use efficiency. The East-West cardinal orientation of the tree row is quite important to have higher light incidence, temperature and canopy openness (Camargo et al., 2011).

Diameter at breast height under tree competition and cardinal directions

The diameter at breast height (DBH) of *P. dubium* was affected (p<0.01) by tree competition, time after planting and the interaction between tree competition *vs*. cardinal directions (Table 4). DBH of 5.0 cm was achieved in 24 months after planting (Figure 3A), which may be considered a profitable time for grazed animal introduction in the integrated crop-livestock-forestry systems. In order

Table 5. Pearson´s correlation between dendrometric variables.

Correlation	North-South				East-West			
	TH	DBH	CVT	CVH	TH	DBH	CVT	CVH
TH	1	0.965	0.936	0.696	1	0.965	0.948	0.710
DBH	-	1	0.965	0.710	-	1	0.954	0.684
CVT	-	-	1	0.774	-	-	1	0.788
CVH	-	-	-	1	-	-	-	1

TH: Tree height (m); DBH: diameter at breast height (cm); CVT: cylindrical volume per tree (m^3); CVT: cylindrical volume per hectare (m^3 ha^{-1}).

to introduce cattle, DBH needs to be above 5.0 cm to avoid significant damage caused on tree trunk in DBH lower than 5.0 cm (Sanchez-Velasquez and Pineda-Lopez, 2010). Even with this recommendation, the improvement in researches related to *P. dubium* associated to stocking rate, cattle weight and damage caused on tree through different DBH with the introduction of livestock might be investigated.

The tree competition (1144 trees ha^{-1}) in East-West cardinal direction showed higher DBH (5.24 cm) than North-South cardinal direction, which showed 4.88 cm of DBH in 1,040 trees ha^{-1} (Figure 3B). The results showed by *P. dubium* in relation to DBH were not expected because in lower tree competition, DBH decreased, however, these results can be associated with other factors as wind and lower tree competition that affected negatively the growth in diameter and tree height.

The cardinal directions North-South and East-West of the tree rows did not affect the correlation between DBH and tree height (Figure 3C and D). In both cardinal directions, the *P. dubium* showed ratio of tree height and diameter at breast height close to 1:1 (0.97:1); this way DBH of 5.0 cm is going to reach a height of 5.0 m, which is crucial to determine the livestock initiation into the integrated livestock-forestry system.

The greatest challenge in introducing *P. dubium* or other Brazilian native species in integrated livestock-forestry systems is the slow growth in comparison to *Eucalyptus* spp. Tree height and DBH are traits that most affect the time of livestock introduction in the production systems. But, in comparison to *Eucalyptus* spp., the results showed by *P. dubium* are promisor due to the DBH achieved 5.0 cm at 24 months after planting (Figure 3A). Nevertheless, this recommendation of 5.0 cm of DBH was established by *Eucalyptus* spp. and not for *P. dubium*. This way, it is quite important in further research to evaluate the possibility to introduce livestock with other DBH of *P. dubium* to know if higher wood density of *P. dubium* in comparison to *Eucalyptus* spp. can be a positive point to reduce the introduction time of livestock in the integrated livestock-forestry systems.

Based on the equation adjusted for time after planting and DBH, in a scenario with the possibility to introduce livestock with ≥5.0 cm DBH, the animal grazing might be introduced in 24 months after tree planting, which is not much longer than *Eucalyptus* spp.

Cylindrical volume of *P. dubium* under tree competition

In respect to cylindrical volume per tree (CVT), significant effects ($p<0.01$) were obtained by tree competition, time after planting, cardinal directions of the rows and their interactions (Table 4). The cardinal direction East-West resulted in higher CVT in tree competition above 408 trees ha^{-1}, these results showed very high positive correlation with DBH and TH in both cardinal directions (Table 5). In cardinal direction East-West, the CVT was 0.014 and 0.013 m^3 for North-South, resulting in 6.27% of CVT higher in cardinal direction East-West (Figure 4A). The cardinal direction East-South can have higher use efficiency of sunlight (Camargo et al., 2011), occasioning in higher carbon dioxide assimilation and consequently higher CVT.

Based on significant ($p<0.01$) interactions between time after planting *vs.* tree competition, the response surface was adjusted and showed in higher tree competition increase in CVT (Figure 4B). The highest CVT (0.0248 m^3 tree) was obtained in the extreme values of tree competition (1324 trees ha^{-1}) and time after planting (36 months after planting) (Figure 4B). The highest tree competition did not affect negatively the CVT, which is quite important to use *P. dubium* in ICLS due to the absence of decreasing in higher tree competition and the possibility to increase the cylindrical volume per hectare. Usually, the increase in tree competition decreases CVT, which depletes the capacity of increasing the number of trees per hectare without decreasing CVT. In the case of *Eucalyptus* spp., the increase in tree competition reduced CVT due to higher tree competition (Ferreira et al., 2016), as well as observed for *P. dubium* (Matos et al., 2015).

Interactions of tree competition *vs.* cardinal directions on cylindrical volume per hectare

The interaction of times after planting *vs.* tree competition was observed by cylindrical volume per hectare (CVH) in

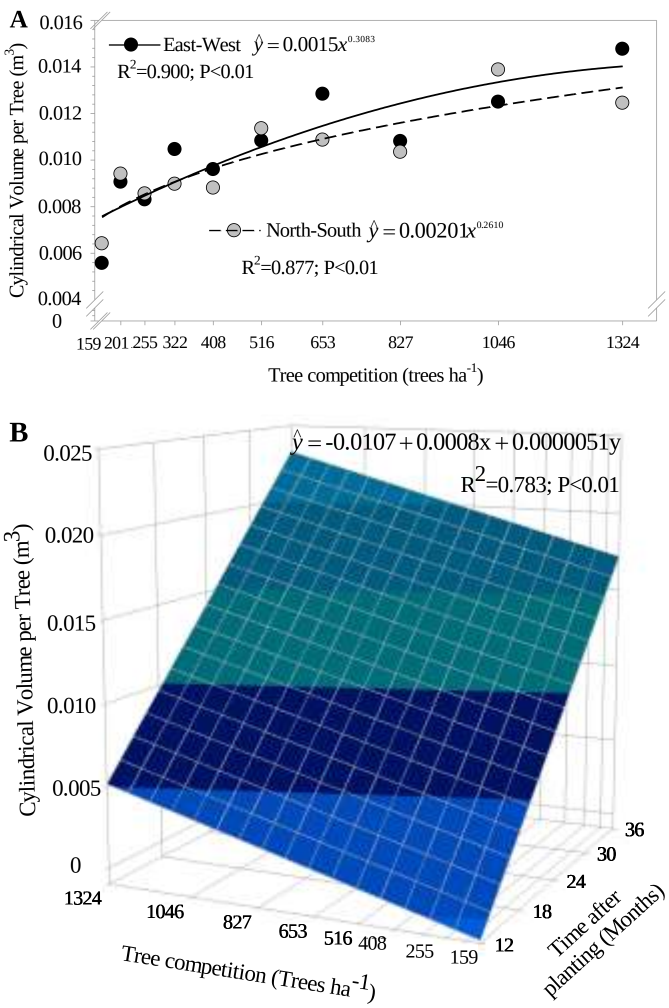

Figure 4. Cylindrical timber volume per tree (CVT) of *Peltophorum dubium.* (A) CVT in function of cardinal directions and tree competition; (B) CVT surface response in function of tree competition and time after planting (months).

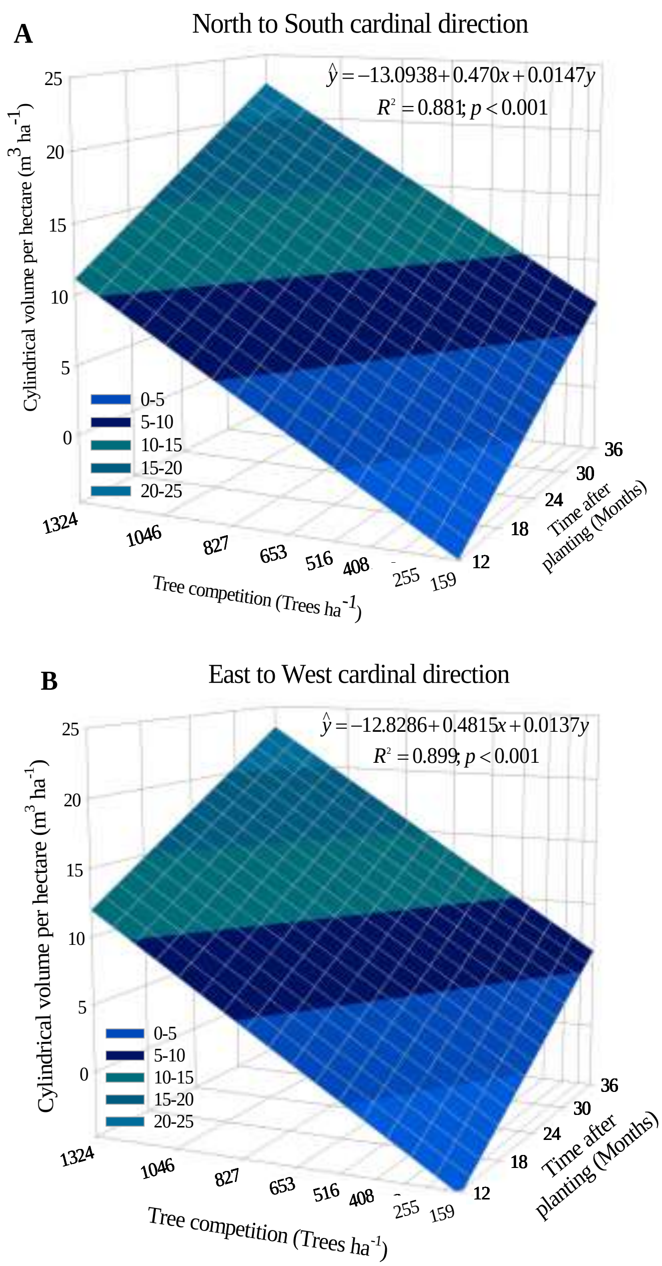

Figure 5. Cylindrical volume per hectare (CVH) of *Peltophorum dubium.* (A) CVH surface response in function of tree competition *vs*. time after planting in North-South cardinal direction; (B) CVH surface response in function of tree competition *vs*. time after planting in East-West cardinal direction.

both cardinal directions (Table 3). The CVT was higher in 1,324 trees ha^{-1} in both cardinal directions (Figure 5A and B). The initial development of the *P. dubium* until 36 months after planting showed this species quite interesting to be introduced in integrated crop-livestock-forestry systems or single forestry, due to its flexibility to adapt under variable tree competition without negative effect on cylindrical volume produced.

The expected is decreasing CVT and CVH under higher tree competition, due to reduction of light use efficiency (Nelson et al., 2016), soil nutrients competition (Dong et al., 2016), and water limitation (Pezzopane et al., 2015). Nevertheless, this absence of limitation in higher tree density showed by *P. dubium* indicates this native Brazilian species as a quite promising native forestry species for woody production in higher tree density. Furthermore, the highest wood density (0.75 g cm^{-3}) for mature trees of *P. dubium* in comparison to *Eucalyptus* spp. (0.45 g cm^{-3}) as forestry component in integrated system may be a decision point to be evaluated to choose which species to use. Nevertheless, the woody density depends on the tree maturity and is expected increase through time (Richter, 2015). The possibility to increase the number of trees per hectare without decrease in CVT which is quite preferably due to the opportunity of increasing economic gain with the timber.

Conclusion

The *P. dubium* showed potential to be inserted in integrated livestock-forestry system due to the absence of negative effect in tree competition on dendrometric tree parameters. The diameter at breast height and tree height showed very high positive correlation; the increase in tree height resulted in increase in DBH in a proportion of 1.0 cm (DBH) for 1.0 m of tree height.

The highest tree competition of 1,342 trees ha^{-1}, in 36 months after planting, promoted increase in cylindrical volume per tree in both cardinal directions North-South and East-West. Nevertheless, the cardinal direction East-West was profitable to increase tree height, DBH, and cylindrical volume per tree.

DBH measured in 24 months after planting was 5.0 cm, which is considered the minimum DBH to introduce livestock into the integrated system without significant damage on trees. Concerning these preliminary results in the first three years, *P. dubium* was promising as Brazilian native species to be inserted in an integrated crop-livestock-forestry system or single forestry as an option for *Eucalyptus* spp.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Fertilizer use optimization approach: An innovation to increase agricultural profitability for African farmers

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Farmers in Africa have not been fully convinced to invest in fertilizer due to uncertainty on returns to investment. This is despite the fact that, more than two-thirds of people in Sub-Saharan Africa rely on agriculture for employment with many living on small farms earning less than $1 per day. Usage of fertilizer has remained low and stagnant hence the yields in have been persistently lower than other parts of the world. To address the plight of the smallholder farmers' inability to optimize productivity in fertilizer use, a collaborative research project Optimization of Fertilizer Recommendations in African (OFRA) 2013 to 2016 was designed through the Alliance for Green Revolution in Africa (AGRA) funding, implemented by Centre for Agriculture and Biosciences International (CABI) and science support by the University of Nebraska Lincoln (UNL). Broad objective was to develop AEZs and crop specific fertilizer recommendations for 13 SSA countries and development of fertilizer optimization approach for smallholder farmers to maximize returns to investment. This paper describes the optimization approach and how it works for farmers benefits. About 65 agro ecological zones (AEZs) specific fertilizer optimization tools (FOTs) for 14 important SSA crops (54%) were developed. Two complimentary tools the paper too and nutrient substitution table were developed to work concurrently with the FOT.

Key words: Optimization, profitability, farmers, approach, Africa, fertilizer optimization tools (FOTs), agro ecological zones (AEZs).

INTRODUCTION

Farmers in Africa have not been fully convinced to invest in fertilizer owing to the uncertainty of returns to investment. This is despite the fact that, more than two-thirds of people in Sub-Saharan Africa rely on agriculture for employment and many of them live on small farms and earn less than $1 per day and as such, the usage of fertilizer remains low and stagnant hence the yields in have been persistently lower than other parts of the world. Moreover, there has been a mixture of trend from high soil nutrient deficits and very low fertilizer use (3% of global fertilizer consumption; 7 kg/ha versus > 150 kg/ha in Asia) World Bank (2007). It is as a result of this the average yield of cereals and average intensity of modern inputs has stagnated in contrast to what has been observed in most developing regions, World Bank (2007). Most often when they apply fertilizer, the smallholder

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farmers in Africa, do it to only a small part of their farm, since they have to make choices that maximize the benefit-to-cost ratio; where fertilizer applications is based on the crop-nutrient rate combinations that gives the greatest net returns for their investment (Kaizzi et al., 2012a). However, existing fertilizer recommendations do not allow farmers to maximize net returns on their investment. In addition to these issues a lack of information about correct application rates, timings, and the use of the correct products for different crops compounds the problems associated with blanket fertilizer recommendations (Rware et al., 2014).

Responding to these myriad of problems facing the farming community in Africa, several governments in SSA in partnership with international and regional bodies introduced inputs subsidy programs as immediate solutions with a view to fostering the use of contemporary inputs and increase agricultural productivity (Druilhe and Barreiro-Hurlé, 2012; Jayne and Rashid, 2013). However, Tavneet Suri (2007), found that on the realization that the fertilizer requires many other complementary inputs, or be risky, many countries have either withdrawn or scaled back fertilizer subsidies, in part because of fiscal constraints and other related barriers such as corruption and inefficiency in the administration of fertilizer subsidies.

The objective fertilizer recommendations in Africa are maximizing yield or profit per hectare. This only satisfies the need for farmers with good financial abilities to apply fertilizer across their entire crop land to maximize net returns per hectare (Kaizzi et al., 2015). Most initiatives have focussed advocating for use of fertilizer to increase crop yields for the food security in Africa. However, with the recent clarion call for farmers to practice farming as a business, there is a paradigm shift on the real focus by farmers is maximising returns to fertilizer investment. The small holder farmers are more focused in using fertilizer for increased yields and get increased returns to investment given their little income for farming inputs (Rware et al., 2014). This paper therefore, addresses the process of developing a fertilizer use optimization approach with the extension workers using the optimization approach to advice farmers to optimize returns from fertilizer use.

To further come up with tailor made solutions to the plight of the small holder farmers' inability to optimize productivity, a collaborative research project optimization of fertilizer recommendations in African (OFRA) 2013 to 2016 was designed jointly funded by AGRA, implemented by CABI and science support by the University of Nebraska Lincoln was designed. The project collaborated with the Africa Soil Health Consortium (ASHC) and Africa Soil Information Services (AfSIS) and National Research Organizations in Burkina Faso, Ethiopia, Ghana, Kenya, Malawi, Mali, Niger, Nigeria, Mozambique, Rwanda, Tanzania, Uganda and Zambia. The broad objective was to develop agro ecological zones (AEZs) specific and crop specific crop response functions for the 13 African countries and development of the fertilizer use optimization approach to address the plight of the smallholder farmers who have little to invest in fertilizer purchases and help them maximize returns to investment on fertilizer and be able to make better choices on which crops would give most returns if fertilizer is applied. This paper discusses the use of the optimization approach to maximize net returns for the farmer's in the economic and agronomic context. The real problem why the approach is needed, and the relevant basic principles and describes how the approach was developed, the tools that support the approach, the requirements in the roll out of the approach and the sustainability considerations of the approach.

METHODOLOGY

Development of the fertilizer use optimization approach in OFRA involved reviewing of past literature on crop nutrient, soil fertility, fertilizer recommendation and general crop response research. This was to identify gaps in relation to crop nutrient response functions and fertilizer recommendations and given an indication to where OFRA trials could be located. The gaps were filled through establishment of trials across 13 different countries in the SSA. The countries were picked because they fell in the list of the chore countries focused into by the AGRA (financing organization). UNL provided quality control of the data and the science aspects of the project. Further, the choice of crops in countries was informed by the position of this crops in the policy whether an important food crop or source of income for the country as well as for the small holder farmers. A standard protocol for establishing the trials and soil and foliar sampling was developed by the science leader in consultation with the country PIs from the 13 countries to guide the PIs in conducting their trials. Selection of trial sites was guided by use of the GIS tool and AEZs, areas considered as country food basket, areas where past research was done and the knowledge of the country teams in relation to the AEZs. AfSIS ensured that the comprehensive database is linked to geo-referenced soil, climate and remote sensing data. Spatial information from AfSIS enhanced accurate and efficient use of the agronomy field research data in the OFRA/SHC database.

Optimization approach

There is generally low use of fertilizer by the smallholder in Africa which largely affects their productivity levels. This is exacerbated by the fact that many of these farmers do not have the financial capacity to use enough fertilizer to maximize net returns per hectare. This is addition to the high fertilizer costs and low commodity prices, associated with costly input supply and inefficient marketing channels; the farmers profit potential is highly reduced. These smallholder farmers have more than one competing needs for the little cash they have and the most pressing need often take priority especially when profitability of fertilizer use is not convincing. These farmers therefore need high net returns on their investments in fertilizer use if they have to embrace the fertilizer use technology in their farms (Kaizzi et al., 2013). Optimization approach is achieved by allocating fertilizer to an optimized choice of crop-nutrient-rate combinations where the farmer gets good returns to investment.

The profitability of different crop-nutrient combinations varies with the relative value of crops, the costs of fertilizer nutrients, the magnitude of each crop's response to an applied nutrient, and the shape of the response curve. Nutrient application continues in a

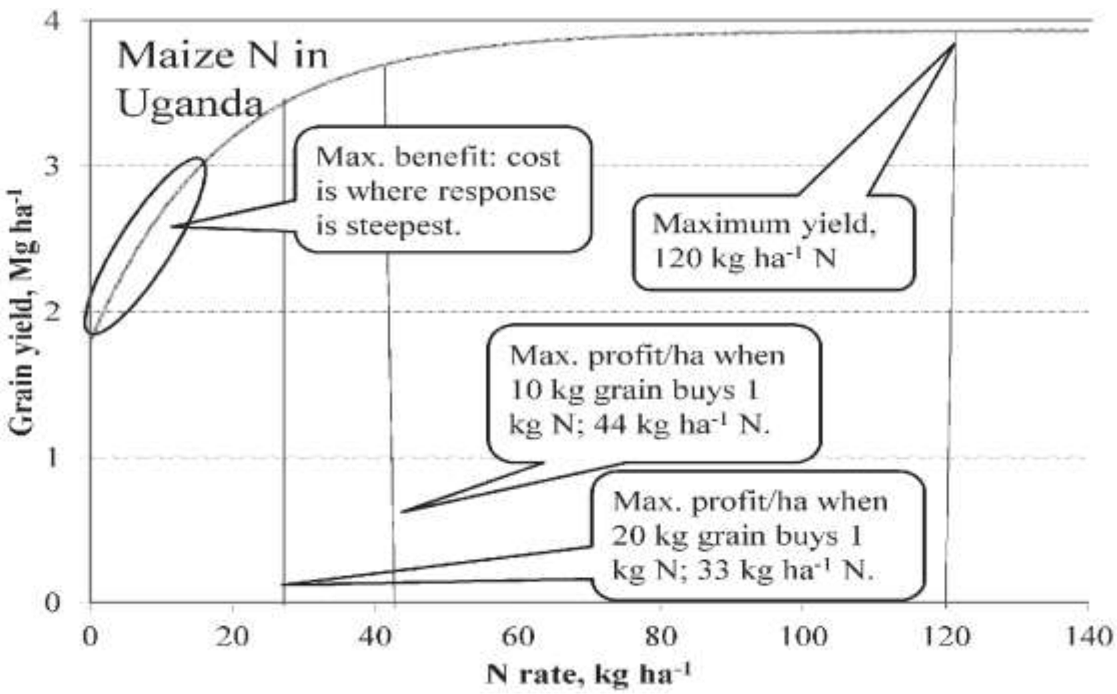


Figure 1. Maize yield response to N fertilizers and effect of change in cost of nutrient to farm gate price on the economically optimum amount of fertilizers (Jansen et al., 2013).

curvilinear trend until yield reaches a plateau. However, yields sometimes does decrease from a peak with high application rates may be due to localized fertilizer salt effects, severe soil water depletion at a critical growth stage following a period of vigorous growth, increased disease, or more lodging (Kaizzi et al., 2015). A method of optimizing across these response functions was developed to determine the allocation of fertilizer investment to the crop-nutrient-rate combinations that maximize net returns on investment. Whereas large scale farmers have adequate finance may strive to maximize net returns per hectare resulting from fertilizer use, majority of the smallholder farmers have meagre opportunities for improvement and are largely vulnerable to agricultural shocks.

Reliable crop–nutrient response functions for an agro-ecological zone are essential for estimating likely profitability of crop–nutrient rate decisions. These functions can be determined using results from locally conducted research and extrapolation of results from other locations with similar growing conditions. Smallholder farms often produce four or more substantial crops and optimization of fertilizer use may involve 12 or more crop–nutrient combinations to determine the optimal rate for a farmer's situation. A computer-based fertilizer optimization tool and its paper version are described (Charles and Kaizzi, 2015). In Figure 1, the crop–nutrient response is represented by an asymptotic function such as $Y = a - bc^N$, where Y is yield, a is yield at the plateau, b is the possible yield increase resulting from nutrient application, c is a curvature coefficient giving an abrupt response at low c values and having a value of 1 with a linear response, and N is the nutrient application rate (Kaizzi et al., 2012c). If cash is not a constraint, the amount of fertilizer that maximizes returns per area of land Economically Optimum Rates (EOR) can be applied. But if financially constrained, the amount of fertilizer that maximizes returns per shilling invested, which is less than the EOR, should be applied. If farm gate prices of produce decline or fertilizer prices increase, farmers have to apply less fertilizer to maintain the EORs. For example, in trials carried out in Uganda (Figure 1), the EOR for maize decreased from 44 to 33 kg N/ ha as C: P increased from 10 to 20. For cash constrained farmers, maximum benefits, that is, highest benefit-to-cost ratio (region indicated with oval) could be obtained by applying less than 30 kg N/ha (Jansen et al., 2013).

RESULTS AND DISCUSSION

The fertilizer optimization tool (FOT)

Fertilizer Optimization tool is the main tool in the optimization approach that was developed. It is available in three versions (computer excel, the paper and the mobile app version). In OFRA project the mobile app was developed and tested in Uganda in partnership with Grameen Foundation. Despite the fact that it was the most preferred and going by the mobile role in extension in the current world, the mobile app did not continue due to funding challenges. A total of 65 AEZs and crop specific FOTs were developed across 13 countries as shown in Table 1, in consideration of the pats research and field trials data. Important to note is that the crops covered by the project represent about 54% of important crops in Africa.

Optimization working principle

The Optimizer uses linear programming concepts using Excel with the Solver add-in the extension worker and

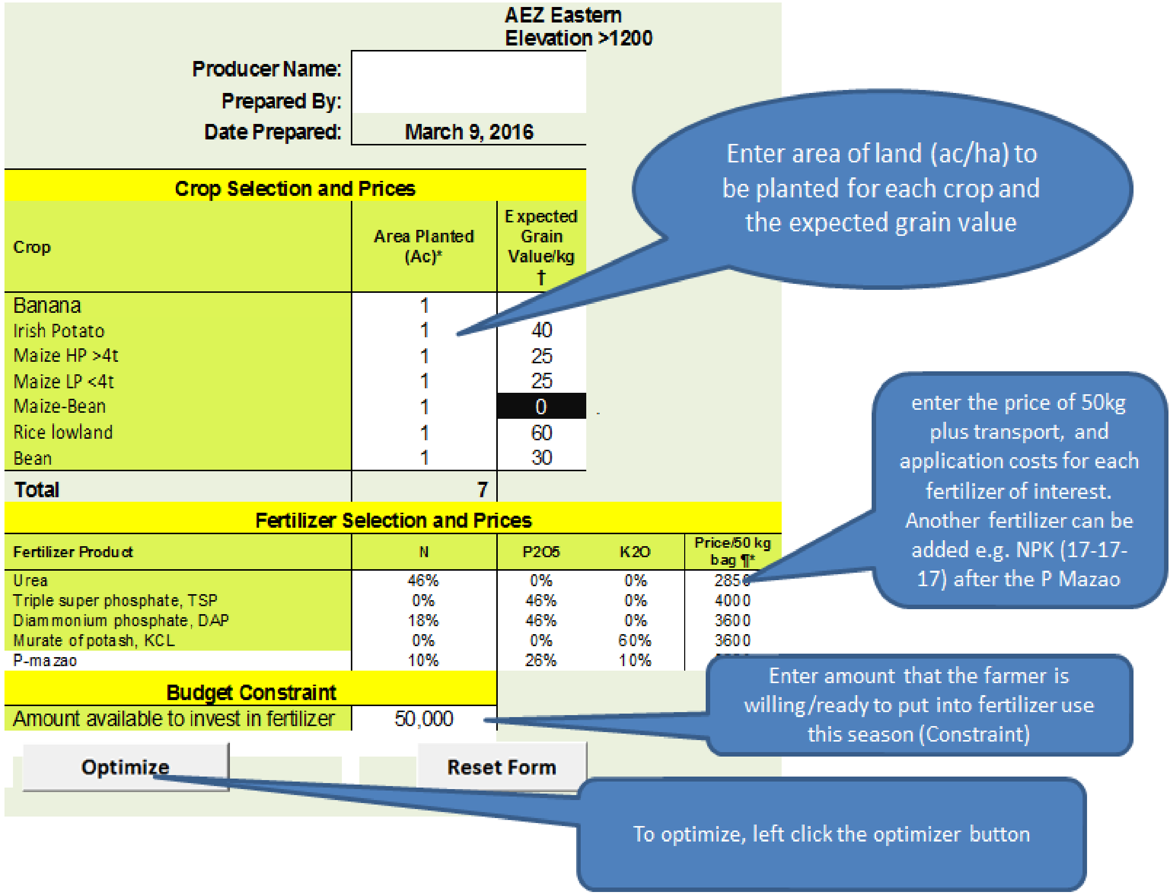
Table 1. FOTs developed for the different Crops and AEZs in 13 OFRA countries.

S/N	AEZ	Elevation (M)	Crops
	Kenya		
1	Western Kenya	>1400 m	Maize, Irish potato, sweet potato, lowland rice, maize-bean, climbing bean, wheat
2	Western Kenya	<1600	Maize, sorghum, finger millet, beans, groundnut
3	Central Kenya		Maize, beans, maize-beans, rice, wheat
4	Coastal area		Cassava, maize, lowland rice, sorghum, finger millet, cowpea
5	Eastern Kenya	>1200 m	Banana, Irish potato, maize, maize-bean, lowland rice, bean
6	Eastern Kenya	<1200 m	Maize, beans, sorghum, Irish potato
7	Rift Valley	>2000 m	Maize, Irish potato, beans, wheat, maize-bean, green gram
8	Rift Valley	<2100 m	Maize, soybean, Irish potato, beans, wheat, maize-bean, green gram
	Rwanda		
9	Eastern region	<1800	Banana, maize, sorghum, lowland rice, beans, soybean
10	Northwest highlands	>1800 m	Wheat, Irish potatoes, maize, climbing bean, soybean, banana, bean
11	Southern Rwanda		Wheat, maize, climbing bean, lowland rice, beans, soybeans, sweet potato
	Tanzania		
11	Central zone		Lowland rice, maize, sorghum, sweet potato, cowpea
12	Eastern zone		Lowland rice, cassava, maize, sorghum, cowpea
13	Lake zone	>1300 m	Maize, Irish potato, finger millet, sweet potato, bean, banana
14	Lake zone	<1400 m	Lowland rice, maize, sweet potato, sorghum, beans, finger millet
15	Northern		Lowland rice, maize, wheat, beans, finger millet
16	Southern zone		Lowland rice, maize, sorghum, cowpea, cassava, groundnut
17	Southern highlands		Lowland rice, maize, sorghum, bean, wheat
18	Western zone		Lowland rice, maize, sorghum, sweet potato, soybean, groundnut
	Uganda		
19	Eastern Uganda	1400-1800 m	Maize, banana, Irish potatoes, beans, soybeans, groundnut
20	Eastern Uganda	>1800 m	Maize, banana, wheat, beans, soybean, groundnut
21	Eastern Uganda-Lake Kyoga basin	>1800	Upland rice, maize, sorghum, soybean, finger millet, beans, groundnut
22	Western highlands Kamwenge, Ibanda, Bushenyi, Kyenjojo		Maize, banana, Irish potato, beans, finger millet, soybean
23	Western highlands	>1800 m	Maize, Irish potato, wheat, beans
24	Central Uganda		Maize, banana, upland rice, beans, soybean, groundnut
25	North, Midwest & West		Upland rice, maize, sorghum, soybean, finger millet, beans, groundnut
	Ethiopia		
26	Cold to very cold sub-afro Alpine	>2500	Barley, wheat, faba bean
27	Hot to warm moist lowlands 9N degrees		Maize, sorghum, lowland rice, teff, beans, soybean
28	Hot to warm moist lowlands 9S degrees		Maize, sorghum, lowland rice, teff, beans, soybean
29	Hot to warm sub-humid and drier lowlands	<1000	Rice, sorghum, maize, teff
30	Hot to warm sub-moist and drier lowlands	1000-1800	Maize, sorghum, rice, finger millet, teff, soybean
31	Tepid to cold humid mid high lands	1700-2200	Maize, sorghum, teff, barley, faba bean
32	Tepid to cold humid mid highlands	2000-2700	Maize, wheat, barley, faba bean
33	Tepid to cold moist mid high lands	1700-2200	Rice, teff, maize, bean, faba bean, sorghum
34	Tepid to cold moist mid highlands	2000-2700	Barley, teff, wheat, maize, faba bean
35	Tepid to cold sub-humid mid highlands	1700-2200	Sorghum, teff, maize
36	Tepid to cold sub-humid mid highlands	2000-2700	Barley, wheat, faba Bean, Irish potato
37	Tepid to cold sub-moist mid highlands	1700-2200	Maize, teff, sorghum, bean, faba bean

Table 1. Contd.

38	Tepid to cold sub-moist mid highlands	2000-2700	Barley, maize, teff, wheat, faba bean
	Zambia		
39	Zone 1		Maize, soybean, sorghum, groundnut, cowpea
40	Zone 2		Maize, cowpea, beans, soybean
41	Zone 3		Maize, cowpea, beans, sorghum, soybean
	Malawi		
42	Lakeshore, middle & upper Shire	200-760 m	Maize, cowpea, bean, soybean, pigeon pea
43	Mid-elevation, upland plateau	760 -1300 m	Maize, cowpea, beans, soybean, pigeon pea
44	Highlands	>1300 m	Maize, cowpea, beans, soybean, sorghum, pigeon pea
	Mozambique		
45	Western	900-1300 m	Maize, cowpea, bean, soybean
46	Western	>1300 m	Maize, sorghum, beans, cowpea, soybean
47		<900 m	Maize, cowpea, beans, sorghum, soybean, pigeon pea
	Burkina Faso		
48	North Sudan Savana		Lowland rice, maize, sorghum, cowpea, groundnut, soybean, pearl millet
49	Sahel		Pearl millet, sorghum, groundnut, cowpea, maize, rice
50	South Sudan Savana		Maize, upland rice, sorghum, lowland rice, cowpea, groundnut, pearl millet
	Nigeria		
51	North Guinea Savannah		Cowpea, groundnut, maize, lowland rice, upland rice, sorghum
52	South Guinea Savannah		Upland rice, lowland rice, groundnut, cowpea, sorghum, soybean
53	Sahel		Pearl millet, sorghum, groundnut, cowpea, maize, rice
54	Sudan Savannah		Maize, groundnut, lowland rice, sorghum, cowpea, soybean, pearl millet
55	Derived Savannah		Maize, sorghum, upland rice, lowland rice, groundnut, soybean
56	Mid-altitude		Lowland rice, maize, sorghum, cassava, groundnut, soybean, upland rice
	Niger		
57	Sahel		Pearl millet, sorghum, groundnut, cowpea, maize, rice
58	North Sudan Savannah		Lowland rice, maize, sorghum, cowpea, groundnut, soybean, pearl millet
	Mali		
59	Sahel		Pearl millet, sorghum, groundnut, cowpea, maize, rice
60	North Sudan Savannah		Lowland rice, maize, sorghum, cowpea, groundnut, soybean, pearl millet
61	South Sudan Savannah		Maize, upland rice, lowland rice, sorghum, cowpea, soybean, pearl millet
	Ghana		
62	Derived Savanna/ Transitional		Maize, sorghum, upland rice, lowland rice, groundnut, soybean
63	North Guinea Savannah		Cowpea, groundnut, maize, upland rice, lowland rice, Sorghum
64	North Sudan Savannah		Lowland rice, maize, sorghum, cowpea, groundnut, soybean, pearl millet
65	South Sudan Savannah		Maize, upland rice, lowland rice, sorghum, cowpea, soybean, pearl millet

farmer together enter the acreage of the various crops being grown, the price of fertilizers available, the expected price for crop outputs and how much money the farmer has to invest in fertilizer. They then press the ‘optimize’ tab. The output includes the recommendations for fertilizers to be applied to each crop, the expected mean effect on yield and net returns for each crop, and the expected total net returns. Figures 1 to 3 gives a stepwise process of how the FOT runs the optimization process. The extension worker (any) intermediary asks the farmer the amount of land the farmer want to set aside for each crop in that particular season. In the input section of the optimizer, the intermediary using the knowledge of the farmer, or market trends information puts the expected grain value. Then the extension asks the farmer how much he/she want to spend or invest in fertilizer purchases that season. The amount to invest is the constraint in this case and then click optimize button. The output section of the FOT shows the mean yield increase and the net returns per crop and the total net



AEZ Eastern
Elevation >1200

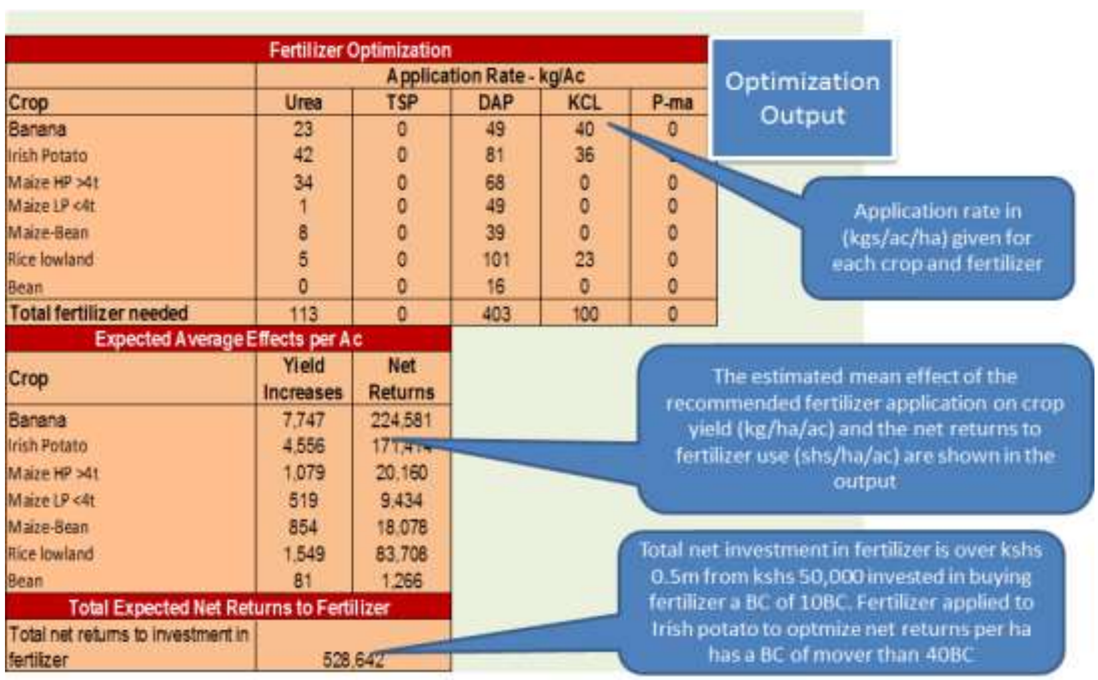
Producer Name:	
Prepared By:	
Date Prepared:	March 9, 2016

Crop Selection and Prices		
Crop	Area Planted (Ac)*	Expected Grain Value/kg †
Banana	1	
Irish Potato	1	40
Maize HP >4t	1	25
Maize LP <4t	1	25
Maize-Bean	1	0
Rice lowland	1	60
Bean	1	30
Total	7	

Fertilizer Selection and Prices				
Fertilizer Product	N	P2O5	K2O	Price/50 kg bag ¶*
Urea	46%	0%	0%	2850
Triple super phosphate, TSP	0%	46%	0%	4000
Diammonium phosphate, DAP	18%	46%	0%	3600
Murate of potash, KCL	0%	0%	60%	3600
P-mazao	10%	26%	10%	

Budget Constraint	
Amount available to invest in fertilizer	50,000

Figure 2. Input into the fertilizer optimizer tool.



Fertilizer Optimization					
	Application Rate - kg/Ac				
Crop	Urea	TSP	DAP	KCL	P-ma
Banana	23	0	49	40	0
Irish Potato	42	0	81	36	
Maize HP >4t	34	0	68	0	0
Maize LP <4t	1	0	49	0	0
Maize-Bean	8	0	39	0	0
Rice lowland	5	0	101	23	0
Bean	0	0	16	0	0
Total fertilizer needed	113	0	403	100	0

Expected Average Effects per Ac		
Crop	Yield Increases	Net Returns
Banana	7,747	224,581
Irish Potato	4,556	171,4[illegible]
Maize HP >4t	1,079	20,160
Maize LP <4t	519	9,434
Maize-Bean	854	18,078
Rice lowland	1,549	83,708
Bean	81	1,266

Total Expected Net Returns to Fertilizer	
Total net returns to investment in fertilizer	528,642

Figure 3. Output from FOT with Kshs. 50,000 invested.

Fertilizer Optimization					
	Application Rate - kg/Ac				
Crop	Urea	TSP	DAP	KCL	P-ma
Banana	23	0	49	40	0
Irish Potato	42	0	81	36	0
Maize HP >4t	34	0	68	0	0
Maize LP <4t	1	0	49	0	0
Maize-Bean	8	0	39	0	0
Rice lowland	5	0	101	23	0
Bean	0	0	16	0	0
Total fertilizer needed	113	0	403	100	0

Key decision making issues:

1. The susgested fertilizer rate may be very low e.g. the 16kgs/ha DAP for bean to make any economical sense or able to apply (you have two options)

i) Advise the farmer to allocate this money to other profitable crops nutrients

ii) You can also ask the farmer to mix with other fertilizer for ease of application

2. Rates are often low with small investments. However returns on investments is high. The increased net returns is expected to enable

Figure 4. Decision making considerations.

return from fertilizer investment. The computer based excel version of the FOT is as shown in Figures 2, 3 and 4.

Tools supporting optimization approach

Optimization approach cannot function independently. To support the roll out of the approach a set of three complementary tools, based on the principle of fertilizer optimization, were developed for use in over 65 agro-ecological zones in 13 OFRA countries. The tools are based on data generated from a series of crop-nutrient response trials carried out 13 countries and legacy data from previous research, both prior to and under the auspices of the OFRA project.

The tools are intended to be used by extension workers who have gone through series of training and practical's to understand and be able to use the three tools concurrently to advice the farmers. Working with a farmer, the extension worker uses the set of tools to generate fertilizer recommendations which reflect that farmer's specific circumstances, including acreages of the different crops grown, fertilizer prices, expected crop output prices, how much the farmer can afford to spend on mineral fertilizer that growing season and other relevant farming practices, such as use of manure. The recommendations generated for each farmer will be different, 'tailor-made', but in all cases the solution provided will result in the greatest return on the farmers' investment in fertilizer.

The nutrient substitution table

It is also referred to as integrated soil fertility management (ISFM) framework is used to adjust the output of the FOT to take into account other ISFM practices the farmer is using that impact on nutrients supply. New fertilizer rate recommendations must consider cropping systems and the effects of ISFM practices on fertilizer need, such as: crop rotations; application of manure or other organic material; compost and annual and perennial green manure/cover crops (Kaizzi et al., 2004, 2006, 2007a,b; Wortmann et al., 2000; Kaizzi and Wortmann, 2001). Estimating fertilizer substitution effects of various ISFM practices requires interpretation of results of numerous relevant studies in consideration of the AEZ, soil type, other agronomic practices, and the farmer's economic decisions. For OFRA, a generic model was developed for Uganda which was adaptable to the diverse cropping systems in the 12 OFRA countries.

The ISFM practices to be included in the look-up table are the use of various types of organic matter (manure, compost, and crop residues), intercropping and rotations with legumes, fallows and the results of selected soil tests. For each practice, Table 2 suggests how the fertilizer recommendations generated by the FOT should be adjusted; for example, for every one ton of farmyard manure applied per hectare, fertilizer equivalent to 6 kg N per hectare could be spared. The extension worker will need to convert the values expressed as amount of each nutrient (N, P or K) into the equivalent amount of fertilizer.

Fertilizer calibration tool

The calibration tool is used to convert the recommendation, expressed as kg of fertilizer per acre or hectare of land, to a more farmer-friendly measure. Few if any small-scale farmers will have access to scales to

Table 2. Nutrient substitution table.

ISFM practice	Urea or CAN	DAP or TSP	NPK 23-21-0+4S or 23:10:5+6S+1.0Zn
	Fertilizer reduction, % or kg/ha		
	N	P	K
Previous crop was a green legume manure (Mucuna, Crotalaria and Lablab) crop	100%	8 kg	28 kg †
Early incorporation of a green legume manure (Mucuna, Crotalaria and Lablab) crop	57 kg	3 kg	11 kg †
Use of agroforestry technologies (*e.g.* leaf prunings of Gliricidia, Leucaena, Sesbania, Senna spectabilis) applied, per 1 t of fresh material	10 kg	1 kg	6 kg††
Farmyard manure per 1 t of dry material	2 kg	1 kg	1 kg
Residual value of FYM applied for the previous crop, per 1 t	1 kg	0.4 kg	0.4 kg
Dairy or poultry manure, per 1 t dry material	24 kg	7 kg	14 kg
Residual value of dairy and poultry manure applied for the previous crop, per 1 t	5 kg	1.4 kg	3 kg
Compost, per 1 t/ha dry wt.	20 kg	1 kg	20 kg
Doubled-up legume-technology (pigeon pea)	In the second year of rotation a mean reduction of over 50 kg N †††		
Cereal-bean intercropping	Increase DAP/TSP by 18 kg/ha, but no change in N & K compared with sole cereal fertilizer		
Cereal-other legume (effective in N fixation) intercropping	Increase DAP/TSP by 20 kg/kg, reduce urea by 30 kg/ha, & no change in K compared with sole cereal fertilizer		
If Mehlich III P >18 ppm	Do not apply P		
If soil test K < 0.25 cmol/kg	Apply 20 kg KCl/ha		

weigh out the fertilizer and not all will know the size of the plots they are using to grow each crop. To overcome this problem, the calibration tool is based on the use of items that are freely available and that can be adapted and used as calibrated measuring scoops. These include cut-down, discarded plastic bottles that previously held water or some other liquid; bottle tops, such as the crimped metal bottle-tops commonly used to cap beer or soda (known as crown corks); or rectangular containers, such as empty match boxes. The calibration tool runs on a laptop but the paper version of the FOT has the calibration factored is shown in Figure 5.

To make it work, first the dimensions of the container to be used are entered; the tool uses these to calculate the volume of the cylindrical or rectangular container. Secondly, the type of fertilizer being used is selected from a drop-down menu: not all fertilizers have the same density, so one bottle-cap full of one fertilizer will have a different weight to the same bottle-cap full of another type of fertilizer. Next the number of kg of fertilizer to be applied per acre or hectare is entered, along with the method of application (broadcast, banding or point placement, also known as micro-dosing) and the distance between rows and plants within rows. Based on the information provided, the calibration tool provides a user-friendly fertilizer recommendation; for example, instead of 40 kg DAP per hectare it might suggest a plastic water bottle lid full of DAP applied as a band 2.1 m long.

Where there are not computes? Paper version of the FOT

In the optimization approach, the FOT is either in the computer excels solver format, paper format and mobile application (developed and tested in Uganda). One of the major challenge that bedevil sub Saharan Africa extension work is poor resourcefulness and majority do not own laptops and besides, the desktop cannot be effectively be used by the extension. Further, African governments have not financed well the extension arm of the agricultural ministries. In this regard, usage of computer based excel format of the FOT is a big challenge. To address that challenge and continuity of extension services, OFRA developed a paper version of the FOT which considers three levels of farmer financial ability and fertilizer use guidelines are provided based on marginal rate of return. If the farmer has one or more crops in the cropping system, some fertilizer application options have priority over other options as shown in Figure 6. The paper FOT does need to be updated when there are substantial changes in the costs of fertilizer use relative to commodity values. About 20% of the profit potential in the decision making is lost with the paper

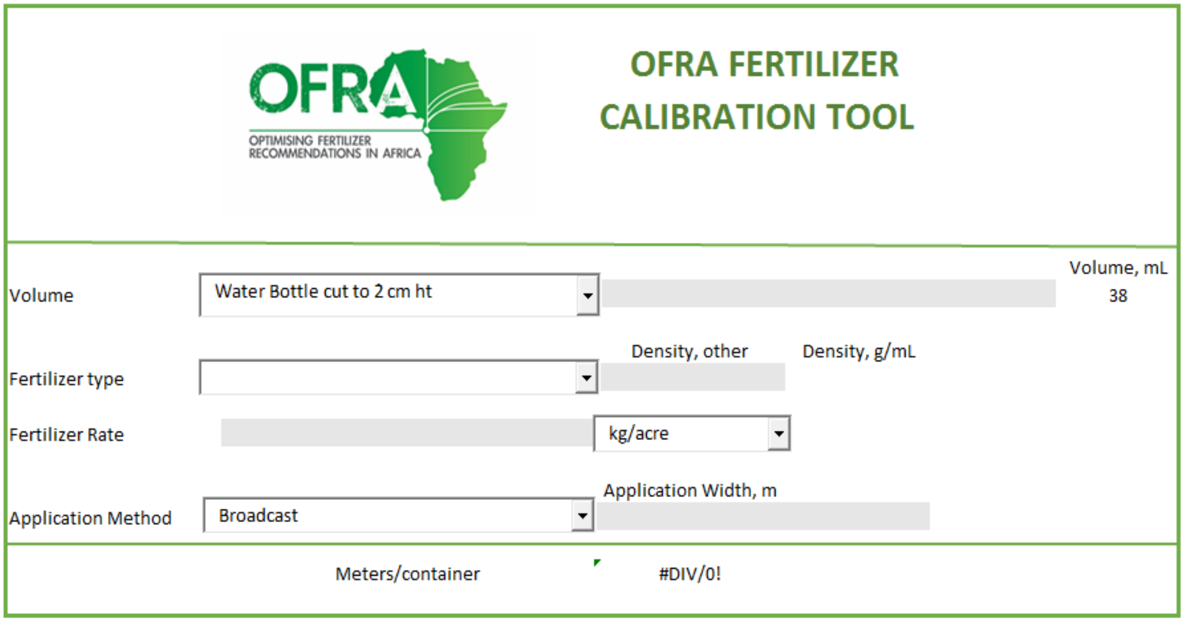


Figure 5. OFRA fertilizer calibration tool.

FOT than the Excel FOT because of the generalization for farmer's budget constraint and selection set choices with financial capability levels (Charles and Kaizzi, 2016), Figure 6 show the example of paper version of the FOT for lower eastern Kenya.

Rolling out the optimization approach

Rolling out the optimization approach was an important step in delivering OFRA. Initial steps for the roll of were to develop of stakeholder's engagement plan and roll out strategies that were country. This was from the understanding that there are different player/stakeholders in each country and hence requiring a specific approaches. In the modern world overcoming barriers to rolling out an agricultural innovation is next to impossible if broad involvement of all key and relevant stakeholders is not engaged. This is to ensure their buy in, appreciation and eventual ownership of the innovation. At this stage it will be important to develop the demand and supply side to ensure a balanced flow. Players in this stage include extension workers and other government agencies that are vital in mobilizing of smallholder farmers. They should be the first people to be trained to understand the approach and how to use the approach in advising farmers. They are also better placed to linking the optimization approach with government's fertilizer subsidy and distribution channels including the agro-dealers. In Uganda MAAIFS expressed interest to use the approach in its fertilizer subsidy program for 2016/2017 financial year.

Given the diversity in the stakeholders involved in rolling out of the optimization approach communication support is critical to ensure that there harmonized messages are passed to all the stakeholders as per their levels. Communication materials need to be packaged for different audiences, researchers, extension, agro-input dealers, fertilizer manufacturers, policy makers and the farmers. Experience in Uganda showed that when different stakeholders were involved in the roll out without proper packaging of the messages, there was contradictory given advice optimization approach.

Once the optimization approach and the supporting tools (FOT tools) are fully developed the next step of deploying them was considered critical. This was made possible through greater involvement of key stakeholders in researchers, extension, policy makers, and end users farmers through a process called taking research into use. Three major entry points were identified in this step. First was the participatory identification and engagement of key stakeholders in the fertilizer industry who are also strategic to create awareness and promote the Optimization approach. The country partners identify key stakeholders to be targeted in the stakeholder's engagement process and draw plans of action on activities. The stakeholders include development agencies e.g. NGOs, District Agricultural offices, Extension workers (Public and private), Agro dealers and fertilizer companies, Farmer groups-Cooperatives, Higher officials of Agriculture at regional and federal levels, Learning/research institutions , Researchers (international, regional and national) institutions (Table 3).

Communication is a critical component of rolling out the

Kenya Eastern Lower Fertilizer Use Optimizer: paper version Feb 2016

The below assumes:

- Calibration measurement is with a i) Water bottle lid (lid) that holds about 6.2 g urea, 7.2 g DAP, and 8.25 g MOP and ii) A 500mls water bottle of 5 cm diameter cut to height of 4m has approx 80 ml to hold 94 g urea, 113 g DAP, and 130 g MOP.
- It is assumed maize, Irish potatoes and sorghum are planted with 75 cm row spacing, and beans with 50cm row spacing.
- It is assumed grain prices per kg (Ksh): 25 maize; 60 beans, 25 sorghum and 30 Irish potato.
- It is assumed 50 kg of fertilizer costs (Ksh): 2850 urea and 3600 DAP.
- Level 1 financial ability needs a benefit: cost ratio (BC) of 0.1-2; Level 2: BC **2 - 4**; Level 3: BC >4.
- Application rates are in kg/ac.
- If any fertilizer rate application is less than 10 kg/acre it is not economical and should be converted to another fertilizer.

Level 1 financial ability

- Irish potato: Apply 53 kg DAP (1 lid for 0.6m) by banding at planting.

Level 2 financial ability

- For maize, sidedress with 28 kg urea (1 lid for 1.0m) at 6 WAP.
- For beans, at planting band 21 kg DAP (1 lid for 2.4m) and sidedress with 12 kg urea (1 lid for 3.4m).
- For Irish potato, at planting band 61 kg DAP (1 lid for 0.5m).

Level 3 financial ability (maximize profit per acre).

- For maize, sidedress with 43 kg urea (1 lid for 0.6m) at 6 WAP.
- For beans, at planting band 30 kg DAP (1 lid for 1.7m) and sidedress with 17 kg urea (1 lid for 2.4m).
- For sorghum, at planting band 11 kg DAP (1 lid for 3.0m).
- For Irish potato, at planting band 61 kg DAP (1 lid for 0.5m).

Figure 6. Kenya Lower Eastern Paper based FOT.

optimization approach. OFRA partnered with ASHC in packing and communicating the optimization approach and the accompanying tools. Thirteen flyers specific to each of the 13 countries were developed to create awareness on the approach and the tools. Over 2000 of this were distributed targeting researchers, extension (public and private) agro dealers and progressive farmers. Consideration was made to adopt specific platforms for awareness creation in order to gunner maximum impact in terms of reach and clarity of the message. As such, we employed different platforms were used in awareness creation. Radios, print media (Daily Nation in Kenya, Daily Monitor in Uganda, Seeds of Gold in Kenya and Uganda), TV, Radio Africa in Tanzania, video documentaries of scientists discussing the preliminary results and potential impacts of the approach, lessons and case study development, national and international stakeholders forums, Country Soil Health Consortia (CSHC), Regional and international conferences.

CONCLUSION AND RECOMMENDATIONS

The optimization approach and supporting tools were developed for different AEZs in 13 countries in SSA. It is an innovation approach with the potential to change smallholder and resource poor farmers to be able to maximize their returns to investment in fertilizer. The approach will go a long way in creating a case of farmers' ability to make profits through farming as well as the policy formulators in planning for fertilizer subsidy

Table 3. Stakeholders for the roll out of the optimization approach.

Stakeholder	Geographical coverage	Major Roles/Mandate
CABI	International	Communications, research†, data management, extension, coordination
University of Nebraska-Lincoln	International	Training, research, geo spatial analysis, science development, scientific reporting
Universities	National/regional	Training, research, education
IFDC, CGIAR, IPNI, AGRA	International	Funding, research, further development of the approach, improvement of the FOTs
NARS	National	Research, training, extension
African research associations	Regional	Research, further development of the approach, improvement of the FOTs
AFSIS	Regional	Research and geospatial analysis
Government	National	Policy, extension, resource mobilization
Private sector	National/regional	Extension, enterprise development, credit facilities
Farmer organizations	National/regional	Extension, credit facilities, resource mobilization, training farmers
NGOs	National and international	Adaptive research, extension and training

programmes. However, it is recommended that researchers to work closely with farmers to validate the approach comparing the predicted versus actual. There is also need to further explore options of integrating the optimization approach with the input supply chain and also bringing in agricultural loan institutions as the approach provides the evidence that investment in fertilizer is profitable and would help in building a case to advance farmers loans. In other words the approach acts as a mini business plan to convince the bank to advance the loan.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Root-knot nematodes on tomatoes in Kyenjojo and Masaka districts in Uganda

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In Uganda and elsewhere in the world, tomato is an economically important vegetable and a good source of vitamins A and C. Being an important horticultural crop, its production is threatened by root-knot nematode infections that make plants susceptible to wilting, growth reduction and infection by other pathogens like bacteria and fungi. Identifying the root-knot nematode species on the crop is paramount in designing proper management strategies especially crop rotation and resistance. In this study, tomato roots infected with *Meloidogyne* spp. were collected from fields in Kyenjojo and Masaka districts in Uganda. Using perineal patterns and molecular diagnostics, the three most common *Meloidogyne* species were identified. *Meloidogyne javanica* omit and was the most common followed by *Meloidogyne arenaria* and *Meloidogyne incognita.*

Key words: Identification, root-knot nematodes, tomatoes, Uganda.

INTRODUCTION

In Uganda, tomato is an economically important vegetable mainly grown by small scale farmers (Tumwine, 1999). It is grown as a sole crop all year round with the major commercial varieties being Money maker, Marglobe, Henz and Roma (Tumwine et al., 2002). Nutritionally, tomato is a good source of vitamins A and C, with recent studies indicating lycopene which has antidioxidant properties to protect against cancer and heart diseases in red tomatoes (Rao and Agarwal, 2000). Tomato growing is one of the areas looked on for horticultural development in Uganda (Osiru et al., 2001) but yields per hectare are still very low, that is, 10 ton/ha (Ssekyewa, 2006). The Agricultural industry in Africa is at stake because of *Meloidogyne* populations, worsened by the fact that farmers have limited awareness or information on *Meloigogyne* spp in their gardens (Kagoda et al., 2010; Onkendi and Moleleki, 2013a). Heavy galling and rotting of roots on dying tomato plants infected by root-knot nematodes in the nine studied districts of Uganda was reported by Bafokuzara in 1996. *Meloidogyne* spp are considered to be the most wide spread and destructive plant-parasitic nematodes and

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Figure 1. Map of Uganda showing district locations of Kyenjojo and Masaka from where samples were taken.
Source: Google earth

can cause an estimated yield loss of 25 to 50% over wide areas of cultivated land (Taylor and Sasser, 1978). A loss of 24 to 38% has been reported in tomatoes (Sikora and Fernàndez, 2005). Speijer and Kajumba (2000) revealed that *Meloidogyne* spp and other plant parasitic nematodes cause banana yield losses of 50% in Uganda. So far, more than 100 species of root-knot nematodes have been described (Hunt and Handoo, 2009*). Meloidogyne incognita, Meloidogyne javanica* and *Meloidogyne arenaria* are the most common in the tropical regions while *M. hapla, M. fallax* and *M. chitwoodi* are successful in temperate and cooler regions (Karssen and Van Aelst, 2001). Under severe infections, plants wilt and die because of alterations in nutrient and water uptake (Kaloshian et al., 1996), an example of *M. enterolobii* on guava in Vietnam (Iwahori et al, 2009). Damage on plants is more pronounced in tropical climates than in temperate because of the favourable conditions for nematode survival and multiplication (De Waele and Elsen, 2007; Kaloshian et al., 1996).

Meloidogyne spp. lifecycle can take three weeks or some months depending on environmental factors such as availability of a suitable host, temperature and moisture (Taylor and Sasser, 1978). The infective second-stage juveniles move in the soil and penetrate the root tips of the host plant using their stylet (Karssen, 2002). The juveniles (J2) feed and undergo three molts (J3, J4 and adult). Sometimes, vermiform males develop and migrate out of the root while the females remain sedentary (Moens et al., 2009). Several management strategies, such as host plant resistance, destruction of residual crop roots, rotation with non-host plants, sanitation and avoidance, and careful use of nematicides, have been reported to effectively control root-knot nematodes (Barker and Koenning, 1998). However, the use of resistant varieties remains the most promising option, especially for small-scale farmers having limited resources. With species identification, the design of the above management strategies can be realized (Blok et al., 2002). This article present species of root-knot nematodes associated with tomatoes in the districts of Masaka and Kyenjojo in Uganda.

MATERIALS AND METHODS

Sample collection

Samples of tomato roots and soil infected with root-knot nematodes were collected from two districts; Kyenjojo in western Uganda (coordinates 00 37N, 30 37E) at an elevation of 1,400 m above sea level, and Masaka district in south western Uganda (coordinates 00 30S, 31 45E) at an average altitude of 1,115 m above sea level (Figure 1). For Kyenjojo, we collected samples from 3 villages; Katooke, Kinogero and Kyeniga and from Masaka district, samples were collected from two villages; Kyabakuza and Kimanya. Fruiting tomato plants were uprooted, and plants with galls were selected to constitute the samples. The whole rooting system of a plant and about 500 g of soil around the plant were collected and placed in a plastic bag, labeled carefully and kept in a refrigerator at 8°C until nematode extraction.

Nematode extraction and pure culture establishment

Nematodes were extracted from tomato roots with the Baermann funnel technique (Hooper, 1990). Therefore, roots were washed in running water, chopped into small pieces and placed on a filter paper (Ederol Rundfilter, 40 g/m^2, Munktell Filter AB, Falun, Sweden) lined in a 2 mm sieve. The sieves were then placed on glass funnels filled with water to a level that just covered the bottom of the sieve and roots. The stem of the funnels connected to a rubber tube was closed with two metal clips. Second-stage juveniles hatched, moved through the filter paper and concentrated at the bottom of the rubber tube. Water was daily added to the funnels to prevent drying of roots as water evaporated. To culture the nematodes, newly hatched J2s tapped from Baermann funnels were inoculated on 3 weeks old tomatoes (*Solanum lycopersicon* cv. Marmande) that were earlier transplanted in 17cm diam. pots filled with sterilized soil (100°C, 16 h). Plants were maintained at 22 to 28°C in the green house with a 14 h light period.

After 6 weeks, infected tomato plants were removed carefully from their pots and the roots were washed to remove excess soil. Individual egg masses were picked at random using a stereoscopic microscope and made to propagate on tomato cv. Marmande to obtain pure cultures. Plants were kept in the green house with the same conditions mentioned previously for 6 weeks.

Collection of materials for perineal patterns and molecular identification

Infected roots from pure cultures were collected and washed. Adult females for perineal patterns were carefully teased out of the roots and kept in a solution of 0.9% sodium chloride. The rest of the root systems were put on Baermann funnels to collect juveniles and males for DNA analysis.

Identification

Perineal patterns

Perineal patterns were prepared according to a method described by Taylor and Netscher, (1974). Females were teased out of the root and placed in a solution of 0.9% sodium chloride. For each pure culture, five perineal patterns were cut from live egg-laying females in 45% lactic acid and mounted in glycerin. They were viewed under a light microscope (magnification1000×), and pictures were taken using a biological imaging soft-ware (cell D).

DNA extraction

DNA of J2s isolated from roots was extracted based on Holterman et al., (2006). Therefore, single to five J2s were picked up from water that was tapped from the funnels using a picking needle and added to 25 µL sterile water in a 0.2 ml-PCR tube. 25µL of worm lysis buffer containing 0.2M Nacl, 0.2M Tris-Hcl (Ph8.0), 1% (v/v) beta-mercaptoethanol and 800 µg/ml Proteinase K was added. The samples were then incubated for 1 h and 30 min at 65°C followed by 5 min. incubation at 99°C was in a thermcycler. The DNA was immediately stored at -20°C waiting further analysis.

PCR amplification

Primers used for DNA amplification are given in Table 1. The quality of the extracted DNA was checked using a universal primer D2a and D3b (De Ley et al., 1999), amplifying the D2 and D3 expansion region of the 28S rDNA nuclear gene. Positive samples with the D2a and D3b primers were further amplified using species-specific primers for *M. incognita*, *M. javanica* and *M. arenaria* (Zijlstra et al., 2000) based on the given specifications. The SCAR amplification reactions were performed in 0.2 ml PCR tubes with the reaction volumes of 50 µL. A master mix containing 34.6 µL double distilled water, 5 µL10 x PCR buffer, 6 µL $MgCl_2$, 1 µL of dNTPs mix, 0.5 µL reverse primers, 0.5 µL forward primers and 0.4 µL Taq DNA polymerase (Phamarcia) was prepared. Each volume was multiplied by the number of samples to be analyzed and brought into a 1ml tube and vortexed. Finally 48 µL of the master mix was pipetted into 0.2 ml Eppendorf tubes containing 2 µL of DNA. The tubes were then transferred to a thermcycler and the cycles ran according to (Zijlstra et al., 2000).The PCR products were then used immediately or kept at -20°C until use.

Mitochondrial DNA analysis

The primers C2F3 and 1108 (Powers and Harris, 1993) were used to amplify the mtDNA region between the cytochrome oxidase sub unit II (COII) and the 16S rRNA genes. These primers were used to further confirm the results of the species-specific primers. Amplifications were done in 50 µL reaction volumes. A master mix containing 34.6 µL ddH_2O, 5 µL 10x PCR buffer, 6 µL $MgCl_2$, 1 µL of dNTPs mix, 0.5 µL reverse primers, 0.5 µL forward primers and 0.4 µL Taq DNA polymerase (Phamarcia) was prepared. Finally 48 µL of the master mix were pipetted plus 2 µL of DNA into a 0.2ml tube. The temperature for the reaction was 94°C for 2 min. followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 2 min. The final elongation followed at 72°C for 8 min. JMV primers (Wishart et al., 2002), were used to detect *M. hapla, M. chitwoodi* and *M. enterolobii.*

Electrophoresis

The reaction products were resolved by electrophoresis on a 1.5% agarose gel in 1 × Tris borate EDTA buffer. For each PCR sample, 1 µL loading buffer-blue orange x6 was added to 5 µL PCR product in 0.2 ml Eppendorf tubes. The mixture was then added to the gel wells. A DNA marker (ladder 100 bp or 1 kb) was added into the first and the last well of gels. Electrophoresis was run at 100 v for 40 min. The gels were then stained in ethidium bromide bath for 30 min Visualization of gels was done on a UV-trans-illuminator and the picture taken.

RESULTS

Pure culture establishment

A total of 30 egg masses were collected from tomato plants to set up pure cultures. From these, 24 egg masses were successfully cultured and used for species identification.

Identification

Using molecular and perineal patterns methods of identification, *M. javanica*, *M. arenaria* and *M. incognita* was identified (Table 2). Surprisingly one of the cultures had a mixture of *M. javanica* and *M. arenaria*, and some of the cultures remained unidentified.

Perinneal patterns

Meloidogyne javanica

The perineal patterns (Figure 2) were typical for *M.*

Table 1. Primers used for molecular diagnosis of *Meloidogyne* species.

Primer code	Primer sequence 5' -3'	Source and specificity	
D2A D3B	ACAAGTACCGTGAGGGAAAGTTG TCGGAAGGAACCAGCTACTA	28S rDNA región, De Ley et al. (1999)	
Far Rar	TCGGCGATAGAGGTAAATGAC TCGGCGATAGACACTACAAACT	*M. arenaria*-specific SCAR, Zijlstra et al. (2000)	
Fjav Rjav	GGTGCGCGATTGAACTGAGC CAGGCCCTTCAGTGGAACTATAC	*M. javanica*-specific SCAR, Zijlstra et al. (2000)	
Finc Rinc	CTCTGCCCAATGAGCTGTCC CTCTGCCCTCACATTAGG	*M. incognita* specific SCAR, Zijlstra et al. (2000)	
C2F3 1108	GGTCAATGTTCAGAAATTTGTGG TACCTTTGACCAATCACGCT	COII/16SrRNA region of mtDNA, Powers and Harris (1993)	
JMV 1	GGATGGCGTGCTTTCAAC	5S gene	Wishart et al. (2002)
JMV2	TTTCCCCTTATGATGTTTACCC	IGS (*M. chitwoodi* and *M. fallax*)	
JMV hapla	AAAAATCCCCTCGAAAAATCCACC	IGS (*M. hapla)*	
JMV tropical	GCKGGTAATTAAGCTGTCA	IGS (*M. incognita, M. arenaria, M. javanica* and *M. enterolobii*)	

javanica with a rounded to flattened dorsal arch and conspicuous lateral lines that clearly separated the dorsal and ventral regions of the patterns.

Meloidogyne arenaria

The *M. arenaria* females' perineal patterns had the characteristic rounded to flattened, low dorsal arch, with some of the striae forming a small shoulder marked by bifurcations and curved to close striae in the lateral field and occasionally with a slight lateral line (Figure 3).

Meloidogyne incognita

Females showed characteristic oval to rounded perineal patterns with a high dorsal arch and wavy striae which bend towards the lateral lines and the absence of distinct lateral line incisures typical of this species (Figure 4).

Molecular identification

Ribosomal DNA amplification with D2A D3B primers

DNA obtained from single to five J2s from the 24 cultures was used. The D2D3 extension fragment of the 28S rDNA was used in order to check the quality of DNA and prevent false negatives in the species-specific PCRs. The samples checked showed the correct band size of 750 bp (data not shown).

PCR amplification of sequence characterized amplified region (SCAR)

The SCAR primer pairs (Table 1) were used for the diagnosis of *M. incognita*, *M. javanica* and *M. arenaria*. PCR with specific SCAR primer Fjav/Rjav produced a 670 bp product for *M. javanica* in cultures Kbz1, Kbz2, Kbz3, Hr205, Hr202, Hr101, Hr201, Hr103, Ky303, Msk1, and Msk6 (Table 2 and Figure 5). The species specific primer pair Far/Rar produced a scar product of 420 bp in cultures Msk1, Msk5, Msk2 for *M. arenaria* (Figure 6). Of the 24 cultures, only one was identified as *M. incognita* producing a 1200 bp product with the Finc/Rinc primer pair (Figure 7). However, the primer produced other weaker bands of the same length for cultures Ktk7, Ktk5, Ktk4, and Ky106 compared to the positive control.

PCR amplification of COII/16SrRNA of the mitochondrial DNA

The primer set C2F3 and 1108 was used to amplify the COII/16S rRNA gene of the mitochondrial DNA. It clearly

Table 2. Sample codes and identification results.

Egg mass codes	Village	District	Species detected
Kbz1	Kyabakuza	Masaka	*M. javanica*
Kbz2	Kyabakuza	Masaka	*M. javanica*
Kbz3	Kyabakuza	Masaka	*M. javanica*
Kbz4	Kyabakuza	Masaka	unidentified
Hr205	Kinogero	Kyenjojo	*M. javanica*
Hr202	Kinogero	Kyenjojo	*M. javanica*
Hr101	Kinogero	Kyenjojo	*M. javanica*
Hr201	Kinogero	Kyenjojo	*M. javanica*
Hr103	Kinogero	Kyenjojo	*M. javanica*
Hr206	Kinogero	Kyenjojo	*M. javanica*
Ktk2	Katooke	Kyenjojo	Unidentified species
Ktk4	Katooke	Kyenjojo	Unidentified species
Ktk5	Katooke	Kyenjojo	Unidentified species
Ktk7	Katooke	Kyenjojo	Unidentified species
Ky107	Kyeniga	Kyenjojo	Unidentified species
Ky2 01	Kyeniga	Kyenjojo	Unidentified species
Ky106	Kyeniga	Kyenjojo	Unidentified species
Ky303	Kyeniga	Kyenjojo	*M. javanica*
Ky301	Kyeniga	Kyenjojo	*M. incognita*
Msk1	Kimanya	Masaka	*M. arenaria, M. javanica*
Msk2	Kimanya	Masaka	*M. arenaria*
Msk4	Kimanya	Masaka	Unidentified species
Msk5	Kimanya	Masaka	*M. arenaria*
Msk6	Kimanya	Masaka	*M. javanica*

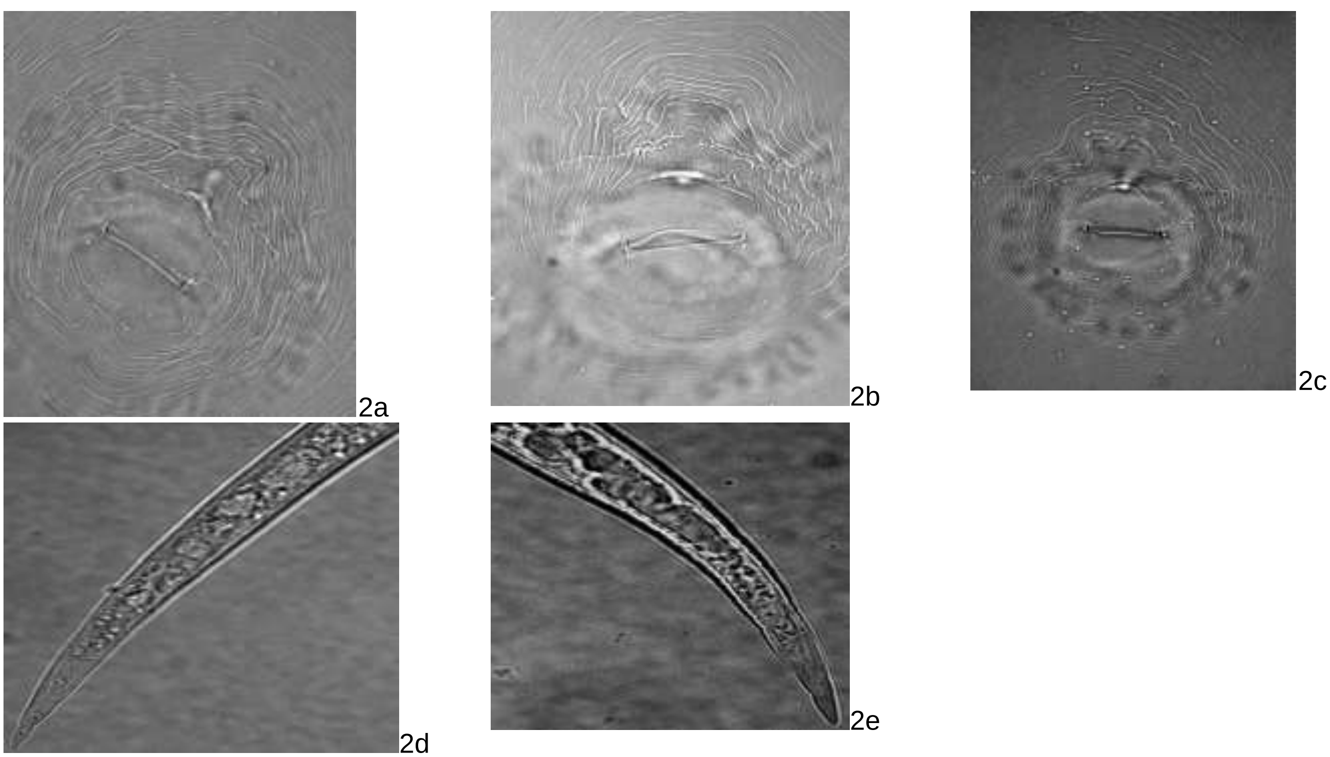

Figure 1. *M. javanica* perIneal patterns (photos 2a to 2c) and J2 tails (photos 2d and 2e).

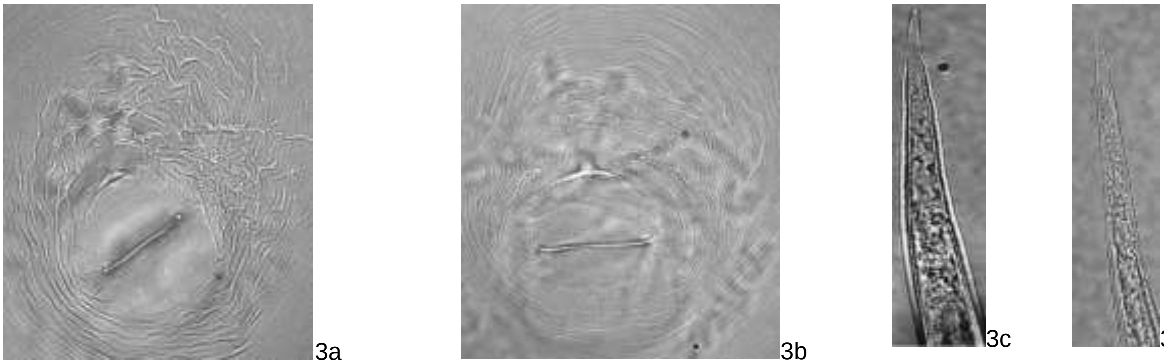

Figure 3. Perineal patterns (photos 3a and 3b) and J2 tails for *M. arenaria* (photos 3c and 3d).

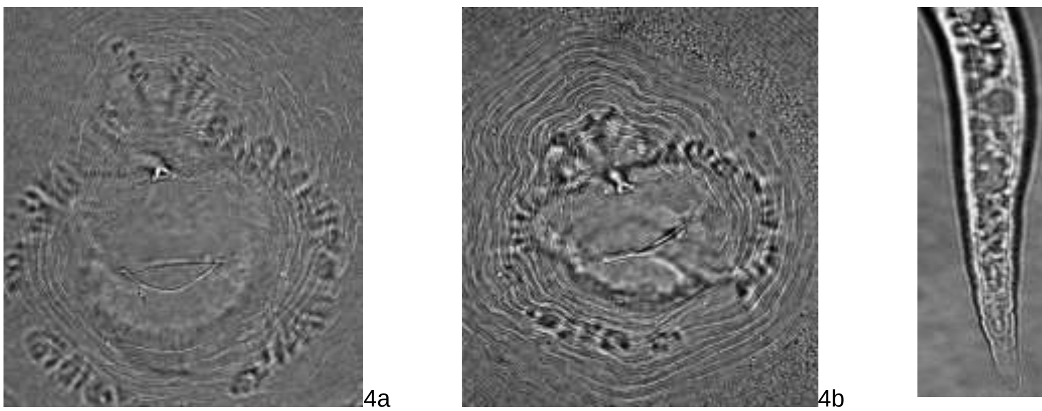

Figure 4. Perineal patterns (photos 4a and 4b) J2 tail for *M. incognita* (photos 4c).

amplified *M. arenaria* cultures with a base pair product of 1100 bp, but did not amplify for *M. javanica* and *M. incognita* as expected. On the same gel, weak bands of around 600 bp were detected for samples Ktk5, Ktk4, Ky107, Ky106, Kbz1 and Hr103 (Figure 8). To check if these bands could be allocated to *M. chitwoodi* or *M. hapla,* we used a one step multiplex PCR using JMV primers (table 1). Neither *M. chitwoodi*, *M. hapla* nor *M.* fallax were detected. To detect the presence of *M. enterolobii* in our samples, primers JMV1 and JMV tropical were used. An amplification product of 615 bp in all the tested samples indicated the absence of *M. enterolobi.*

DISCUSSION

Accurate and careful identification of *Meloidogyne* species infecting crops is a core for efficient use of plant resistance and successful management of root-knot nematodes. The severe infections on tomato plants and growth impairment observed in fields during sampling, requires immediate attention and implementation of feasible control strategies. The purpose of this study was to identify root-knot nematode species found in the districts of Kyenjojo and Masaka in Uganda. This gave light on the *Meloidogyne* species present in the areas. Results from the sampled areas indicate the presence of *M. incognita*, *M. arenaria* and *M. javanica* which is not a surprise as they are mentioned as the most common *Meloidogyne* species in tropical regions (Taylor and Sasser, 1978; Moens et al., 2009) like Uganda where annual temperatures are between 15 to 30°C. *M. javanica* dominated the presently studied samples with 45%, followed by 37.5% of unidentified species, 12.5% *M. arenaria* and lastly *M. incognita* with 4%. A similar finding was reported by Nono-womdin et al., (2002) in Tanzania and Naz et al., (2012) in Pakistan where *M. javanica* was widely distributed than *M. incognita*. These results are in disagreement with Eisenback et al. (1981) where *M. incognita* was the most prevalent among all *Meloidogyne* species in the studied areas of the international *Meloidogyne* project. As reported by Oliveira et al. (2011), the undescribed species in the samples was expected with the present movement of plant-derived materials around

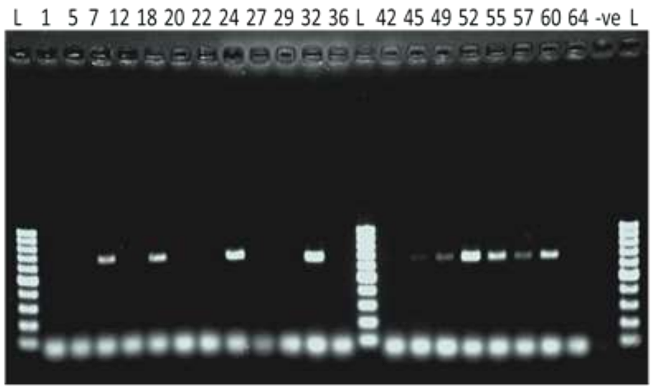

Figure 5. Amplification products of PCR reactions using Fjav/Rjav SCAR primer for *M.javanica* with a 1000bp ladder.

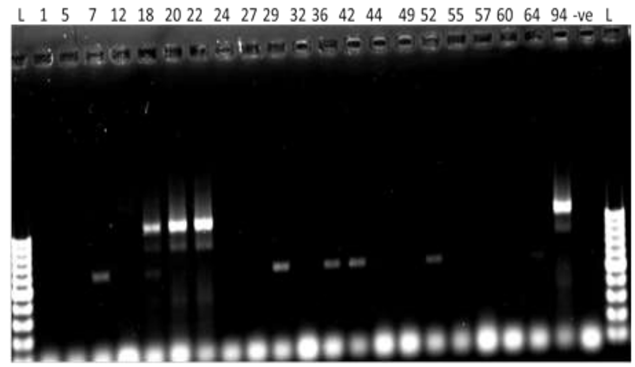

Figure 8. Amplification products generated from 1 to 5 Js with mitochondrial primer sets C2F3/ 1108 with a 1000bp ladder.

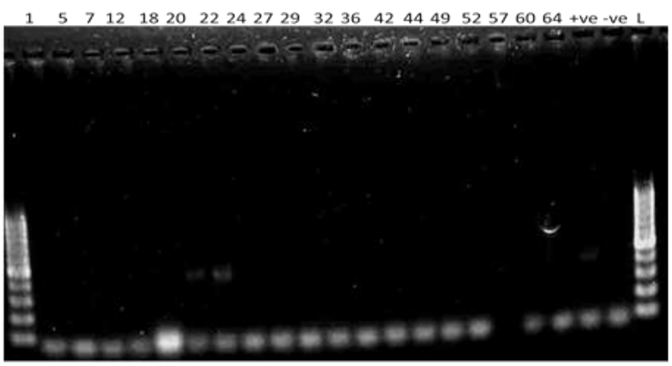

Figure 6. Amplification products of PCR reactions using specific primer of *M.arenaria* with a 1000bp ladder.

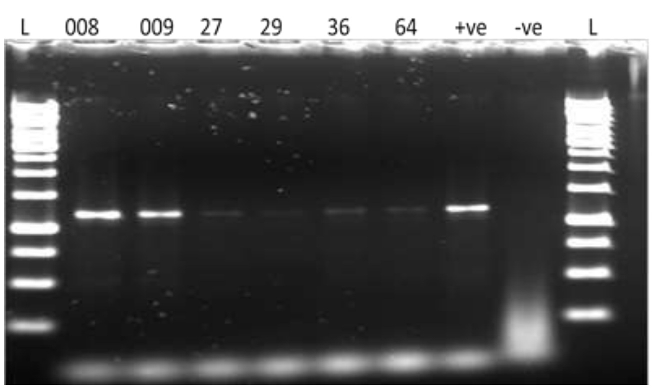

Figure 7. Amplification products of PCR reactions using Finc/Rinc SCAR primer for *M. incognita* with1 kb ladder.

the world, climate change, continuing growth of human population and consequent changes in land use and agricultural practices. There are also high possibilities of new species being introduced into the continent because of limited quarantine regulations. These come with implications on the agricultural sector like reduced crop yields, high costs of control and loss of revenue. Perineal patterns are considered important for differentiating *Meloidogyne* species. However, in this study, they were found to be variable making it difficult to separate species based on them alone. This was evident in some perineal patterns of *M. arenaria, M. incognita* and the species not identified having visible lateral lines and could easily be confused to be patterns of *M. javanica*. The same confusion was reported by Rammah and Hirschmann (1990), García and Sánchez- Puerta (2012).

SCAR primer sets were used to detect tropical *Meloidogyne* species; Fjav/Rjav, for *M. javanica*, Finc/Rinc for *M. incognita* and Far/Rar primers for *M. arenaria* successfully gave PCR products of 670 bp, 1200 bp and 420 bp, respectively. These results showed agreement with earlier studies (Zijlstra et al., 2000; Adam et al., 2007). However, Finc/Rinc for *M. incognita* formed other weak amplification products of the same length with the positive control. This suggeststhat probably, the species had some relationship with *M. incognita* though female perineal patterns which did not match exactly. Failure of the primer to give a strong band in the other isolates could also have been because of low copy in the amounts of the corresponding targeted regions of the primer sets (Adam et al., 2007). Moreover, according to literature studied, no information has been given in regard to such behavior of the primer.

The mitochondrial DNA is a good source of genetic markers for species and population genetics identification (Blok and Powers, 2009). It is important in molecular phylogenetic and diagnostic studies of root-knot nematodes (Stanton et al., 1997) because of its high copy number and fast evolution rate (Moritz et al., 1987). The primers C2F3/1108 were previously tested and reported as effective for amplifying the COII/LrRNA region of mtDNA for *M. javanica, M. arenaria* and *M. incognita (*Powers et al., 2005; Blok et al., 2002). For this study, they only gave an amplification product of 1.1 kb for *M. arenaria.* The primer did not give amplification products for *M. incognita* and *M. javanica* as it was expected. The *M. arenaria* result was in agreement with that of Blok et al. (2002) and Powers and Harris (1993). With the same primer, an amplification product of approximately 600 bp was observed. Such product size is close to the products given by *M. chitwoodi* and *M. hapla* using the same primer. JMV primers (Wishart et al., 2002) were used to specifically detect *M. hapla* and *M. chitwoodi* but no result was given. Moreover, depending on the annual temperatures of the sampled areas, the presence of these two species is not expected because *M. hapla* and *M. chitiwoodi* are found in cooler climates. Since Powers and Harris (1993) tested the primers for North American species, the general applicability of the primer on all *Meloidogyne* species was not confirmed. This gives some evidence to suggest that the amplification product could be of a new species outside the ones studied. Amplification for other species (*M. incognita* and *M. javanica*) failed, possibly due to inconsistent PCR conditions. Similar abnormalities of the primer were reported by Devran and Söğüt (2009) where the primer did not give any result. According to Adam et al. (2007), the inability to identify the species in some samples could be as a result of changes in priming sites that prevent amplification of the diagnostic product and changes in regions between the primer sites leading to the formation of products different from what is usual or the samples contain species different from the tested ones.

The use of species-specific primers was very helpful and gave confidence in the identification process. However, since the primers are species-specific for *M. incognita, M. javanica* and *M. arenaria*, they could not amplify other nematode species outside that category.

To assist in the identification of *Meloidogyne* species, the use of RAPD technique needs to be considered. Already in use today, random amplified polymorphic DNA are useful in characterizing intra and interspecific variation since there is no need for prior information on the DNA sequence of the targeted genome (Block and Powers, 2009). For example, Adam et al. (2007) found consistent amplification patterns from Males, females, and J2 s of *M. javanica* using RAPDs. Cenis (1993) reported the usefulness of RAPD-PCR to diagnose and address the unsolved questions on genetic variation and population genetics of root-knot nematodes. Furthermore, sequence analysis of rDNA has increased in importance in the identification of *Meloidogyne* spp. Different sets of primers have been reported to amplify different regions of rDNA. For example, the primers designed by Vrian et al. (1992) have been used to amplify the ITS region for *Meloidogyne* spp. to form a product of approximately 800-bp which can then be sequenced to produce species-specific primers or enzyme digested fragments (Blok and Powers, 2009). Blok and Powers, (2009) also reported that species that are difficult to separate using morphological and biological features like *M. hispanica* from *M. arenaria* and *M. incognita* have been separated by the comparisons of their ITS, 18S and D2/D3 a region within the 28S gene. Sequence polymorphisms in the IGS region have been used by Wishart et al. (2002) and Blok et al. (2002) to distinguish *M. chitwoodi* and *M. fallax* from other species like M*. enterolobii*, *M. hapla* and *M. incognita* / *M. arenaria* / *M. javanica* using JMV primers.

A mixture of two species in a culture presumed to be from a single egg mass which could have been caused by two females lying close together in the root and forming an egg mass that can easily be mistaken to be from one female. The same finding was reported by Devran and Söğüt, (2009). Further research should employ other methods like use of sequence-based methods on the ribosomal DNA, mitochondrial DNA, and use of isozymes to describe species that were not identified.

Conclusion

There are new emerging species of root not nematodes and it is difficult to conclude that a single method is sufficient to delineate the species. Generally, there is limited information on the species of root-knot nematodes and the associated yield losses in Africa. Identification of these nematodes will guide management programs because some species are host specific.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Relationship between the sensory attributes and the quality of coffee in different environments

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In order to understand the relationship between the sensory attributes of coffee (including factors such as acidity, flavor, and aftertaste), and given the possible influences of genetic factors on coffee quality, we evaluated the sensory quality of 10 commercial cultivars of *Coffea arabica* in three important coffee regions of Minas Gerais, Brazil, and examined, via path analysis, the interactions of the attributes that determine coffee quality. The experiments were conducted in three cities (Lavras, Patrocínio, and Turmalina) in three separate regions of cultivation in Brazil. Beverage quality was evaluated by assessing the sensory attributes of the cultivars Oeiras MG 6851, Catiguá MG 1, Sacramento MG 1, Catiguá MG 2, Araponga MG 1, Paraíso MG H419-1, Pau Brasil MG 1, Catiguá MG3, Topázio MG1190, and Bourbon Amarelo LCJ10, the last of which was used as the control cultivar. Experiments were designed in a randomized block consisting of 3.5 m × 0.7 m plots containing 10 plants of the various cultivars, with three replicates of each plot. We concluded that cultivation environment greatly influences the quality of the coffee produced by the cultivars. The cultivars Bourbon LCJ10, Araponga MG1, Paraíso MG H419-1, Sacramento MG1, Oeiras MG6851, and Catiguá MG2 exhibiting high potential for cultivation in the regions of Lavras, Patrocínio, and Turmalina.

Key words: *Coffea arabica*, sensory analysis, path analysis, genotype-environment interactions.

INTRODUCTION

Interest in unique cultivars for the production of specialty coffees has increased significantly in recent years due to the increasing global demand for high-quality coffee, leading researchers to focus not only on productivity factors but also the beverage quality produced by these new varieties (Figueiredo et al., 2013).

Environmental factors, dictated by altitude and latitude, and beverage quality are closely intertwined. Coffee is grown throughout the state of Minas Gerais, Brazil, accounting for 70% of *Coffea arabica* production in Brazil and 25% of global production (CONAB, 2016). Due to the quantity and diversity of coffee cultures, genotypic-environmental interactions greatly influence the quality of the final product (Pereira et al., 2010; Sobreira et al., 2015th, 2015b).

Southern Minas Gerais is largely mountainous, with altitudes ranging from 700 to 1,255 m, and features a climate that falls somewhere between the B2 and B3 types (wet) prevalent in most parts of the region (Silva et al., 2008). Cerrados, an area within Minas Gerais, is located on a plateau where altitudes range between 820 and 1,110 m, and experiences a type B1 (wet) climate, factors that potentially favor the production of high-quality coffee (Barbosa et al., 2012). This region is Brazil's first region to have a geographical demarcation internationally recognized; it also has a certificate of origin, traceability, and a sustainability program (Barbosa et al., 2010). Specifically the region of Jequitinhonha Valley has relatively non-humid climate and great potential for quality coffee production (Farnezi et al., 2010).

Evaluation of coffee quality in Brazil is performed by coffee cupping sessions, in which professional coffee tasters ascribe grades to several attributes, with the sum of the grades providing the final score (Chalfoun et al., 2013). This coffee cupping session can detect whether the coffees from different cultivars have special nuances, generally related to chocolaty, caramelly, fruity and flowery tastes (Sobreira et al., 2015a).

Our goal here was to gain a better understanding of the relationship between these sensory attributes and the influence of genetic factors on coffee quality by evaluating the sensory quality of 10 commercial cultivars of *C. arabica* grown in three prominent coffee regions of Minas Gerais state, and using path analysis to examine the interactions between these attributes and how they affect the coffee-beverage quality.

MATERIALS AND METHODS

Description of the experimental area

Experiments were conducted in January 2006 in three significant coffee regions of Minas Gerais state, consisting of Lavras (Experimental Campus of Federal University of Lavras, southern Minas Gerais), Patrocínio (Experimental Farm of the Agricultural Research Company of Minas Gerais, in Alto Paranaíba), and Turmalina (Capão da Estiva Farm, in the Jequitinhonha Valley). The average altitude of these three municipalities are 966, 920, and 820 m, respectively; soils in all three locations are classified as dark red latosols, as they have low saturation (V < 50%) and a Fe_2O_3 (for H_2SO_4) content among 180 and 360 $g \cdot kg^{-1}$, which mostly occurs in the topmost 100 cm of the B horizon (Santos, 2006).

Experimental design and treatments

We evaluated 10 cultivars of *C. arabica* L.: Oeiras MG6851, Catiguá MG1, Sacramento MG1, Catiguá MG2, Araponga MG1, Paraíso MG H419-1, Pau Brasil MG1, Catiguá MG3, Topázio MG1190, and Bourbon Amarelo LCJ10, the last of which was used as the control cultivar, as it is known to produce high-quality beans for use in specialty coffees (Ferreira et al., 2012; Figueiredo et al., 2013.).

We used a randomized block design with three replicates, consisting of plots containing 10 plants, six of which composed the core plot. We used a spacing pattern of 3.5 m between rows and 0.70 m between plants, equivalent to 4,081 plants ha^{-1}.

Variables analyzed

Analyses of the sensory attributes were performed annually for the first three crops. After harvesting, fruits in the cherry and nut stages were separated via a water tank and a 3.0 mm × 3.0 mm wire-mesh-screen sieve. Separation of fruits in cherry from those still in green, which remained in the sample, was performed using a coffee peeler, ensuring that only ripe fruits were peeled; in total, seven gallons of peeled cherries was obtained. These samples were equally distributed in sieves of 1 m² (composed of a wooden frame and a 2.00 mm × 1.00 mm screen mesh, manufactured with polyethylene yarn) and turned 12 times daily until the coffee grains attained levels of approximately 11 to 12% water content (wb). After drying, the samples were processed and prepared for use in the sensory analyses.

Sensory analysis was performed by members of the Brazilian Specialty Coffee Association (BSCA), and followed established BSCA procedures. Attributes consisted of clean cup (Ccp), sweetness (Swt), acidity (Acd), body (Bod), flavor (Fla), aftertaste (Aft), balance (Bln), and overall impression (Ove), with each attribute assigned a score ranging from 0 to 8 based on the intensity of the sample, thus ensuring greater objectivity than conventional "cup tests". The sum of the scores was considered to be the final score (Fsc) of the beverage, from which was determined the final classification. Each sample was given a baseline score of 36 points, which was incorporated into the scoring of each attribute; specialty coffees were those for which final scores exceeded a value of 80 (BSCA, 2014).

Statistical analysis

The averages of the quality attributes of the first three crops were used in the statistical analyses. The data obtained were adjusted to the following linear mixed model (Smith et al., 2005):

$$y_{ijk} = \mu + c_i + b_{j(k)} + a_k + ca_{ik} + \epsilon_{ijk},$$

where y_{ijk} is the observed value of the variable response related to the *i*th cultivar of the *j*th block in the *k*th environment; μ is a constant inherent to all observations; c_i is the effect of the *i*th cultivar (expressed as $i = 1, ..., 10$); $b_{j(k)}$ is the effect of the *j*th block within *k*th environment ($j = 1, 2, 3$; $b_j \sim N(0, \sigma_b^2)$); a_k is the effect of the *k*th environment (where k = 1, 2, and 3); ca_{ik} is the effect of the interaction between the *i*th cultivar and the *k*th environment; and ε_{ijk} is the experimental error associated with the y_{ijk} observation ($\varepsilon_{ijk} \sim N(0, \sigma^2)$).

A fixed effects model is a statistical model were analyzed with a

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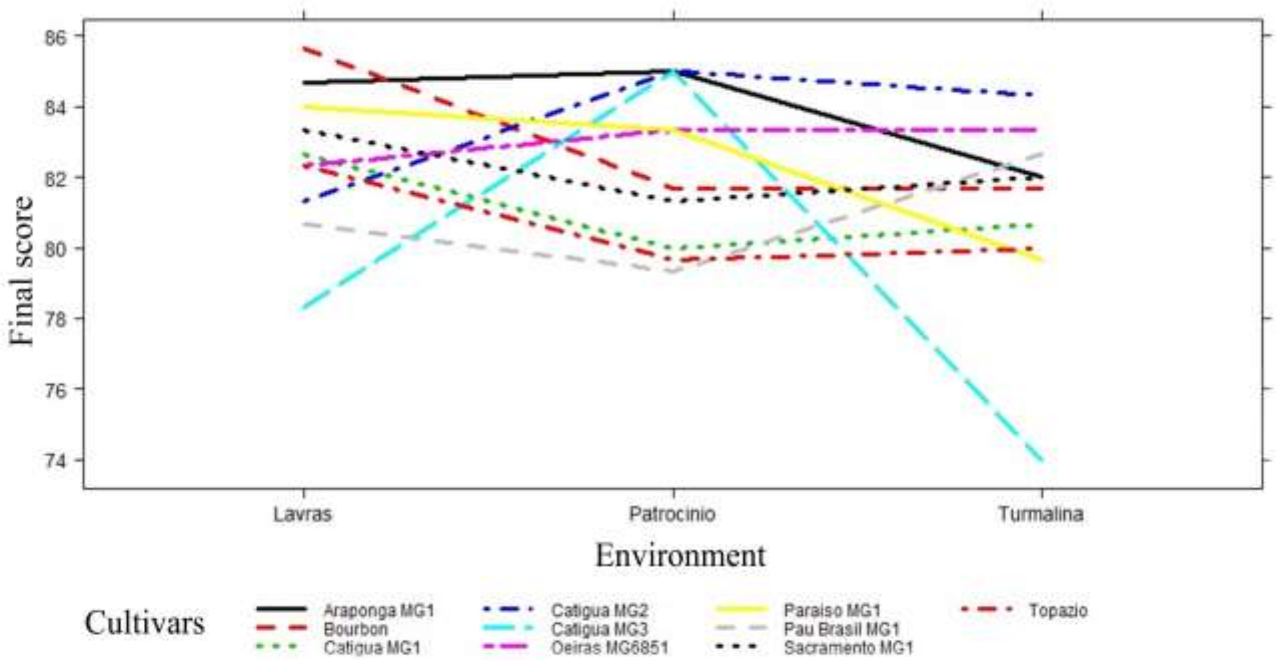

Figure 1. Cultivar performance relative to the final scores in the three cultivation regions of Lavras, Patrocínio, and Turmalina.

Wald type II test. For this study, the average performance of the cultivars was calculated as the adjusted means of the cultivars for each environment and their respective confidence intervals (95%), according to the statistical model described above, using the LSmeans procedure of the library "doBy" (Hojsgaard and Halekoh, 2014) in R (R Development Core Team, 2013).

Path analysis was used to examine both direct and indirect correlations between the quality attributes and the final score (Wright, 1921). The final score (Nsc) was considered to be the main variable, with the attributes Ccp, Swt, Acd, Bod, Fla, Aft, Bln, and Ove set as the explanatory variables.

Data were standardized by dividing the deviation of each observation between the averages of the attributes by the standard deviation for that attribute. Standardized observations were assigned an average of zero and a variance of one; in our analysis, Fsc was considered to be the basic variable (Y), with Ccp, Swt, Acd, Bod, Fla, Aft, Bln, and Ove as the primary factors. It was necessary to ensure that no multicollinearity existed among the explanatory variables in order to maximize reliability of the estimates of the direct and indirect effects obtained via the path analysis. As such, analysis of the correlation matrix between the explanatory variables model and the condition number of the matrix was obtained, that is, the relation between the largest and smallest self-value of the matrix. The condition number (CN) for the correlation matrix was CN = 17, indicative of a slight multicollinearity (CN < 100), but one too weak to pose a problem for path analysis (Montgomery; Peck, 1992). All statistical procedures were performed using R software (R Development Core Team, 2013).

RESULTS AND DISCUSSION

We detected a genotype × environment interaction effect (χ^2 91,440), indicating that beverage quality of the cultivars varies with environmental factors such as altitude, and the genetic diversity of the cultivated material-a result that accords with the findings of previous research (Mendonça et al., 2005; Pereira et al., 2010; Ferreira et al., 2012; Chalfoun et al., 2013).

In general, beverage quality varied for all cultivars among the three regions included in this experiment (Figure 1), a result that supports recommendations that coffee production be concentrated in specific regions. According to the BSCA methodology, final scores must be higher than 80 for classification as specialty coffee (Chalfoun et al., 2013). Scores between 71 and 75 were assigned to hard beverage, 75 to 79 for only soft drink, 80 to 84 for soft drink, and above 85 for strictly soft drink (Martinez et al., 2014). The Bourbon LCJ10 cultivar, a genotype with good potential for specialty coffee production, had final average scores of 85.67 in Lavras and 81.67 in Patrocínio and Turmalina.

The Catiguá MG3 cultivar exhibited the greatest degree of variation in response to different environmental conditions. This cultivar scored high (85) in Patrocínio and was thus classified as a specialty coffee in that region, but scored considerably lower in both Lavras (78.33) and Turmalina (74).

Overall, the averages of the final scores of the 10 cultivars were very similar among the three municipalities, ranging from 82.36 in Patrocínio to 82.53 in Lavras to 81.03 in Turmalina. The latter site is at a much lower elevation than the other two locations-an indication that beverage quality tends to improve with increasing elevation (Bertrand et al., 2012). The cultivar Bourbon had the highest final score of all in Lavras, with

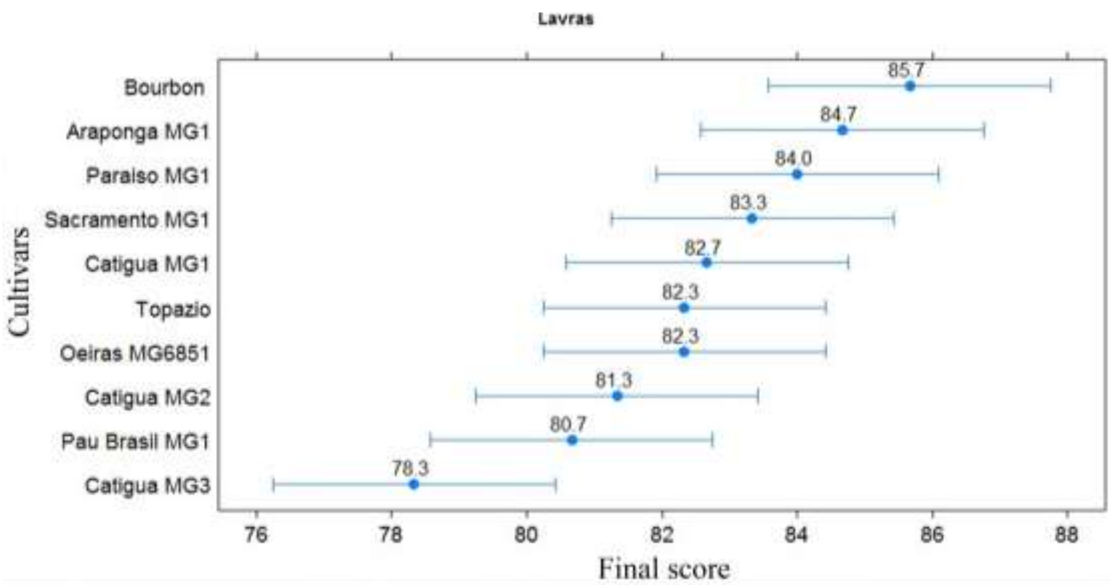

Figure 2. Averages of the final scores of the coffee cultivars in Lavras.

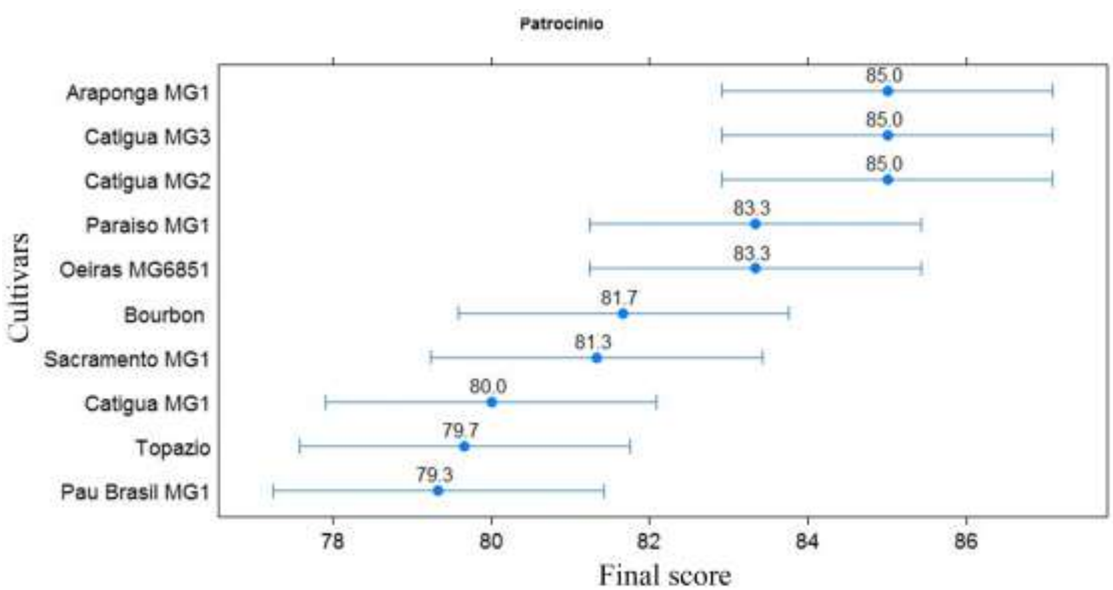

Figure 3. Averages of the final scores of the coffee cultivars in Patrocínio.

no overlap with the confidence intervals of the cultivars Catiguá MG2, Pau Brasil MG1, and Catiguá MG3, indicating a clearly superior performance of Bourbon over the others (Figure 2). Similarly, Araponga MG 1, the final score of which was only a single point below the Bourbon control, overcame the Catiguá MG3 cultivar, highlighting the potential of this cultivar for producing high-quality coffee. Most of the cultivars assessed in Lavras scored higher than 80, with the exception of Catiguá MG3. These results corroborate those of Barbosa et al. (2012), who suggested that many of the environmental conditions prevalent in southern Minas Gerais, including average temperatures, precipitation levels, and elevation, are ideal for the production of high-quality coffee.

In the Alto Paranaíba region, represented by the city of Patrocínio, the cultivars Araponga MG1, Catiguá MG3, and Catiguá MG2 scored higher than the cultivars Catiguá MG1, Topázio MG1190, and Pau Brasil MG1 (Figure 3). Moreover, the cultivars Araponga MG1, Catiguá MG3, Catiguá MG2, Paraíso MG H419-1, and

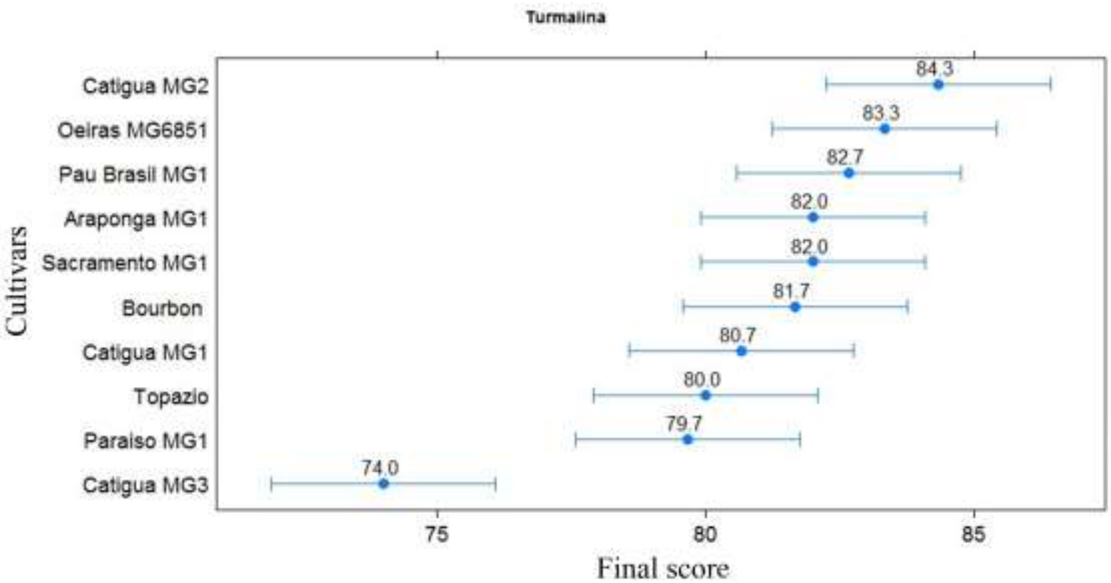

Figure 4. Averages of the final scores of the coffee cultivars in Turmalina.

Oeiras MG6851 had final scores higher than the control Bourbon cultivar, demonstrating that the combination of genetic and environmental factors affects the chemical composition of coffee, corroborating the findings of other authors (Ferreira et al., 2012; Figueiredo et al., 2013, 2015). In this location, only Topázio MG1190 and Pau Brasil MG1 failed to attain the minimum score for classification as specialty coffees. The experiment was performed at 820 m of altitude.

In Turmalina, the cultivar Catiguá MG3 was graded as having little potential for specialty coffee production compared to the other cultivars, with a final score of 74 and no overlap between the confidence intervals for this cultivar and the other nine cultivars (Figure 4). This result shows the influence of the environmental conditions in the Turmalina region on the qualitative characteristics of this cultivar whereas 'Catiguá MG3' had the highest score in Patrocínio. The cultivars Catiguá MG2, Oeiras MG6851, Araponga MG1, Sacramento MG1, Bourbon, and Catiguá MG1 also scored higher than 80, with final scores ranging from 80.7 to 85.

In addition to the Bourbon control, the cultivars Araponga MG1, Paraíso MG H419-1, Sacramento MG1, Oeiras MG6851, Catiguá MG2, and Catiguá MG1 scored the highest in all three regions, which allows us to infer that genetics has a more dominant influence than environmental conditions in these cultivars. It is worth noting here that all cultivars, with the exception of the control, derive from crosses between the cultivars Timor Hibryd and Catuaí, which are widely used in the specialty coffee market (Kitzberger et al., 2011; Scholz et al., 2013).

The quality of these cultivars has been reported elsewhere. Pereira et al. (2010) showed that Catiguá MG2 had sensory quality scores superior to the Bourbon cultivar at an altitude of 900 m for two consecutive years, as did the Sacramento MG1 and Catiguá MG1 cultivars, results similar to those presented by Chalfoun et al. (2013). Fassio et al. (2016), while assessing these same cultivars in Lavras and Patrocínio, gave Catiguá MG2, Paraíso MG H419-1, and Araponga MG1 the highest sensory scores, and Sobreira et al. (2015b) found that cultivars deriving from Timor Hybrid de scored higher than traditional and Bourbon cultivars.

Simple correlations between the explanatory variables (Ccp, Swt, Acd, Fla, Bod, Aft, Bln, Ove) and the Fsc were performed in order to better understand the relationships between the various attributes, which could then be used to guide sensory analyses (Table 1). The Fla attribute had the strongest positive correlation with Fsc (0.80), followed by Ccp and Swt (0.74). Whereas the final grade is obtained by determining the sum of the individual characteristics, these correlation analyses indicated that improvement in the quality of the other sensory attributes could be obtained via selection for these three factors.

The attribute of flavor represents the intensity, quality, and complexity of the combination of all of the attributes, whereas Ccp refers to the absence of negative impressions during ingestion and Swt refers to the pleasantness of the taste, which is the result of the presence of certain carbohydrates. Bitterness (or "green" flavor) in this context is the opposite of Swt (SCAA, 2014). Such terms are commonly used by both professional coffee tasters and researchers involved in analyses of the sensory qualities of coffee (Kitzberger et al., 2011; Gamboa et al., 2013). All of the correlations both between the main variable and the explanatory variables and among the various explanatory variables were positive, indicating that all of the sensory attributes

Table 1. Correlations between the sensory characteristics of coffee cultivars in the three regions of Minas Gerais assessed in this study.

	Sensory characteristics of beverage[1]								
	Fsc	**Ccp**	**Stw**	**Acd**	**Bod**	**Fla**	**Aft**	**Bln**	**Ove**
Fsc	1.00***								
Ccp	0.74***	1.00***							
Dcr	0.74***	0.60***	1.00***						
Acd	0.53***	0.22***	0.27***	1.00***					
Bod	0.61***	0.40***	0.40***	0.38***	1.00***				
Fla	0.80***	0.57***	0.64***	0.26***	0.31***	1.00***			
Aft	0.61***	0.26***	0.32***	0.22***	0.29***	0.41***	1.00***		
Bln	0.71***	0.69***	0.41***	0.24***	0.35***	0.57***	0.30***	1.00***	
Ove	0.73***	0.43***	0.40***	0.29***	0.44***	0.53***	0.40***	0.52***	1.00

[1]Final score (Fsc), clean cup (Ccp), sweetness (Stw), acidity (Acd), body (Bod), flavor (Fla), aftertaste (Aft), balance (Bln) e overall impression (Ove).**(value - $p < 0.05$) e ***(value - $p < 0.001$).

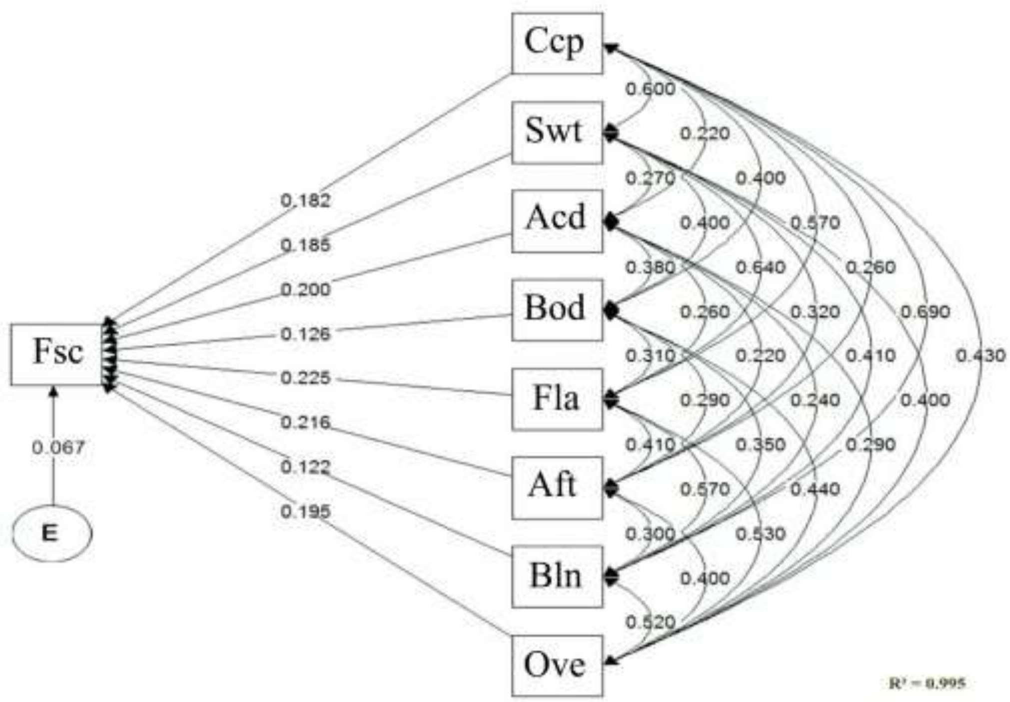

Figure 5. Graphical representation of direct and indirect effects of the sensory attributes on the final score of beverage quality, where: final score (Fsc), clean cup (Ccp), sweetness (Stw), acidity (Acd), body (Bod), flavor (Fla), aftertaste (Aft), balance (Bln) and overall impression (Ove).

are important components in determining beverage quality, corroborating the results of several other studies of *C. arabica* genotypes (Dessalegn et al., 2008; Kathurima et al., 2009; Sobreira et al., 2015). However, these correlations are only indicative of associations among the various attributes and do not reveal cause-and-effect relationships. For this, analysis of the direct and indirect effects among the variables is required, in order to identify the most effective selection criteria.

The ranks, direct effects (pyx_i) of the explanatory variables (x_i) on the main variable (y), and correlations (r_{xixi}) among the explanatory variables are shown in Figure 5. The indirect effect of an explanatory variable (x_1) on the main variable (y) via a second explanatory variable (x_2) can be expressed as pyx_2*r_{12}. Note that the variables explained 99.50% of the total variation of the final scores, according to the determination coefficient (R^2). All estimates of the ranks of the direct effects were higher than the residual effect (0.067), indicating that all of the attributes are important and influence the final score, and corroborate the results shown in Table 1. Of the attributes, Fla was the most influential, followed by Aft, Acd, Ove, Swt, Ccp, Bod, and Bln.

The estimated rank for Fla was 0.225; estimates of the indirect effects of Fla on Fsc were higher than the residual effect for only five variables, of which the indirect effect of Fla via Swt was greatest (pyx_2*r_{12} = 0.185*0.640 = 0.1184), due to the highly positive correlation between flavor and sweetness (0.640), followed by indirect effects on Ccp (0.1038), Ove (0.1034), Aft (0.0885), and Bln (0.0693).

Aftertaste had a direct effect of 0.216 on Fsc, and indirect effects of 0.0924 on Fla and 0.0780 on Ove. Other indirect effects were lower than residual ones. Acidity had a direct effect of 0.2002 on Fsc, and no indirect effect was greater than the residual effect. As such, it would seem that Acd does not have any relationships with other attributes that may influence Fsc but exerts only a direct influence on the final score, which can be attributed to the low correlations between Acd and the other attributes (Figure 5).

Sobreira et al. (2015b) divides the acidity category into the three subcategories alive, sweet, and undefined/medium, flavor into chocolaty, fruity, and caramel, and aftertaste into long, refreshing, and pleasant. In this paper, these three categories – acidity, flavor, and aftertaste – were considered to be decisive in determining the final score of the beverage. Kathurima et al. (2009), in a study of 42 genotypes of *C. arabica* in Kenya, observed that aftertaste, acidity, and flavor correlated strongest with quality, a result similar to ours. Likewise, in a similar study, Sobreira et al. (2015a) observed that aftertaste and flavor correlated highly with quality for the germplasm Timor Hybrid.

The direct effect of Ove on the Fsc was 0.195, and the indirect effect of Ove via Fla was 0.119, the highest level of indirect effect on Ove of all attributes. Although the correlation between Ove and Bln (0.520) was similar to the correlation between Ove and Fla (0.530), the indirect effect of Ove via Bln was lower than the residual effect due to the direct effect of Bln being quite low (0.122) compared to the direct effect of Fla on the Fsc (0.225). Sweetness had a direct effect of 0.185 on the Fsc, with indirect effects of 0.109 via Ccp, 0.144 via Fla, 0.069 via Aft, and 0.078 via Ove, with the indirect effects via the other attributes lower than the residual. Clean cup had a direct effect of 0.1821 on the Fsc, and indirect effects of 0.111 via Swt, 0.128 via Fla, 0.084 via Bln, and 0.084 via Ove, all higher than the residual effect.

The attributes Bod and Bln had the least amount of influence on the Fsc, with estimated direct effects of 0.126 and 0.122, respectively. For both features, the effects of Swt, Fla, and Ccp were higher than the residual, and the effects of the latter two attributes were superior to the direct effect of Bln on the Fsc.

Conclusions

1. The cultivation environment greatly influences the quality of the beverage of coffee cultivars.
2. The cultivars Bourbon LCJ10, Araponga MG1, Paraíso MG H419-1, Sacramento MG1, Oeiras MG6851, and Catiguá MG2 exhibited potential for the production of high-quality coffee in the regions included in this study.
3. The attributes acidity, flavor, and aftertaste exert a strong influence on coffee quality.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Distribution, incidence and farmers knowledge of banana *Xanthomonas* wilt in Burundi

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Banana Xanthomonas wilt (BXW) is a devastating bacterial disease caused by *Xanthomonas campestris* pv. *musacearum.* The disease was simultaneously reported in Cankuzo and Bubanza provinces, Burundi, in November 2010. However, the extent to which the disease has spread to other banana growing regions in the country is unknown. Therefore, to ascertain the distribution and incidence of the disease and farmers' knowledge on measures to control the disease, a survey was conducted in all 16 banana growing provinces of Burundi in August 2011. A total of 208 farms were sampled, selecting six farms per surveyed commune, three affected and three non-affected. The survey was conducted using a structured questionnaire. The disease was present in 10 out of 16 provinces constituting all agricultural lands in Burundi. The highest incidence was recorded in Ruyigi province (34%), where the Kayinja system is dominant and the lowest in Muyinga (3%), where the East African Highland bananas (EAHB) dominate. Awareness of BXW symptoms, modes of spread and control measures was generally low, ranging from 8 to 30% of households surveyed. The limited knowledge of the disease among farmers was thought to be largely responsible for driving the epidemic in Burundi.

Key words: Debudding, disease incidence, *Musa* species, *Xanthomonas campestris* pv. *Musacearum*.

INTRODUCTION

Banana (*Musa* species) is the first crop in Burundi in terms of production with 1,848,727 tonnes in 2011, followed by sweet potatoes and cassava (FAOSTAT, 2016). Plantains, which are also *Musa* spp., are not common in Burundi. Banana is used for beer, cooking the and for dessert and therefore contributes significantly to food security and income. The crop protects land against soil erosion especially in Burundi, where the landscape is hilly with steep slopes (Rishirumuhirwa, 1997). However, banana production is threatened by wilt (BXW) caused

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by *Xanthomonas campestris* pv. *musacearum* (Xcm). BXW is one of the recent threats to banana production in East and Central Africa. The disease was first reported in Ethiopia on Ensete and banana (Yirgou and Bradbury, 1974; Smith et al., 2008). It was first reported in East and Central Africa in Uganda and the Democratic Republic of Congo (DR Congo) in 2001 and since then has continued to spread to most countries in the region (Tushemereirwe et al., 2003; Karamura et al., 2008). Other banana production constraints include soil fertility, poor management, inaccessibility to fertilizers, diseases and pests such as banana bunchy top disease, *Fusarium* wilt and Sigatoka (Tinzaara et al., 2007). BXW is a devastating disease with the potential to drastically reduce banana production and negatively impact farmers' livelihoods. At the vegetative stage, symptoms of BXW are mainly yellowing and wilting of leaves while flowering banana shows withering and rotting of male buds and premature ripening of the fruits (Tushemereirwe et al., 2003; Karamura et al., 2008; Ssekiwoko et al., 2010). The disease is spread by insects, birds, infected planting materials, cutting tools and animals which can browse on healthy banana after feeding on diseased mats (Biruma et al., 2008, Tinzaara et al., 2009). Recommended cultural control measures for the disease include destruction of affected plants, disinfection of farm tools, using clean planting material and early removal of male buds using a forked stick and keeping animals out of the fields (Tinzaara et al., 2016).

In Burundi, the disease was first confirmed in November 2010 in the provinces of Cankuzo and Bubanza which are bordering with Tanzania and Rwanda, respectively (Niko et al., 2011). In Cankuzo (East of Burundi), the disease was reported in Gisagara Commune, Gitanga colline, about 25 km from the Tanzania border whereas in Bubanza province (West of Burundi), the hotspot was observed in Musigati commune at Rushiha colline about 60 km from DR Congo border (Niko et al., 2001). A few months later, the disease spread rapidly to other provinces in the country and new pockets were identified in seven provinces. However, little is known on the actual disease distribution, incidence and farmer awareness on the disease in the country. This study, therefore, was aimed at determining BXW distribution, incidence and farmers' knowledge of disease symptoms, mechanisms of spread and control in Burundi.

MATERIALS AND METHODS

A survey was carried out in August 2011 covering all 16 provinces of Burundi. Two communes per province were surveyed, except in Makamba province where five out of six affected communes were surveyed. The number of communes per province in Burundi varies from 5 to 9 and the country has a total of 117 communes. Six farms per commune were selected randomly (three affected by BXW and three unaffected) from the 35 communes visited out of 117 countrywide.

Data from 208 farms were recorded using a structured questionnaire. The questionnaire was designed to capture information on banana production constraints in general but with more emphasis on BXW. Farmer interviews were conducted and then observations made in banana fields to record data on banana plantation management, disease symptoms, and incidence of the main banana diseases and pests and the affected cultivars. Enumerators were selected based on the working experience on banana management and were trained to distinguish banana diseases, especially those that are present in Burundi. They further were further trained to use the questionnaire theoretically in a meeting as well as practically on farm level by simulating data collection from farmers.

The incidence of BXW, which consisted of percentage of diseased plants in a field, was calculated based on observation and counting of 30 plants selected using diagonal walks in the field. Geographical coordinates were also recorded using GPS unit to map the disease distribution. Data were subjected to descriptive statistics (means and percentages) and analyzed using SPSS 11.0, ArcGis 9.3 software.

RESULTS

The results of the farmer knowledge about cultural practices that contribute to BXW management are presented in the Figure 1. Banana plantations (57.2%) in Burundi are more than 20 years old while 59.6% are poorly managed. Forty-nine percent of the banana grown in Burundi is used for beer, 28.4% for cooking, 20.9% for dessert types and 0.7% for plantains. Banana plantations are mostly established in a mixed cultivars system in which farmers use at least two cultivars. The most common varieties observed include cooking banana Igisahira gisanzwe- AAA–EA, 19.4%), beer bananas Igitsiri (AAA-EA, 16.3%) and Kayinja (ABB, 13.6%), dessert banana Kamaramasenge (AAB) whereas hybrids FHIA (AAAA), which are currently the most widely promoted in the country, are not yet widespread (0.9%) on farm level.

BXW was observed in 10 (Bujumbura, Cibitoke, Bubanza, Makamba, Rutana, Ruyigi, Cankuzo, Karuzi, Muyinga and Ngozi) out of the 16 provinces surveyed (Figure 2). The province of Makamba (South) was the most affected with the disease found in five communes. Cibitoke, Cankuzo, Bubanza and Ruyigi provinces had each two affected communes while in Ngozi, Karuzi and Muyinga, the disease was observed at least in two sites (Figure 2). The incidence recorded varied in the different communes and is indicated in Table 1. The highest incidence was recorded in Ruyigi (34%) while the lowest was registered in Muyinga (3%). The incidence ranged from 17 to 25% in the most affected province (Makamba).

Farmers' awareness of symptoms, modes of spread and control measures of BXW was low (Figure 3). A small percentage of farmers ranging from 22.6 to 31.3% recognize BXW symptoms. Premature fruit ripening and wilting and yellowing of leaves were the most widely known symptoms, probably because they are visible by external observations. However, some farmers were still confusing BXW with *Fusarium* wilt due to the common

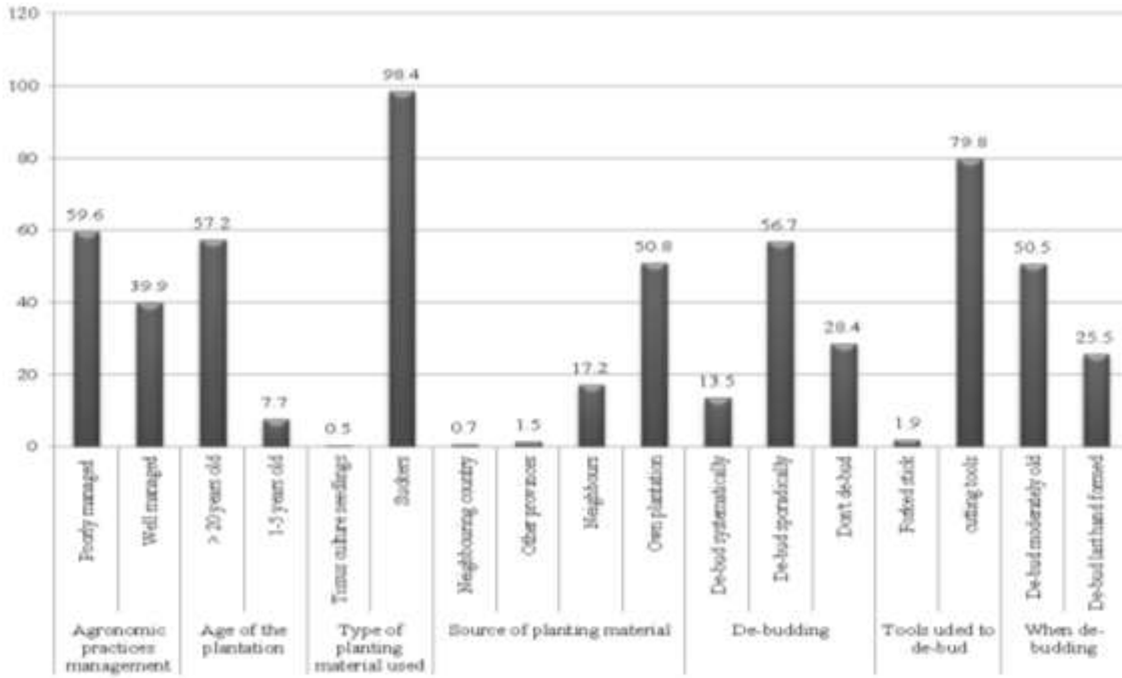

Figure 1. Cultural practices that contribute to BXW management.

yellowing and wilting of leaves. Very few of the interviewed farmers (1.9 to 26.4%) had knowledge of the mechanisms of BXW spread. Moreover, the percentage of the interviewed farmers who were aware of and implementing disease control was extremely low (ranging from 1.9 to 14.9% of farmers interviewed). Those who were aware reported that their main sources of information on BXW were radio/TV (22.6%), friends/neighbours (14%), extension (12.8%), research (2.3%), schools/churches (0.8%) and NGOs (0.4%). However, certain control practices were less known to farmers (or farmers did not have sufficient information on the practices). For instance most of farmers were not aware of the removal of male bud when the last hand has formed.

Most farmers (98.4%) also used suckers from their own field or neighbours to establish new plantations. The use of tissue culture and macropropagated materials is not yet widespread (Figure 1). Some households exchange suckers of improved varieties between provinces (1.5%) and even from neighbouring countries (0.7%). This was particularly true for the provinces of Muyinga and Ngozi. Farmers are also unaware of agronomic practices that contribute to disease control.

DISCUSSION

BXW has spread rapidly within the Burundi since its outbreak reported in two provinces in November 2010. This study showed that 10 out of the 16 provinces were already affected in less than a year with an incidence ranging from 3 and 34%. The low incidence in the Mabanda and Bubanza commune reflects the recent infection of these areas. Disease transmission was suspected to be driven by insects in Kayinja-based banana systems although tool-infection was locally important in several sites. Kayinja, also known as Pisang Awak, is preferred by farmers for its different uses but it is considered as one of the susceptible cultivars to BXW (Biruma et al., 2007). The only affected field found in Ngozi belongs to a farmer who owns another infected banana plantation in Karuzi. It is believed that the farmer involuntarily contaminated his plantation in Ngozi with non-disinfected tools after his passage in Karuzi. Additionally, some farmers admittedly reported that they were not aware about tools disinfection when they cut down a diseased banana, contributing involuntarily to the spread of the disease. The rapid spread of BXW is also influenced by farmers' limited knowledge and use of management measures such as timely removal of male buds and decontaminating tools. De-budding and decontamination of farm tools are known to be effective means of eliminating existing sources of inoculum and reducing opportunities of further spread of Xanthomonas wilt by insect vectors (Eden-Green, 2004; Tinzaara et al., 2006; Nakakawa et al., 2016). Considering that BXW can spread up to a radius of 75 km per year (Kwach et al., 2013), the lack of awareness and small size of a province in Burundi (from 830.60 to 2465.64 km^2), one can understand why the disease moved rapidly from the two affected provinces to the closest provinces.

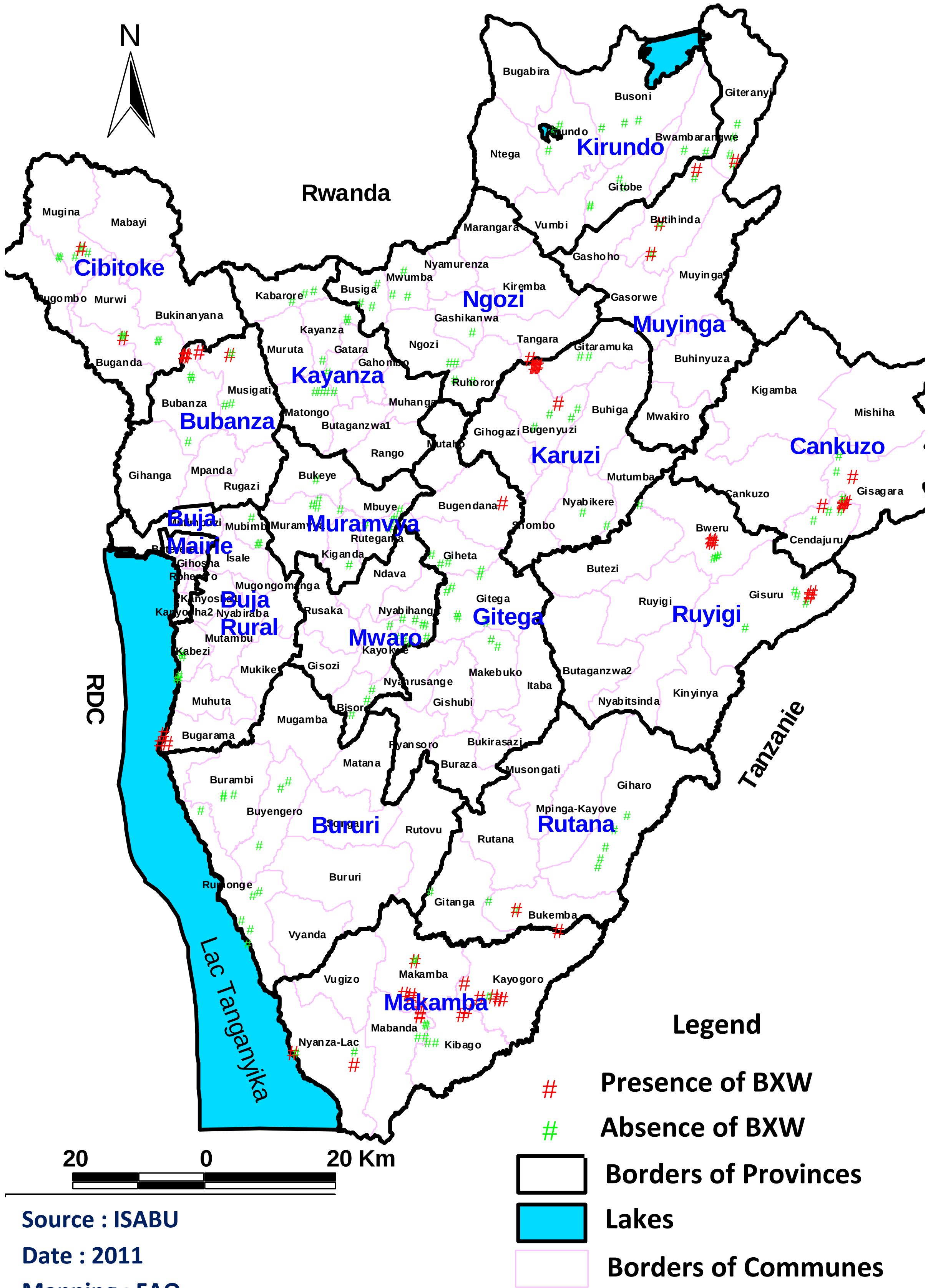

Figure 2. Distribution map of BXW in Burundi, August 2011 (ISABU, 2011).

Table 1. Incidence of BXW in different communes in Burundi, August 2011.

Province	Commune	Incidence (%)
Bubanza	Musigati	27
	Bubanza	3
Bujumbura	Bugarama	10
Cankuzo	Gisagara	14
	Cendajuru	13
Cibitoke	Murwi	14
	Mugina	6
Karuzi	Gitaramuka	14
Ngozi	Tangara	9
Rutana	Bukemba	21
Makamba	Nyanza-lac	25
	Makamba	22
	Kayogoro	19
	Kibago	18
	Mabanda	17
Muyinga	Giteranyi	10
	Butihinda	3
Ruyigi	Bweru	34
	Gisuru	33

The entry of the disease in Burundi is still a puzzle, though disease is suspected to have come on infected planting material or contaminated tools. The provinces of Makamba and Ruyigi which border the Kigoma region of Tanzania were the most affected. The Tanzania border region was reported to be affected by BXW in 2010 (Mgenzi et al., 2010) although there is a quite distance in between occupied by forests. However, it is likely that farmers on either side of the border exchange planting materials and trans-boundary workers can spread the disease using their working tools. The disease probably came to those provinces from Tanzania through either planting material or tools. In Makamba, Bujumbura and Rutana provinces, the most dominant symptoms are the shrivelling of inflorescences and premature ripening of banana fruits. This indicates that the disease was transmitted by insects as Kayinja, dominant in these provinces, is more susceptible to insect-mediated infections (Mbaka et al., 2009; Tinzaara et al., 2006).

Farmers' knowledge of the disease symptoms, spread mechanisms and control options is very critical in the management of the disease (Nkuba et al., 2015; Tinzaara et al., 2016). Farmer awareness of the disease was generally low in all provinces of Burundi. Additionally, farmers' management practices are not conducive to eradication of the disease once a field is infected as they prefer to abandon infected fields. This constitutes a major threat to farmers who are implementing control measures as abandoned banana fields act as a permanent source of the inoculum. This suggests the need for mobilization and sensitization at community level. The approach of engaging the community to own the problem contributed to the success in BXW management in Uganda (Kubiriba et al., 2012; Tinzaara et al. 2013; Ochola et al., 2015). This would include engaging locals in the bid for zero tolerance of Xanthomonas wilt by eliminating any sources of inoculum through effective implementation of control measures backed-up by community by-laws that most efficiently brings to book errant farmers who hesitate to manage the disease. The farmers need to understand that the disease is extremely devastating but can be controlled. This encourages farmers to aggressively fight the disease.

Conclusion

The rapid spread of BXW and its high incidence in affected fields in Burundi poses a serious threat to food security and incomes of rural communities in banana

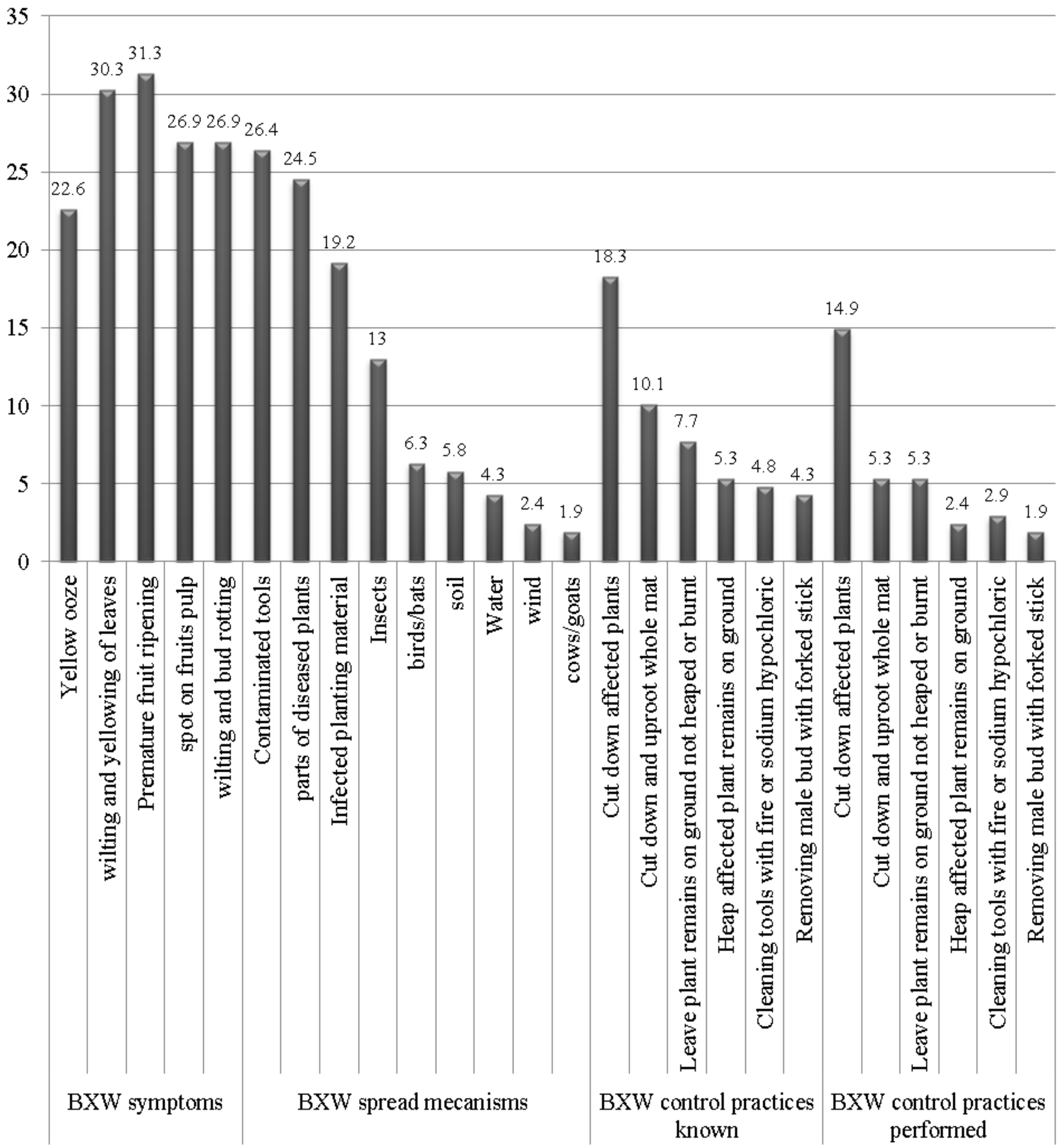

Figure 3. Farmers' knowledge on BXW symptoms, spread mechanisms and controls measures.

based cropping systems. The limited knowledge of the disease by the farmers and other stakeholders in the country makes it difficult to control. Farmers have limited awareness about the control measures, in particular when to remove the male bud. In the infected areas, farmers who are following control measures such as removing the male buds and disinfecting tools are harvesting bananas. BXW is also spreading by contaminated tools, with workers involuntarily transmitting the disease because they are not aware of tools disinfection. Poor management of banana plantations in Burundi makes control measures very difficult when bananas are infected. To remove mats constituting of up to ten plants makes control labour intensive and farmers are discouraged when they have to uproot poorly managed plantations which are no longer profitable. Farmers' knowledge is particularly low on control measures and the proportion of farmers using control practices is also low. There is therefore a need of improving awareness of farmers on banana cultural practices and how to make banana a profitable crop while controlling BXW and other diseases and pests.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Morphological diversity and identification of accessions of melon

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Family agriculture is a rich germplasm source wherever it takes place; however, information on genetic variability of this type of culture in Brazil is scarce. Samples of melon (*Cucumis melo* L.) accessions grown by family agriculture were rescued and this study aimed at characterizing the genetic variability in one sample of these accessions so as to identify the melon subspecies and the corresponding varieties. Fifteen accessions and their S_1 progenies were characterized (quantitative and qualitative descriptors) in two field experiments carried out in randomized blocks. Data on the parental and S_1 generations were compared and it was possible to identify the subspecies *Cucumis melo* subsp. *agrestis* and their varieties *C. melo* var. *makuwa* and *C. melo* var. *momordica*, and the subspecies *C. melo* subsp. *melo* and its variety *C.melo* var. *cantalupensis*, although some sub-accessions remained unidentified. A total of 26 subaccessions were found. UPGMA grouping method showed a high genetic diversity among and within accessions and sub-accessions. Clusters were formed by the melon subspecies, although there were discrepancies. Nonetheless, there is indication of trait introgression from the two melon subspecies and their varieties in the material grown by the family farmers of the state of Maranhão.

Key words: *Cucumis melo,* botanical variety, traditional agriculture.

INTRODUCTION

Melon (*Cucumis melo* L.) is a vegetable belonging to the family Cucurbitaceae A. Juss, subfamily Cucurbitoidae with great economic importance in several parts of the world. It is originated in tropical Africa (Burger et al., 2010) although there are studies indicating that the genus *Cucumis* L. is originated in Asian-Australian (Renner et al., 2007; Schaefer et al., 2009; Sebastian et al., 2010). In Brazil, the Northeastern region is responsible for 94% of the national melon production (Agrianual, 2014) and the most frequently produced melon is the Yellow type (*Cucumis melo* var. *inodorus*), to which several cultivars and hybrids commonly recognized as AF-682, Tropical, AF-646, Gold Mine, Vereda, Goldex, and Jangada. In addition, there are other types that belong to the

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botanical variety *cantalupensis*: Piel de Sapo, Galia, Charentais, Cantaloupe, and Honey Dew (Costa, 2008). Since they are genetically improved products, these cultivars are uniform and have good plant and fruit traits despite some limitations regarding tolerance or resistance to biotic and abiotic stresses. Some breeding programs have been conducted at Embrapa units and at the Federal Rural University of the Semiarid Region (UFERSA); consequently, there is a demand for germplasm in order to progress further in the development of new cultivars (Aragão, 2015, personal communication).

On the other hand, there is a high variability between melon accessions grown by small farmers, mainly in the states of Piauí and Maranhão, from which, a sample was rescued and is stored in the Active Germplasm Bank of Cucurbitaceae of the Brazilian Northeast (BAG) (Silva et al., 2010) and this sample has more than 150 accessions. The species *C. melo* shows high polymorphism, mainly in fruit traits, such as size, shape, color, texture, and flavor, and is considered the most diverse species in the genus *Cucumis* (Bates and Robinson, 1995).

Cucurbit species were introduced in the Brazilian Northeast through different routes (Correa, 2010), and to date, have been dry farmed in small-sized agricultural establishments where farmers use their own seeds in the rainy season every year, thereby constituting traditional varieties or populations. Variability studies on the rescued cucurbits have fairly contemplated watermelon accessions (*Citrullus lanatus* (Thunb) Matsun & Nakai). However, few studies have been conducted with *C. melo* accessions. Neitzke et al. (2009) studied genetic variability in melon landraces in the South of Brazil, but, the authors do not allude to the subspecies or their varieties of the studied accessions. On the other hand, Torres Filho et al. (2009) characterized 42 accessions of melon plants grown by traditional agriculture in the Brazilian Northeast, and found a high variation among accessions, as well as different botanical varieties, following the classification by Munger and Robinson (1991). Furthermore, Aragão et al. (2013) evaluated the genetic diversity among melon accessions from traditional agriculture of the Brazilian Northeast using data from the morphological characterization conducted by Torres Filho et al. (2009), and they also performed molecular characterization. They observed a wide variability among accessions and different botanical varieties using the classification by Robinson and Decker-Walters (1997). Recently, a study was conducted on genetic diversity following the botanical classification by Pitrat et al. (2000), where a sample of melon accessions grown by traditional agriculture in the state of Maranhão (data not published). Despite its relatively small size, the sample was large enough to identify the occurrence of both melon subspecies (*C. melo* subsp. *agrestis* and *C. melo* subsp. *melo*) and some varieties (*C. melo* var. *conomon*, *C. melo* var. *momordica, C. melo* var. *chandalak* and *C. melo* var. *cantalupensis*). Nonetheless, there are over 100 accessions in the melon Germplasm Bank that have not been studied in depth, out of which approximately 80 accessions have also been collected from traditional agriculture, which emphasizes the need for furthering this research.

Hence, this study aimed at morphologically characterizing a second sample of melon accessions from the Cucurbitaceae Active Germplasm Bank of the Brazilian Northeast, which were collected in the state of Maranhão, using part of IPGRI (2003) descriptors as a basis, as well as the descriptors used by Pitrat et al. (2000) to define the melon subspecies and their botanical varieties.

MATERIALS AND METHODS

15 accessions collected from traditional agriculture of the state of Maranhão between 1996 and 1998 were evaluated (Table 1); the accessions went through a multiplication phase and were stored in cold chamber with temperature of 10°C and relative humidity of 40%, in the Cucurbitaceae Active Germplasm Bank of the Brazilian Northeast, located at the Embrapa Semiárido, Petrolina – PE.

Experiments were conducted at the Department of Technology and Social Sciences of the State University of Bahia (DTCS/UNEB), located in the municipality of Juazeiro-BA, situated at 09°24'50'' South latitude and 40°30'10'' West longitude, with an altitude of 368 m, in the years of 2014 and 2015.

To deploy the first experiment seeds of the accessions derived from open pollination (parental generation) were used, and in the second experiment, seeds of the S_1 generation were used. Progenies were obtained through controlled pollination (self-fertilization).

For seedling production, 20 seeds from each accession were sowed on polystyrene trays filled with commercial substrate *Plantmax®* in a greenhouse covered with shade cloth with 50% light penetration, and irrigated twice a day. After 15 days of sowing, seedlings were transplanted to a definitive site where soil had been previously prepared by ploughing, harrowing, and furrowing. Experimental design was a completely randomized block with four replicates in the first experiment and two replicates in the second experiment. Five plants were used per plot with a spacing of 2.5 m between rows and 0.8 m between plants, watered by furrow irrigation.

In order to perform self-fertilization, one pistillate flower bud and two staminate flower buds from each plant were protected using disposable 200-mL cups and wooden support, and after 24 h, pollination was performed (Figure 1). While conducting the experiments, weeding was performed and the phytosanitary status of the plant was monitored.

Characterization was conducted in both experiments using IPGRI's (2003) quantitative and qualitative descriptors of plants and fruits, and including the descriptors proposed by Pitrat et al. (2000). Quantitative descriptors were evaluated when fruits were harvested between 30 and 45 days after they were fixed, and were as follows: MF - mean mass of fruits (kg), SND – blossom scar diameter (mm), SIL - blossom scar length (mm), SPT - pulp thickness in the side part of the fruit (cm), UPT - pulp thickness in the upper part of the fruit (cm), LPT - pulp thickness at the lower part of the fruit (cm), CL - fruit cavity length (cm), CD - fruit cavity diameter (cm), PL - fruit peduncle length (mm), PD - fruit peduncle diameter (mm), FD - fruit diameter (cm), FL - fruit length (cm), LSS - Soluble solids in the side part of the fruit (ºBrix), HSS – homogenized soluble solids (ºBrix),

Figure 1. Representation of pubescence on pistillate flower ovaries. A: Short, appressed hair on the ovary (*Cucumis melo* subsp. *agrestis*). B: Long hair on the ovary (*C. melo* subsp. *melo*); C: Protection of flower buds; and D: Pollination process.

Table 1. Passport data on accessions of the Cucurbitaceae BAG of the Brazilian Northeast.

Accession	Municipality	Municipality headquarters coordinates
BGMEL 63	Colinas	7° 6′ 59″ South, 46° 15′ 26″ West
BGMEL 66	Colinas	7° 6′ 59″ South, 46° 15′ 26″ West
BGMEL 67	Colinas	7° 6′ 59″ South, 46° 15′ 26″ West
BGMEL 68	Colinas	7° 6′ 59″ South, 46° 15′ 26″ West
BGMEL 78	Codó	4° 27' 18'' South, 43° 52' 44'' West
BGMEL 79	Itapecuru Mirim	3° 23' 42'' South, 44° 21' 36'' West
BGMEL 86	Codó	4° 27' 18'' South, 43° 52' 44'' West
BGMEL 87	São Luis Gonzaga do Maranhão	4° 22' 51'' South, 44° 40' 14'' West
BGMEL 97	Caxias	4° 52′ 29'' South, 43° 20′ 49'' West
BGMEL 101	Caxias	4° 52′ 29'' South, 43° 20′ 49'' West
BGMEL 103	Caxias	4° 52′ 29'' South, 43° 20′ 49'' West
BGMEL 108	Caxias	4° 52′ 29'' South, 43° 20′ 49'' West
BGMEL 111	Colinas	7° 6′ 59″ South, 46° 15′ 26″ West
BGMEL 112	Colinas	7° 6′ 59″ South, 46° 15′ 26″ West
BGMEL 115	São Vicente Ferrer	2° 53' 44'' South, 44° 52' 53'' West

and MS - mean mass of 100 seeds (g). Qualitative descriptors were: Fruit shape (globular, flattened, elliptical, pyriform, ovate, elongate, acorn, and malformation), fruit skin color (light yellow, yellow, greenish yellow, bright yellow, light yellow with medium green spots, yellow with dark green spots, pale green, dark green, pale green with dark green stripes, pale green with medium green stripes, and pale green with dark green spots), fruit stripe color (absent, pale green, medium green, and dark green), degree of ribbing in fruits (absent, superficial, intermediate, and deep), fruit skin cracking (present and absent), skin roughness of fruit (absent, superficial, intermediate, pronounced), reticulation (absent, superficial, intermediate, and pronounced), fruit pulp color (white, greenish, orange), fruit placenta color (white, greenish, and orange), fruit aroma (absent, present), fruit abscisson (absent, present), ovary and young fruit pubescence (short, long), and sex expression (monoecious, andromonoecious). To complement the study on fruit phenotypes, a systematic photographic documentation of the fruits (inner and outer parts), of their parental progenies, and respective S_1 generation was performed.

If segregation occurs between the parental and S_1 generations in each accession, an additional code representing the variation should be added to the accession codes in the Cucurbitaceae BAG of the Brazilian Northeast (Table 1), and these variants would thus be labeled sub-accessions. Therefore, a zero (0) should be added to the accession code of offspring that did not have variations between parental and S_1 generations. For instance, if BGMEL63 accession did not show segregation, its new code would be BGMEL63.0, or sub-accession BGMEL63.0. On the other hand, accessions that showed variation between phenotypes of both generations regarding plant, flower, and fruit traits should receive a number according to the number of subdivisions that occurred. Taking accession BGMEL63 as an example again, if three types of offspring occurred in S_1 generation, then sub-accessions should be designated BGMEL63.1, BGMEL63.2, and BGMEL 63.3, and so on.

Diversity analysis was based on qualitative and quantitative descriptors. To determine the distance matrix of joint analysis (quantitative and qualitative descriptors), Gower's algorithm (1971) was used. From the dissimilarity matrix, the grouping analysis using the UPGMA method was obtained. In order to validate the clusters generated by the UPGMA method, a cophenetic correlation coefficient (CCC) was estimated based on Pearson's correlation coefficient between the distance matrix and the cophenetic matrix (distance matrix between genotypes) (Cruz et al., 2011). All these analyses were performed with the help of R program (R Development Core Team, 2012).

RESULTS AND DISCUSSION

Comparing the phenotypes of the parental and S_1 generations, it was observed that some accessions did not show variation in flower and fruit traits, while others showed high variation (Table 2). Hence, the 15 accessions initially studied were subdivided in 26 sub-accessions according to the traits shown in the table.

Comparing data on both generations, four sets were identified. The first one had nine subaccessions; some of them were comprised of *C. melo* subsp. *melo* variety *cantalupensis* (BGMEL63.0, BGMEL78.0, and BGMEL101.0) and the others were comprised of *C. melo* subsp. *agrestis* var. *makuwa* (BGMEL66.0, BGMEL67.0, BGMEL79.0, BGMEL111.0, BGMEL112.0, and BGMEL115.0). All plants in these sub-accessions showed the same phenotype of flower and fruit traits (homozygotes) and they also showed descriptors which allowed for the identification of subspecies and respective botanical varieties (Figure 2 and Table 2).

The second set was comprised of seven sub-accessions derived from variations within accessions (parental generation): BGMEL68, BGMEL87, BGMEL108, BGMEL86, BGMEL 87, BGMEL 97, and BGMEL 103 (Table 2 and Figure 3). Some progenies were homozygous and had plant and fruit traits that also allowed for the identification of their subspecies and respective botanical varieties. Hence, *C. melo* subsp. *agrestis* var. *momordica* (sub-accessions BGMEL68.1 and BGMEL87.1), as well as variety *makuwa* (subaccession BGMEL108.1) was identified. *C. melo* subsp. *melo* was also identified, with the variety *cantalupensis* (sub-accessions BGMEL86.1, BGMEL87.2, BGMEL97.1, and BGMEL103.1) (Figure 3). Therefore, although these sub-accessions were selected based on the offspring that did not show segregation between the parental and S_1 generations, they did show morphological traits in fruits and flowers that allowed the identification of the subspecies and varieties of each sub-accession.

In the third set, a group of offspring showed marked variations in fruit traits between both generations (Figure 4); however, ovary pubescence characters did not vary between the parental and S_1 generations (Table 2) and it was therefore possible to identify the subspecies. There are six sub-accessions in this set: BGMEL68.2, BGMEL86.2, BGMEL108.3, BGMEL86.3, BGMEL97.2, and BGMEL108.2; the first three belong to *C. melo* subsp. *agrestis*, and the three latter belong to *C. melo* subsp. *melo* (Figure 4 and Table 2).

Most of the traits of sub-accession BGMEL68.2, *C. melo* subsp. *agrestis*, fit into variety *momordica*; however, there was no fruit cracking (Figure 4). Accessions belonging to var. *momordica* showed fruit skin cracking when ripe, white, cream, or orange pulp color, and absence of sugar and aroma (Fergany et al., 2010), and therefore, the data in this study do not support the inclusion of this sub-accession in the variety *momordica*. Although fruit cracking is determining in variety *momordica*, it is an undesired trait since it affects appearance and decreases post-harvest durability, compromising commercialization (Neitzke et al., 2009). Nonetheless, melon populations of variety *momordica* were observed with a slight aroma when ripe, little sweetness, and pulp color ranging from cream to orange (Manohar and Murthy, 2012). On the other hand, sub-accession BGMEL 86.2, also belonging to *C. melo* subsp. *agrestis*, showed a variation in traits, and it was therefore impossible to determine its variety. It showed pale green and light yellow skin color, greenish and orange pulp color, absence of furrow and superficial reticulation (Figure 4), possibly a typical trait of groups derived from *C. melo* subsp. *melo*. Sub-accession BGMEL108.3, *C. melo* subsp. *agrestis*, showed fruit traits typical of *C. melo* var. *acidulus*; however, skin color and some data related to fruit pulp did not allow its inclusion in this variety, since accessions belonging to variety *acidulus* are mainly characterized by having a very hard white pulp, and absence of sugar and aroma (Figure 4) (Fergany et al., 2010).

Among the sub-accessions of *C. melo* subsp. *melo*, sub-accession BGMEL86.3 showed all characters of the variety *reticulatus*; nonetheless, its monoecious sex expression does not coincide with this variety (Table 2 and Figure 4). In sub-accession BGMEL108.2 (Figure 4), most traits (roughness, late maturation, fruit shape and color) fit into variety *inodorus*; however, due to sex expression and pulp color this sub-accession was not assigned to this variety. Finally, sub-accession BGMEL97.2 showed fruit traits of variety *chito*. However, the fruits showed pulp color orange, hampering its classification into this variety (Figure 4). On the other hand, the fruit size ranged from 0.4 to 0.8 kg indicating fruit size small to intermediate (Ipgri, 2003) which is not typical of *chito* variety that has very small fruit size, less than 0.4 kg (Ipgri, 2003; Pitrat et al., 2000).

Sub-accessions where subspecies or botanical groups were not identified (sub-accessions BGMEL87.3, BGMEL103.2, BGMEL68.3 and BGMEL 108.4) comprised the last set. Cracking, pulp color and fruit shape in sub-accessions BGMEL68.3 and BGMEL87.3 indicated the variety *momordica* (Figure 5); however, there was segregation of the descriptor ovary pubescence that defines the melon subspecies (Jeffrey, 1980) as shown in Table 2, since some plants had long trichomes, both in the parental and S_1 generations, and others had short trichomes inside the same accession. This made it impossible to determine the subspecies, and consequently, the variety. The same performance occurred in the other sub-accessions of this set (Table 2). These results show trait introgression between the two melon subspecies. Dogimont (2011), when studying melon genes, did not allude to inheritance of ovary pubescence, and therefore, it is important to study this

Table 2. Classification of sub-accessions into their corresponding subspecies (ssp.), variety (V), ovary pubescence (OP), sex expression (SE), furrow on the fruit surface (FS), stripe color (SC), and pulp color (PC) in the parental and S_1 generations (G).

Accession	ssp.	V	G	OP	SE	FS	SC	PC
BGMEL63.0	*melo*	*cantalupensis*	(P)	L	M	1 2 3	0	1 3
			(S1)	L	M	3	0	3
BGMEL66.0	*agrestis*	*makuwa*	(P)	S	A	0	0	1
			(S1)	S	A	0	0	1
BGMEL67.0	*agrestis*	*makuwa*	(P)	S	A	0	0	1
			(S1)	S	A	0	0	1
BGMEL78.0	*melo*	*cantalupensis*	(P)	L	M	1 2	0	3
			(S1)	L	M	1 2	0	3
BGMEL79.0	*agrestis*	*makuwa*	(P)	S	A	0	0	1
			(S1)	S	A	0	0	1
BGMEL101.0	*melo*	*cantalupensis*	(P)	L	M	1 2 3	0	2
			(S1)	L	M	3	0	2
BGMEL111.0	*agrestis*	*makuwa*	(P)	S	A	0	0	1
			(S1)	S	A	0	0	1
BGMEL112.0	*agrestis*	*makuwa*	(P)	S	A	0	0	1
			(S1)	S	A	0	0	1
BGMEL115.0	*agrestis*	*makuwa*	(P)	S	A	0	0	1
			(S1)	S	A	0	0	1
BGMEL68.1	*agrestis*	*momordica*	(P)	S	M	0	0	1
			(S1)	S	M	0	0	1 3
BGMEL68.2	*agrestis*	ND	(P)	S	M	0	0	1
			(S1)	S	M	0	0	1 3
BGMEL68.3	ND	ND	(P)	S	M	0 1	0	1 3
			(S1)	S L	M	0 1	0	1 3
BGMEL86.1	*melo*	*cantalupensis*	(P)	L	M	1 3	0	3
			(S1)	L	M	2 3	0	2 3
BGMEL86.2	*agrestis*	ND	(P)	S	M	0	0	2 3
			(S1)	S	M A	0	0	1 3
BGMEL86.3	*melo*	ND	(P)	L	M	1	0	3
			(S1)	L	M	1	0	3
BGMEL87.1	*agrestis*	*momordica*	(P)	S	M	0	0	1
			(S1)	S	M	0	0	3
BGMEL87.2	*melo*	*cantalupensis*	(P)	L	M	2 3	0	3
			(S1)	L	M	2 3	0	3

Table 2. Contd.

BGMEL87.3	ND	ND	(P) (S1)	S S L	M M	0 1 1 2	0 0	1 3 1 3
BGMEL97.1	*melo*	*cantalupensis*	(P) (S1)	L L	M M	1 3 2	0 0	3 3
BGMEL97.2	*melo*	ND	(P) (S1)	L L	M A M A	0 1 0 1 2	0 0	2 3 1 3
BGMEL103.1	*melo*	*cantalupensis*	(P) (S1)	L L	M M	2 3 2	0 0	2 3 3
BGMEL103.2	ND	ND	(P) (S1)	S S L	M A	1 0 1 2	0 0	3 2 3
BGMEL108.1	*agrestis*	*makuwa*	(P) (S1)	S C	A A	0 0	0 0	1 1
BGMEL108.2	*melo*	ND	(P) (S1)	L L	M M A	0 0	0 0	3 1 3
BGMEL108.3	*agrestis*	ND	(P) (S1)	C C	M M	0 1	0 0	1 1 3
BGMEL108.4	ND	ND	(P) (S1)	C C L	M M	0 0	4 0 4	1 1 2 3

[0]Did not show variation inside the accession.[1,2,3,4]segregation occurred inside each accession. OP -Ovary pubescence: S - short, L - long. SE - Sex expression: M - monoecious, A- andromonoecious. FS - Furrow on the fruit surface (FS): 0 - absent, 1 - superficial, 2- intermediate, 3 - deep. SC - Stripe color: 0 - absent, 1 - pale green, 2 - medium green, 3 - dark green 4- light yellow. PC -Pulp color: 1 - white, 2 - greenish, 3 - orange.

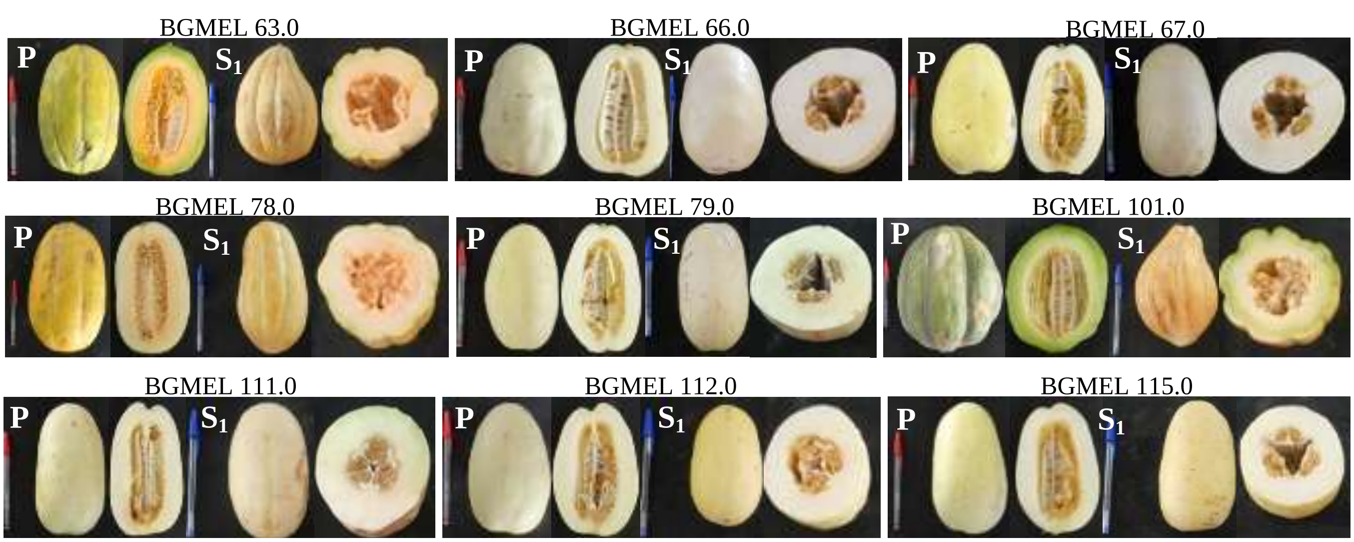

Figure 2. Homozygous sub-accessions for flower and fruit traits in both generations studied (parental and S_1) that allowed the identification the melon subspecies and their varieties.

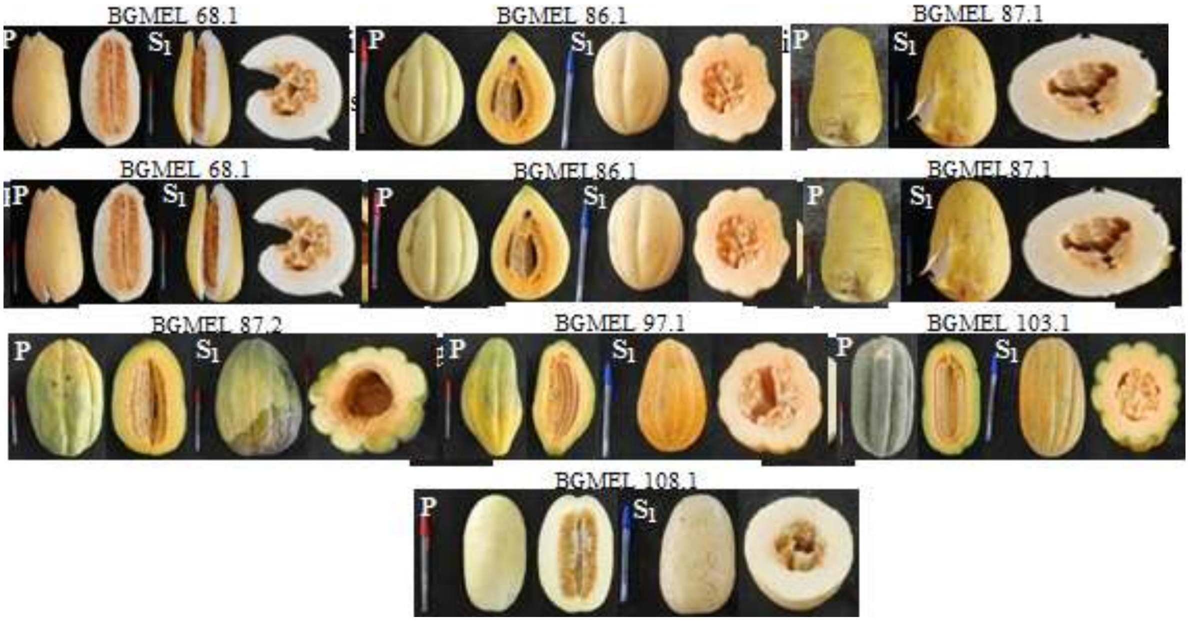

Figure 3. Sub-accessions derived from variations inside the parental generation, which maintained plant and fruit traits in the S_1 generation and showed flower and fruit traits that allowed the identification of the subspecies and varieties of melon.

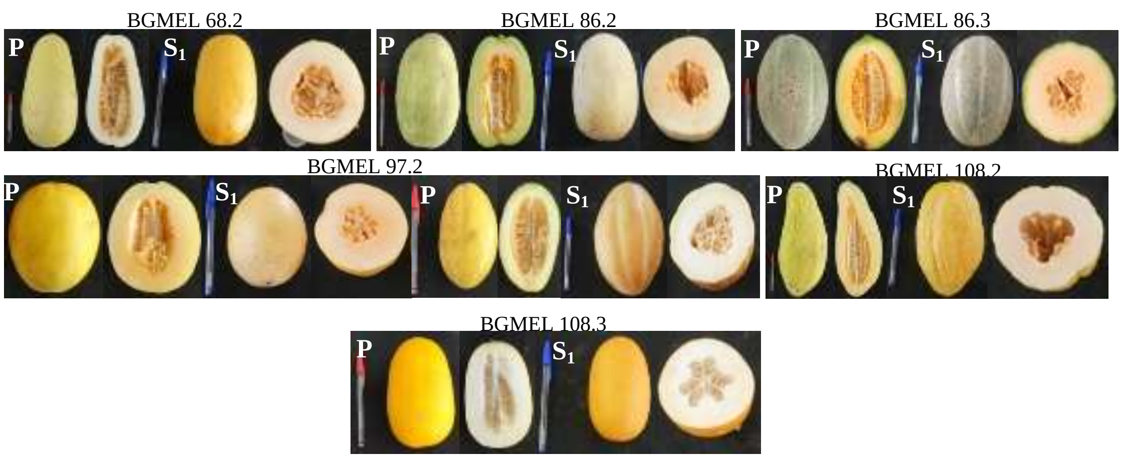

Figure 4. Sub-accessions with variations in fruit descriptors that allowed the identification of the melon subspecies but not the distinction in their respective varieties.

trait to help define melon subspecies. Regarding the defining traits of varieties (Pitrat et al., 2000), the presence of furrow and pulp color in sub-accession BGMEL103.2 fit into variety *cantalupensis*, and it was not possible to associate sub-accession BGMEL108.4 to any variety due to the great variation in traits such as stripes, fruit surface and cracking in the parental generation, and pulp color (Figure 5). Due to the fact that it was not possible to determine the subspecies, therefore, it was not possible to assign sub-accessions to a specific variety. However, these results show strong evidence of trait introgression between different melon varieties.

Torres Filho et al. (2009), conducting the morphological characterization of melon accessions collected in the Brazilian Northeast following the classification by Munger and Robinson (1991), identified 80.9% of the 42 accessions analyzed as to their varieties; however, 19.1% remained indeterminate. Among the varieties identified are var. *conomon*, var. *cantalupensis*, var. *momordica*, and var. *inodorus* (commercial cultivar). In a previous study (data not published), analyzing melon accessions collected in the state of Maranhão following

Figure 5. Representation of the phenotypes of sub-accessions whose subspecies and varieties were not identified.

the classification by Pitrat et al. (2000), it was possible to classify the subspecies and varieties of 44.0% of the studied accessions; 32.0% were identified by subspecies, although their varieties remained not defined, and 25.0% was not defined for both subspecies and varieties. Among the varieties defined are: *C. melo* var. *momordica, C. melo* var. *cantalupensis, C. melo* var. *conomon*, and *C. melo* var. *chandalak*. In this study, despite the marked variations in traits that allowed the distinction between subspecies and varieties, only 61.5% of the studied sub-accessions were classified by subspecies and varieties, while 23.1% had only subspecies defined, and 15.4% remained undefined for both subspecies and varieties. However, data showed that there was trait introgression between the different subspecies and their varieties and these results indicate that the management of seeds of different types of melons by family farmers might have contributed to cross-pollination between subspecies and varieties, since there are no barriers to cross-pollination between different botanical varieties (Decker-Walters et al., 2002). The exchange of seeds between family farmers is quite common, and it is possible that these seeds were mixed through germplasm exchange among family farmers.

In addition to studies conducted in Brazil, other countries have also been developing studies with melon germplasm derived from family farming. Szamosi et al. (2010) observed a wide diversity of morphological characters comparing germplasm of Hungarian melons and Turkish melons of the varieties *reticulatus*, *inodorus*, *cantalupensis*, *dudaim*, *chate*, *chito*, and *flexuosus*. Yildiz et al. (2014), when comparing morphological and molecular characterization of melons of varieties *inodorus*, *cantalupensis*, *reticulatus*, *canomon*, *flexuosus*, *dudaim*, *momordica*, and six unknown accessions, observed a high variation in the traits of Turkish melons. Trimech et al. (2013), following the classification by Munger and Robinson (1991), observed significant differences in Tunisian melons among accessions and among and within sampling sites, as well as in different varieties *reticulatus*, *inodorus*, and *dudaim*, and some accessions remained indeterminate because they did not fit into any melon variety, similar to the study conducted in Brazil. However, all these studies, Brazil included indicated that the variation found in these melon subspecies and their varieties make this germplasm very important to melon breeding. It is important to emphasize that different melon varieties, notably the wild types that generally belong to *C. melo* subsp. *agrestis*, pose great interest from the germplasm standpoint, since they have the majority of genes responsible for controlling biotic stresses caused by fungi such as powdery mildew (*Podosphaera xanthii*), gummy stem blight (*Didymela bryoniae*), alternaria leaf blight (*Alternaria cucumerina*), potyviruses (PRSV-W, WMV, ZYMV), and resistance to insects such as the leaf miner (*Liriomyza* spp.), among other biotic stresses (Dogimont, 2011).

On the other hand, in addition to the high variation found in subspecies and respective varieties, the study on the diversity found in accessions and sub-accessions using morphological descriptors gives a dimension of the existing variability. Clusters formed by the joint analysis of quantitative and qualitative descriptors obtained using the UPGMA method showed that data from the first experiment comprised three groups (Figure 6). The cophenetic correlation coefficient (CCC) was 0.96, indicating a good representation (Cruz et al., 2011), and the cut-off point of dendograms was obtained by Mojena (1977) method. The first cluster was comprised of accessions BGMEL108, BGMEL112, BGMEL79, BGMEL115, BGMEL67, BGMEL66, and BGMEL111. These accessions belong to the subspecies *agrestis* variety *makuwa*. It is worth noting that accession BGMEL 108 showed variation in fruit traits among plants within the accession (Figures 3 to 5), as described previously, although it was found one sub-accession of the variety *makuwa* (sub-accession 108.1) in this variation. Accession BGMEL 68 was the only one comprising the second group and accessions BGMEL 97, BGMEL 101, BGMEL 103, BGMEL86, BGMEL63, BGMEL78, and BGMEL87 comprised the third cluster. This group was comprised of the accessions of subspecies *melo* variety

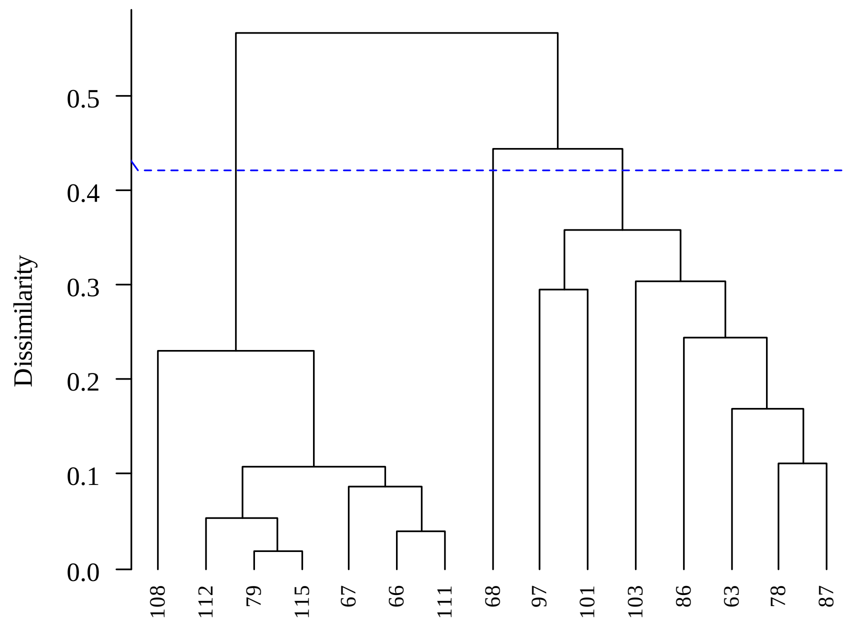

Figure 6. Representation of the clusters of the accessions of parental generation using the UPGMA method.

cantalupensis, although some accessions, such as BGMEL86 and BGMEL87, had variations coinciding with subspecies *agrestis* (Table 2).

The cluster analysis of sub-accessions (Figure 7) showed that four groups were formed with good consistency (CCC = 0.82). Sub-accessions (BGMEL108.3, BGMEL108.4, BGMEL111.0, BGMEL112.0, BGMEL115.0, BGMEL108.1, BGMEL79.0, BGMEL66.0, and BGMEL67.0) comprised the first group. It was possible to identify two subgroups in this first group: one comprised of BGMEL108.3, belonging to subspecies *agrestis* and with indeterminate varieties although it had traits of the variety *acidulus*, and sub-accession BGMEL108.4, whose subspecies was not identified due to segregation of ovary pubescence between the parental and S_1 generations (Table 2). The second subgroup gathered all the other sub-accessions of subspecies *agrestis* variety *makuwa*.

In the second cluster, comprised of seven sub-accessions, there were also two subgroups, the first one comprised of sub-accession BGMEL108.2, belonging to *C. melo* subsp. *melo* and variety not defined yet with characters of var. *inodorus*, and the other one comprised of the other six sub-accessions; two from *C. melo* subsp. *agrestis,* var. *momordica* (BGMEL 68.1 and 87.1), two from *C. melo* subsp. *agrestis* and indeterminate variety (BGMEL68.2 and BGMEL86.2), and two with indeterminate subspecies and varieties (BGMEL87.3 and BGMEL68.3), although both had characters of var. *momordica*. Therefore, the two subspecies and sub-accessions with characters of different varieties were assigned to this group, which indicates again the occurrence of trait introgression both in subspecies and varieties and that this might be a consequence of the management of seeds by farmers, as indicated previously, since there is no cross-pollination barrier between subspecies or between their respective varieties (Decker-Walters et al., 2002).

The third group was comprised only of sub-accession BGMEL 87.2, assigned to *C. melo* subsp. *melo* var. *cantalupensis*, and the last group included nine sub-accessions (BGMEL101.0, BGMEL97.2, BGMEL103.2, BGMEL86.3, BGMEL78.0, BGMEL86.1, BGMEL63.0, BGMEL97.1, and BGMEL103.1), all from *C. melo* subsp. *melo*, except for sub-accession BGMEL 103.2, which remained indeterminate due to segregation in ovary pubescence (Table 2) although it had characters of var. *cantalupensis*. Sub-accession BGMEL 97.2 showed characters of variety *chito*, BGMEL86.3 had characters of variety *reticulatus*, and all the others belonged to var. *cantalupensis*. Thus, this cluster was practically comprised of sub-accessions of *C. melo* subsp. *melo*, with only one exception. Thus, clusters of sub-accessions showed a reasonable agreement, as all accessions of *C. melo* subsp. *agrestis* were in the first group, except for sub-accession BGMEL108.4, which is indeterminate (as detailed previously). The second group had a mixture of sub-accessions of both subspecies, with predominance of *C. melo* subsp. *agrestis*, again indicating introgression of characters of subspecies and their varieties. The third group had only one accession of *C. melo* subsp. *melo* var. *cantalupensis* (BGMEL87.2, Figure 7) and the fourth group had all sub-accessions, with only one exception, of *C. melo* subsp. *melo,* and most of them belonged to var.

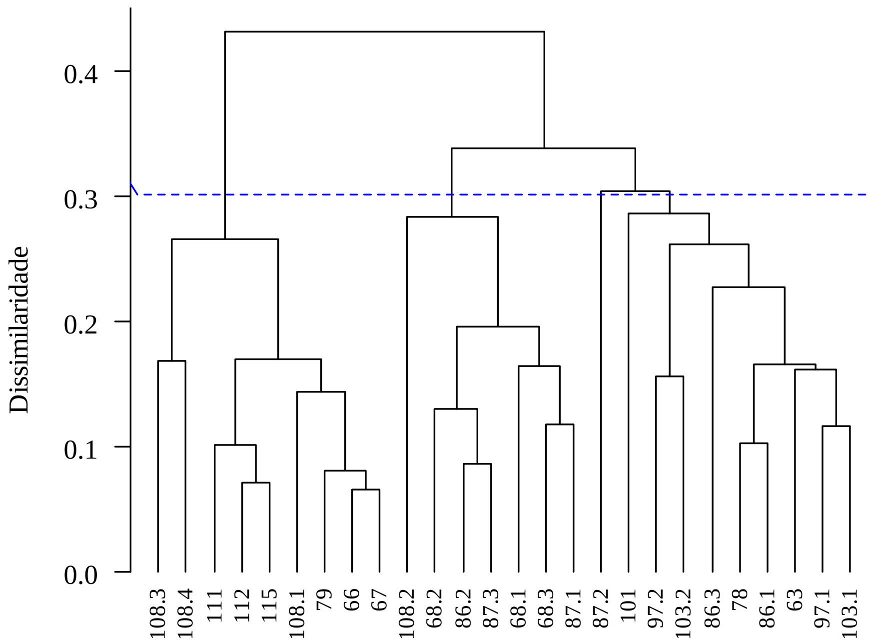

Figure 7. Representation of the clusters of sub-accessions deriving from the second experiment using the UPGMA method.

cantalupensis. It is worth noting that, in addition to the morphological traits that are determining in the identification of subspecies and their varieties, some other morphological traits might also have been important to form the groups. Fruit mass is one example, since fruits were found with a high variation between sub-accessions, ranging from 400 to 2500 g, and there were other very contrasting characters. Therefore, this variation might have been determining to form the third group; even though this group is comprised of *C. melo* subsp. *melo* var. *cantalupensis*, it has been separated from other sub-accessions of the same species and variety of the fourth cluster.

Although there was a trend towards clustering according to subspecies and varieties (groups I and IV, Figure 7), different subspecies were included in group II; moreover, detailed data on subspecies and varieties indicate that there is a high trait introgression, which was more evident in the study of the samples in the present study than in the study performed previously (data not published), in which a sample of accessions from traditional agriculture was analyzed. It is also important to emphasize that some varieties could not be identified only because of the lack of agreement of one or few traits. When these traits are not fixed in the accessions, obtaining new inbred generations might allow the separation of characters that enable the identification of the species and new varieties. Even if such separation is not possible, the existence of characters of different varieties, notably *C. melo* subsp. *agrestis*, indicates that this germplasm is very valuable for the identification of resistant genes to biotic stresses faced by commercial melon plant cultures. Therefore, it should be preserved in the short, medium, and long term, as a treasure that, once unveiled, might help develop new commercial melon cultivars to be used in several melon production systems throughout the country.

Conclusions

1. Several different melon varieties occur in the genetic material of melon grown by family farming in the Brazilian Northeast.
2. There is variability among and within samples of melon accessions and sub-accessions in the melon germplasm bank derived from family farming in the Northeastern Brazil.
3. There is trait introgression between the two melon subspecies and among their different varieties in the germplasm cultivated by small farmers.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Genotypes of conilon coffee can be simultaneously clustered for efficiencies of absorption and utilization of N, P and K

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The objective of this research was to group C. canephora cv. Conilon coffee genotypes of different ripening cycles for both efficient absorption and utilization of N, P and K in contrasting environments. The experiment was arranged in a factorial scheme 13x4, with fours replicates, the factors being: 13 genotypes of Conilon coffee and four types of fertilization (NPK: 0%-100%-100%, 100%-0%-100%, 100%-100%-0% and 100%-100%-100% of the level recommended). The results indicated that conilon coffee genotypes have efficiencies to absorb and use N, P, K modulated by the availability of these nutrients in the soil, furthermore it was possible to assume that conilon coffee genotypes of early ripening cycle have high joint efficiencies of absorption and utilization in environment with adequate NPK supply. To optimize the nutritional management, the genotypes 67 and 76 would be recommended for plantations with low technological potential to better exploit their efficiencies of absorption and use of N, P and K; and the genotypes 02, 48 and 67 would be recommended for crops with high technological potential where, besides the nutritional efficiency, their responsiveness could be explored. For breeding programs, it is recommended the exploitation of conilon genotypes 02 and 67, for presenting simultaneously high absorption and utilization efficiency of NPK.

Key words: *Coffea canephora*, mineral nutrition, fertilization, crop breeding.

INTRODUCTION

In recent years, the coffee world market witnessed significant change with a substantial increase of about

30% of world production (ICO, 2016), driven mostly by *Coffea canephora* Pierre ex A. Froehner cultivars (Robusta type of coffee). This supply growth happened due to the association between greater productivity and lower production costs (Martins et al., 2013a), coupled with an increasing use of coffee in the industry in the manufacture of soluble coffees and blends with *Coffea arabica* L. (Arabica type of coffee) beans, participating up to 50% in the blends proportion.

The *C. canephora* species has a reproduction by allogamy, presenting wide genetic and phenotypic variability (Fonseca et al., 2004; Ferrão et al., 2008). In addition, Conilon, the most used genotypes from this species in Brazil, have different fruit ripening cycles (Bragança et al., 2001). By definition, the maturation period is the time between flowering and fruit ripening, and this period of time can be used to classify the ripening primarily in early, intermediate and late cycle. This characteristic may vary depending on the genotype and environment (Pezzopane et al., 2003). Furthermore, Conilon coffee genotypes of different ripening cycles also show differences in growth and plant vigor, which in turn interferes with the photosynthetic activity and transport of assimilates (Morais et al., 2012), resulting in differential absorption and accumulation of nutrients and biomass, thus possible requiring a different mineral fertilization management (Martins et al., 2013a; Martins et al., 2013b; Prezotti and Bragança 2013; Partelli et al., 2014).

Beside the relationship between genotype and ripening cycle, the supply of nutrients; particularly nitrogen (N), phosphorus (P) and potassium (K); is extremely important. Studies report that nitrogen is the macronutrient that is accumulated in greater quantities in Conilon plants, making the requirement for this nutrient to be very high (Bragança et al., 2008; Bragança et al., 2010; Clemente et al., 2013). There is evidence that Conilon trees cultivated without satisfactory phosphorus supply present uneven and limited development of roots and aerial part (DaMatta et al., 2007; Martins et al., 2013b). Additionally, potassium is the third major macronutrient in order of accumulation by Conilon coffee (Bragança et al., 2008), with importance to control of turgidity and maintenance of fruit (Nogueira et al., 2001). Therefore, its nutritional deficiency is extremely detrimental to plant growth and crop yield.

In this scenario, the study of nutritional efficiency becomes of great importance for research involving genotypes with potential to adapt to conditions of nutritional limitation (Tomaz et al, 2011; Martins et al., 2013c), regarding the plant ability to absorb, translocate and use particular mineral nutrients (Fageria, 1998). However, there are few methods to simultaneously identify genotypes efficient in absorbing and using the mineral nutrient supplied in the soil (Fageria, 1998; Martins et al., 2013a). Thus, we aim at to group Conilon coffee genotypes of different ripening cycles for both efficient absorption and utilization of N, P and K in contrasting environments.

MATERIALS AND METHODS

Local conditions

The experiment was conducted in greenhouse, located in the experimental area of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), in Alegre, ES, Brazil, with coordinates of 20°45' S latitude and 41°33' W longitude, and an average altitude of 277.41 meters over sea level, from September 2012 to March 2013.

The soil was collected at a depth of 10 to 40 cm, with the first 10 cm of the soil being discarded to reduce the effect of the organic matter present on the surface layer. A soil sample was sent to laboratory for chemistry and physic characterization, performing according to the methodology described by Embrapa (2006) (Table 1). The soil was characterized as a clayey red-yellow latosol.

After the characterization, the soil was dried under shade and homogenized in a 2.0 mm mesh sieve. Subsequently, the soil was separated into samples of 10 dm^3, standardized by weighing on a precision balance and placed in sealed plastic pots (with a capacity for 12 dm^3).

Along the assays, the pots were irrigated daily, maintaining the soil moisture at 60% of the total pore volume, obtained by particle density and soil density determination using the cylinder method, according to Embrapa (1997).

Plant material

The genotypes have high compatibility gametophytic, high adaptability, high visual evaluation índex (VEI > 6.5), different ripening cycles (early, intermediate and late), tolerance to drought, moderate resistant to rust of coffee, high yield potential (average 70.4 bags of 60 kg ha^{-1}), large grain and production stability (Fonseca et al., 2004) (Table 2).

The conilon coffee seedlings were provided by the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural – INCAPER, produced at Fazenda Experimental de Marilândia-ES (with coordinates of 19°24' S latitude and 40°32' W); seedlings were planted in pots with 120 days of development, with two pairs of leaves and good phytosanitary and nutritional aspects

Experimental design for the study of nutrition with NPK

The experiment was arranged in a factorial scheme 13×4, with fours replications, the factors being: 13 genotypes of conilon coffee (02, 23, 32, 48, and 67 of early ripening cycle; 22, 31, 73, 77, and 83 of intermediate ripening cycle; 24, 76, and 153 of late ripening cycle) and four types of fertilization (NPK: 0%-100%-100%, 100%-0%-100%, 100%-100%-0% and 100%-100%-100% of the level recommended by Lani et al., 2007), following a completely randomized design. The experimental plot has consisted of one

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Table 1. Physical and chemical characteristic of the soil used as substrate.

Characteristics	Value
Coarse sand (g kg^{-1})[1]	395.30
Fine sand (g kg^{-1})[1]	157.70
Silt (g kg^{-1})[1]	43.60
Clay (g kg^{-1})[1]	403.40
Soil density (kg dm^{-3})[2]	1.20
pH[3]	5.40
P (mg dm^{-3})[4]	2.00
K (mg dm^{-3})[5]	93.0
Ca ($cmol_c$ dm^{-3})[6]	1.70
Mg ($cmol_c$ dm^{-3})[6]	1.10
Al ($cmol_c$ dm^{-3})[7]	0.00
H+Al ($cmol_c$ dm^{-3})[8]	2.10
Sum of bases ($cmol_c$ dm^{-3})	3.37
Potential CEC ($cmol_c$ dm^{-3})	5.45
Effective CEC ($cmol_c$ dm^{-3})	3.37
Base saturation (%)	61.80

[1.]Pipette method (slow mixing); [2.]Graduated cylinder method; [3.]pH in water (relation 1:2.5); [4.]Extracted by Mehlich-1 and determined by colorimetry; [5.]Extracted by Mehlich-1 and determined by flame photometry; [6.]Extracted with 1 mol L^{-1} potassium chloride and determined by titration; [7.]Extracted by oxidation, humid route, with potassium dichromate in sulfuric medium, and determined by titration (Embrapa, 1997).

Table 2. Characterization of ripening cycles, visual evaluation index (VEI) and mean productivity (bags of 60 kg ha^{-1}) of the genotypes of *Coffea canephora.*

Genotypes	Ripening cycle	VEI[1]	Mean productivity[2]
67	Early	6.5	66.0
73	Intermediate	8.3	78.0
83	Intermediate	10.0	83.7
77	Intermediate	6.6	86.1
76	Late	7.1	84.1
48	Early	7.3	72.4
22	Intermediate	6.9	62.3
23	Early	7.3	64.2
24	Late	7.7	69.7
31	Intermediate	7.3	62.3
32	Early	7.8	62.5
02	Early	7.0	62.0
153	Late	7.0	62.0

[1]VEI, Visual evaluation index: Scale from 1 to 9 (1 = poor and 9 = very good) (Fonseca et al., 2004). [2]Annual mean productivity (bags of 60 kg ha^{-1}) of genotypes in eight successive harvests in the different studied environments in the state of Espírito Santo (Fonseca et al., 2004).

plant per pot with three replications.

The study of nutrition with nitrogen (Sub experiment 1 - 0% and 100% of the N) fertilization was performed with NH2CONH2 p.a., diluted in distilled water and applied over the soil surface, distant 10 cm of the plant collar, following levels consistent with the treatments of 0 and 100% of the N (respectively 0.00 and 2.15 g dm^{-3} of N). The fertilization was divided into four applications, performed at 30, 60, 90, and 120 days after planting. In this study, the fertilization with phosphorus and potassium was provided to all parcels in a single application before planting, using KH_2PO_4 P.A. diluted in water and applied in the entire volume of soil, according to the recommendation (Lani et al., 2007).

The study of nutrition with phosphorus (Sub experiment 2 - 0% and 100% of the P_2O_5), consisted of 0.00 and 1.75 g dm^{-3} of P_2O_5,

Table 3. Mean squares, coefficients of variation (CV) and overall means for efficiency of absorption of nitrogen, phosphorus and potassium (EAN, EAP and EUK respectively) and for efficiency of utilization of nitrogen, phosphorus and potassium (EUN, EUP and EUK respectively) for genotypes of conilon coffee grown in environments with discriminating levels of N, P and K in the soil.

Variation source	EAN	EUN	EAP	EUP	EAK	EUK
Genotypes (G)	282.87*	1.71*	39.11*	334.62*	876.33*	263.18*
Fertilization (A)	20591.94*	12.44*	51.49*	2740.45*	14748.25*	8137.74*
Interaction G*A	181.62*	1.02*	39.31*	61.82*	254.17*	160.03*
Residue	9.07	0.02	0.87	2.02	15.37	4.46
CV (%)	6.77	5.13	6.71	41.04	5.87	5.89
Overall mean	44.51	3.08	13.89	3.46	66.74	39.86

*Significant at 5% probability by F test.

were applied using KH2PO4 p.a., diluted in distilled water and homogenized completely the volume of soil in the pot. In this study, fertilization with nitrogen and potassium was performed in four cover applications, the first at 30 days after planting, and the others, at an interval of 30 days. In all fertilizations, the nutrients were supplied via p.a. salts (KNO_3, KH_2PO_4 and NH_2CONH_2), seeking to establish the nutritional balance of the soil, according to the recommendation (Lani et al., 2007).

The study of nutrition with potassium (Sub experiment 3 - 0% and 100% of the K), consisted of 0.00 and 1.5 g dm^{-3} of potassium, were applied using KCl p.a., diluted in distilled water and homogenized completely the volume of soil in the pot. In this study, the fertilization with phosphorus was provided to all parcels in a single application before planting, using $CaHPO_4$ p.a., (recommendation by Lani et al., 2007), and fertilization with nitrogen was performed in four cover applications, the first at 30 days after planting, and the others, at an interval of 30 days, using NH_2CONH_2 p.a., (recommendation by Lani et al., 2007); both diluted in water and applied in the entire volume of soil.

Evaluation of the study and calculate indices

After 150 days of cultivation, the plant materials (leaves, stems and roots) were collected and separated in paper bags, which were then dried in laboratory oven, with forced air circulation at 60.0°C (STF SP-102/2000 CIR), until constant weight. After drying, the plant materials were weighed on analytical balance (SHIMADZU AUW-220D; precision: 0.00001 g) and triturated (CIENLAB EC-430, 8 blades, 1725 rpm, 20 mesh) to obtain a homogeneous powder.

To quantify the nitrogen content, 0.5 g (+/-0.001g) of the prepared material, in triplicate samples, were transferred to Taylor tubes (25 mm × 200 mm) and submitted to the stages of sulfuric digestion (H_2SO_4), distillation (NaOH 40%) and titration (NaOH 0.02 mol L^{-1}) of nitrogen in "Kjeldahl" distillers (Marconi MA-036), according the Kjeldahl method (Ma and Zuazaga, 1942).

To quantify phosphorus and potassium content, 0.5 g (±0.001 g) of the prepared material, in triplicate samples, were transferred to Taylor tubes (25 mm × 200 mm) and submitted to the stages of nitropercloric digestion (HNO_3 and $HClO_4$) in a digestion block (Tecnal, TE-007D) at 180 to 190°C for 3 h; afterwards, 3 mL of ascorbic acid ($C_6H_8O_6$, 0,87M) were added and the determination was done by spectrophotometry at 725 nm (Femto, 700 Plus) (Defelipo and Ribeiro, 1996).

The following indices were calculated: Absorption efficiency = (total nutrient content in the plant)/root dry matter) (Swiader et al., 1994) and use efficiency = (total dry matter)^2/(total nutrient content in the plant) (Siddiqi and Glass, 1981).

For each nutrient (N, P and K), the genotypes were classified in 4 groups according to the efficiencies of absorption (horizontal axis) and utilization (vertical axis). The grouping was made into two different scenarios of nutrient supply (for N, P and K separately), creating a set of contrasting environments. Therefore, there was cluster analyses in scenarios with low supply of N, P and K (0% of the recommended by Lani et al. (2007) and cluster analyses in scenarios with standard supply of N, P and K (100% recommended by Lani et al., 2007). The grouping, in each scenario, was performed with the intersection of the axes, defined with the overall means for each variable.

Statistical analysis

The data were subjected to analysis of variance (p≤0.05) and the analyses were performed using the statistical software SISVAR (Ferreira, 2011).

RESULTS

Effect of genotypes and fertilizations over the nutritional efficiency

The analysis of variance showed significant interaction for the parameters of nutritional efficiencies for N, P and K between genotypes of conilon coffee and scenarios of nutrient supply in the soil (Table 3). This fact shows that the rates of absorption and utilization of N, P and K are influenced by the genetic material as well as by the supply in the soil, which may allow separation of genotypes between distinct groups for efficiencies in each condition of nutrient supply.

Nutritional efficiency for N, P and K of genotypes of conilon coffee

Figure 1 shows the distribution of the genotypes of conilon coffee as function of the efficiencies of absorption (AE) and utilization (UE) of nitrogen in environment with low supply of this nutrient (control level - 0% N), allowing their separation into four groups: Group 1, consisting of genotypes with higher efficiencies for both absorption and utilization of N (67 and 76); Group 2, with lower efficiency of absorption and higher efficiency of utilization of this nutrient (23, 73, and 153); Group 3, with higher efficiency of absorption and reduced efficiency of utilization of this

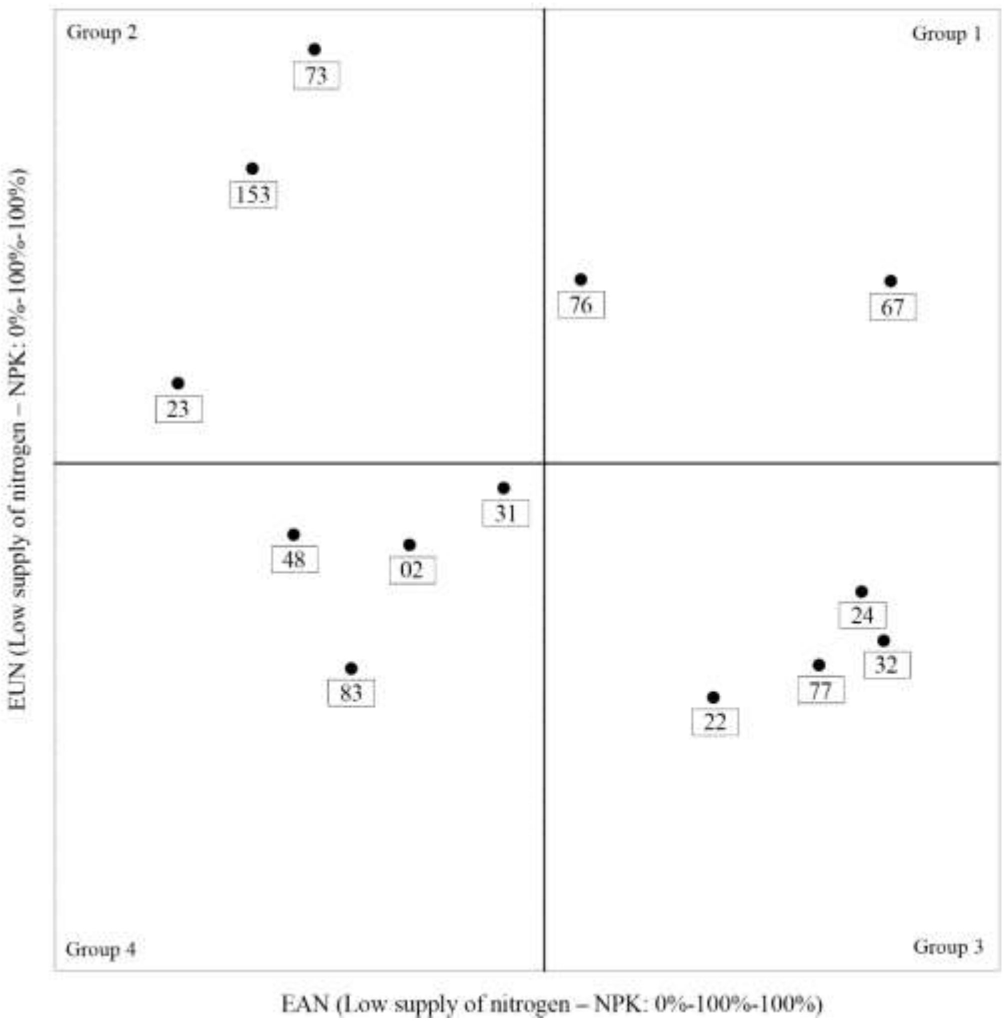

Figure 1. Distribution of genotypes of conilon coffee as function of the efficiencies of absorption (EAN) and utilization (EUN) of nitrogen in environment with low supply of this nutrient (control level - 0% N).

nutrient in the production of dry matter (22, 24, 32, and 77); and Group 4, with low efficiencies for both parameters (02, 48, 31 and 48).

In Figure 2, the distribution of the genotypes of conilon coffee shows the differentiation of the genotypes in an environment with low supply of P (control level - 0% P). In this condition, only a genotype, the 67, showed higher efficiencies for absorption and utilization of P (Group 1); the genotypes 22, 23, 24, 31, 48 and 76 were allocated in Group 2, characterized for reduced efficiency of absorption of P but higher utilization of this nutrient; the genotypes 02, 32, 77, 83, and 153 presented inverse behavior (Group 3), and the genotype 73 presented low absorption and utilization efficiency of P (group 4).

The distribution of the genotypes regarding the efficiencies for potassium, in environment with low supply, is presented in Figure 3. The results show that the genotypes 67, 76, 77, and 83 with higher efficiencies for the nutrition with K (Group 1), the genotype 22 and 73 were placed in Group 2, characterized by lower efficiency of absorption and higher efficiency of utilization of potassium; the genotypes 23 and 48 formed the Group 3, with higher efficiency to absorb and lower to utilize K to produce dry matter. Group 4 was formed by the genotypes 02, 24, 31, 32, and 153, with low absorption and utilization efficiencies for potassium (Figure 3).

The distribution of genotypes as function of the efficiencies of absorption and utilization of nitrogen, phosphorus and potassium in environment with adequate nutrient supply (100% of N, P and K supply) formed the following groups (Figure 4): Group 1, with the genotypes 02, 48, and 67 (high of absorption and utilization efficiency of N, P and K); Group 2, with the genotypes 22, 23, 24, and 76 (low absorption efficiency and high utilization efficiency of N, P and K); Group 3, with the genotypes 31, 32, 83, and 153 (high absorption efficiency

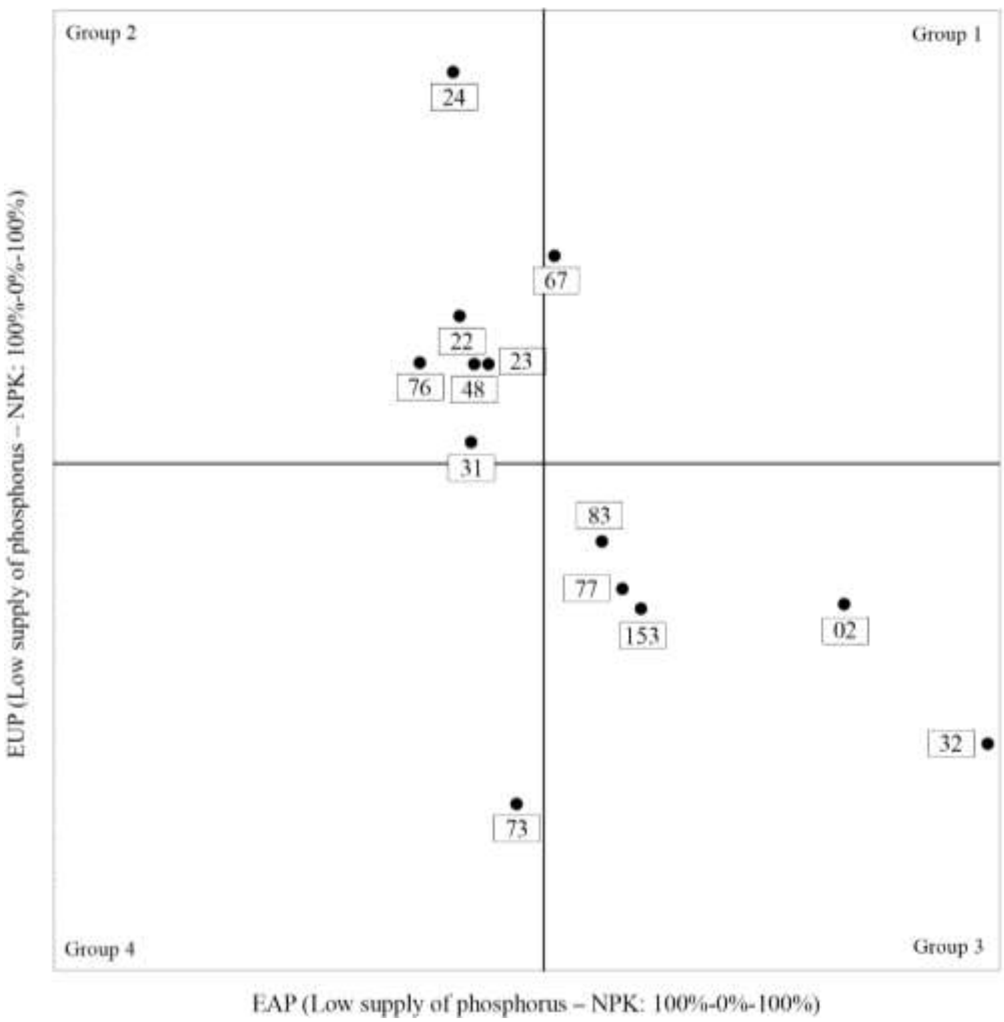

Figure 2. Distribution of genotypes of conilon coffee as function of the efficiencies of absorption (EAP) and utilization (EUP) of phosphorus in environment with low supply of this nutrient (control level - 0% P).

and low utilization efficiency of N, P and K); Group 4, formed with the genotypes 73 and 77 (low of absorption and utilization efficiency of N, P and K).

DISCUSSION

A tendency was observed that conilon coffee genotypes of intermediate and early ripening cycles presented, respectively; low and high joint efficiencies of absorption and utilization of NPK in the environment with adequate supply (Figure 4), and this trend repeated itself in environments with low P supply (Figure 2).

The correspondence between genotypes with low joint efficiencies of absorption and utilization of NPK in an environment with adequate supply, with the characteristic of the cycle of fruit ripening is new, since the behavior of conilon coffee genotypes at low supply of nutrients in soil has always been linked to the morphology of the root system, architecture and diameter of roots (Amaral et al., 2011; Martins et al., 2013c; Colodetti et al., 2014).

Some results suggest that, in environments with adequate nutrient supply, the behavior of genotypes seems to have a relationship with the characteristic of the ripening cycle, indicating that genotypes of early cycle have more efficiency regarding the accumulation of nutrients and dry matter (Partelli et al., 2014, Martins et al., 2015). Higher efficiency of absorption and utilization of nutrients may be linked to earliness of the cycle, since there is some evidence that precocity of fruit maturation may be governed by an larger demand by the metabolic drains, thus creating more transport of photoassimilates to the fruits, and thus increasing the intensity of the sources, which would trigger an increase in net assimilation of carbon, associated with positive changes in stomatal conductance, mainly supported by greater

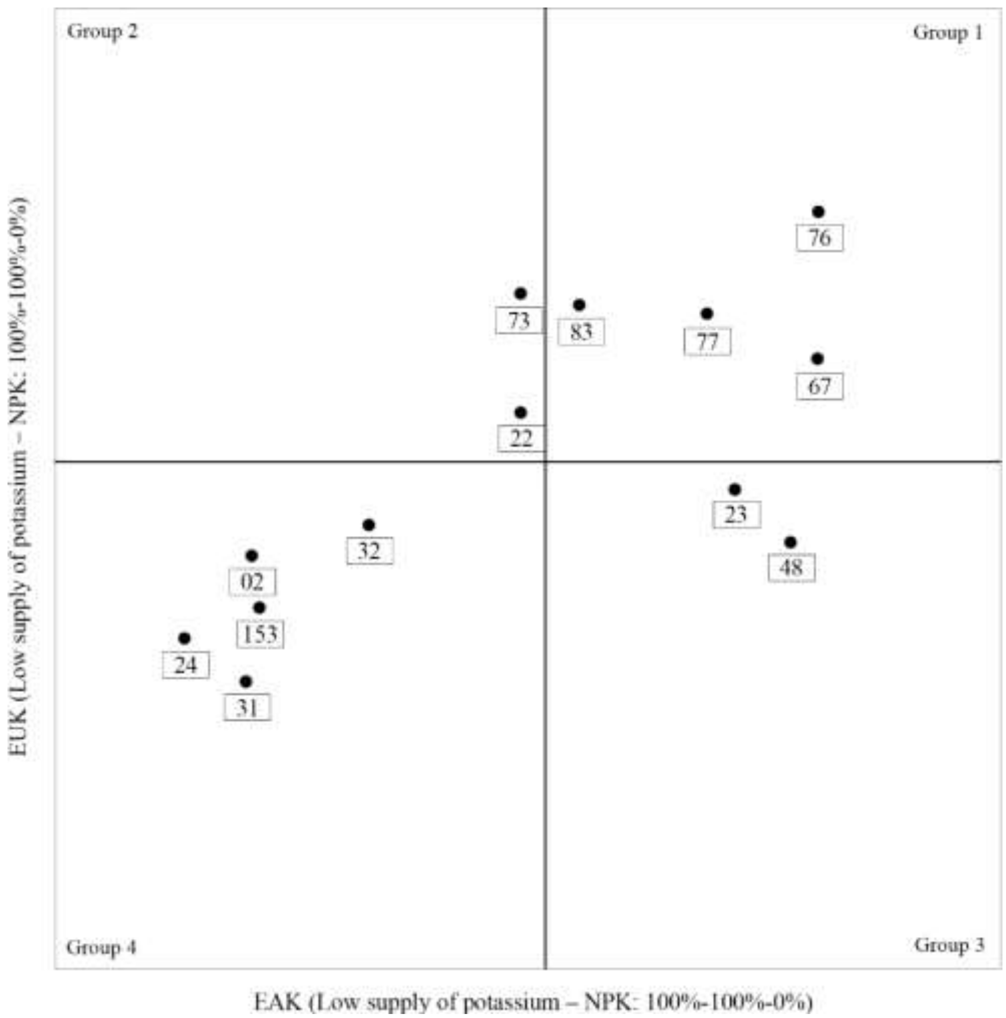

Figure 3. Distribution of genotypes of conilon coffee as function of the efficiencies of absorption (EAK) and utilization (EUK) of potassium in environment with low supply of this nutrient (control level - 0% K).

stomatal aperture (Morais et al., 2012).

As the nutritional efficiency is linked to the accumulation of dry matter, mainly the efficiency of utilization, another factor that gives advantages to early ripening genotypes is the longer period of time that the plant can spend in vegetative recovery compared to their counterparts, resulting in optimization of the process of remobilization of assimilates (DaMatta et al., 2008).

This observation may also explain, at least in part, the responsiveness achieved by genotypes of early cycle, because the increment in the source, with the adequate supply of water and nutrients in the soil, may help stabilizing the sink-source relation of these genotypes, thus meeting the high demand of the fruit, without facing the limitation imposed by the need for metabolic substrates.

Under condition of nutritional limitation, the genotype 02 had low joint efficiencies of absorption and utilization of N and P - Group 4 (Figures 1 and 2), and also low K utilization efficiency - Group 3 (Figure 3), however, in environment with adequate supply, the same genotype showed high joint efficiencies of absorption and utilization of NPK - Group 1 (Figure 4), and this fact indicates a characteristic of responsiveness and may be related to its ripening cycle of this genotype (Table 2).

In general, it has been noted a tendency for genotypes of early cycle being intolerant and inefficient when cultivated with low supply of nutrients in soil, however, they can be highly responsive to a balanced supply of nutrients (Partelli et al., 2014, Martins et al., 2015). Revisiting other results, it was possible to verify that the genotype 02 (also referred as CV-12) is characterized as non-efficient for N and P (Martins et al., 2013a; Machado et al., 2016) and intolerant to the deficit of N and P soil (Colodetti et al., 2014; Martins et al., 2015), but this genotype is responsive to the soil fertilization with N and

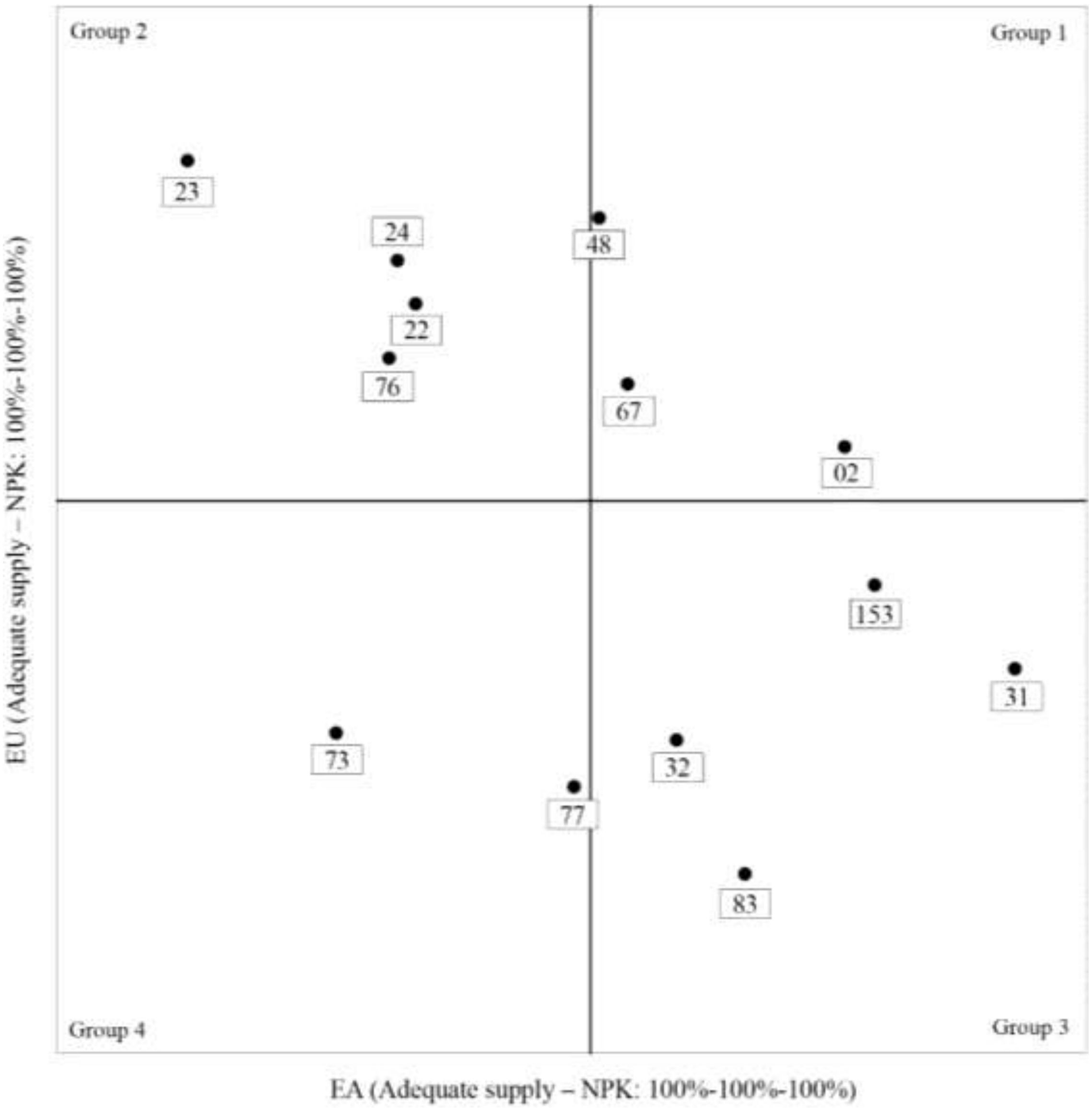

Figure 4. Distribution of genotypes of conilon coffee as function of the combined efficiencies of absorption (AE) and utilization (UE) of nitrogen, phosphorus and potassium in environment with adequate supply of this nutrient (100% of supply N, P and K).

P (Martins et al., 2013a; Machado et al., 2016). Additionally, it has been observed that the genotype 02 has a high yield potential under field conditions (composing three recommended clonal cultivars of conilon coffee), but always linked to an intense demand for nutrients and water.

The behavior of the genotype 67 is also interesting among the displayed cluster, because it has shown high joint efficiency of absorption and utilization for N, P, K, and also NPK, respectively in conditions of low supply of N (Figure 1), P (Figure 2), and K (Figure 3), and also in environment with adequate supply of NPK (Figure 4). This finding was surprising, because it was expected that this genotype had high joint efficiencies of absorption and utilization of NPK in an environment with adequate supply of nutrients, since it is genotype of early ripening cycle, and for not being tolerant to deficit of N in the soil (Colodetti et al., 2014) and not presenting high growth of root system (Martins et al., 2013b; Martins et al., 2013c), which could possibly characterize a different potential to acquire nutrients. However, the results have indicated, at least for N and P, that the genotype 67 (also referred as CV-01) has characteristics of utilization efficiency of N and P under conditions of low supply (Martins et al., 2013a; Machado et al., 2016).

The genotype 76 was clustered, almost in all nutritional scenarios, in groups of high efficiency of utilization, presenting high efficiencies of absorption and utilization for N and K in conditions of low supply of these nutrients (Figures 1 and 3). This genotype also presented low absorption efficiency and high utilization efficiency for P in condition of limitation on the supply of this nutrient (Figure 2), with the same behavior for NPK in conditions of adequate nutrient supply (Figure 4). This fact raises the hypothesis that the absorption efficiency may be compromised by the metabolism that governs the delay in the maturation of genotype of late ripening cycle and that may possibly exist an intensified use of absorbed nutrients, mainly due to its classification as tolerant to a deficit of N and P in the soil, and provide the high efficiency of utilization of N and P when cultivated in environments with nutritional restrictions (Martins et al., 2013a; Colodetti et al., 2014; Martins et al., 2015).

Conclusions

In conclusion, to optimize the nutritional management, the genotypes 67 and 76 would be recommended for plantations with low technological potential to better exploit their efficiencies of absorption and use of N, P and K; and the genotypes 02, 48 and 67 would be recommended for crops with high technological potential where, besides the nutritional efficiency, their responsiveness could be explored. For breeding programs, it is recommended the exploitation of conilon genotypes 02 and 67, for presenting simultaneously high absorption and utilization efficiency of NPK.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Billet metering mechanism of a sugarcane planter

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Mechanical sugarcane planters simultaneously perform ploughing, fertiliser application, seedling metering and furrow covering operations. This study aimed to evaluate the billet metering quality and uniformity of a sugarcane planter and the total damage to buds. The experimental design used was completely randomised in a 2 x2 factorial arrangement totalling four treatments, which consisted of two planting speeds (5.0 and 6.5 km h^{-1}) and two conveyor belt rotation speeds (50 and 100%, which corresponds to 45 and 85 rpm, respectively, in the conveyor belt pulley), with 20 replicates per treatment. The following parameters were evaluated: number of billets m^{-1}, total buds m^{-1}, viable buds m^{-1} and damaged buds (%). The planter metering mechanism exhibited uniform billet metering with low bud damage (5.9%). The increase in working speed decreased the number of billets (9.7 m^{-1}), total buds (22.9 m^{-1}) and viable buds (18.5 m^{-1}). Furthermore, the increase in conveyor belt rotation speed also increased these parameters.

Key words: Statistical process control, agricultural mechanisation, planting speed, *Saccharum officinarum*.

INTRODUCTION

Brazil is expected to produce 654.6 million tons of sugarcane in the 2015/2016 crop year in an area of approximately nine million hectares. The sugarcane production of the country is estimated to increase by 3.1% when compared with the previous crop year. An higher increase in production is precluded by the relatively small increase in planted area in Brazil (0.7%) and by the fact that the yield of sugarcane fields in São Paulo, the largest sugarcane-producing state, is recovering from drought in the previous crop year (Conab, 2015). The Brazilian sugarcane industry encompasses seven states, which are responsible for making Brazil the largest producer and exporter of sugarcane and ethanol in the world (Bottega et al., 2013). The mechanical sugarcane planting system includes a planter that simultaneously conducts the ploughing, fertiliser application, seedling metering, pesticide application and furrow covering operations (Ripoli et al., 2007). This process follows conventional agronomic recommendations, with single-row spacing ranging from 1.0 to 1.60 m and double-row spacing with double rows 0.40 to 0.50 m apart from each other and with 1.40 m between double rows (Coleti and Stupielo, 2006).

The average bud density used is 12 buds per metre of

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furrow and varies among sugarcane farmers and varietal specifications (Beauclair and Scarpari, 2006). Many farmers use between 18 and 22 buds per metre of furrow seeking to improve the plant stand to ensure that the final seedling quality allows a ratio of 12 buds per metre of furrow, with the remaining buds becoming unviable during the process (Raveli, 2013).

Quality indicators of agricultural sugarcane operations have been used in Brazil since the 1990s through studies on tillage, pesticide application, liming, planting and harvesting. Campos et al. (2008), Silva et al. (2008), Barros and Milan (2010), Noronha et al. (2011), Cassia et al. (2014) and Ramos et al. (2014) listed several quality indicators of mechanical sugarcane operations. Monitoring through statistical process control may increase the operational quality levels.

Planting speed is a key factor because it increases working capacity (Melo et al., 2013), which is desired by farmers. Billet metering uniformity is affected by planting speed because the machine has to perform the operations correctly but without hindering performance or damaging vegetative organs during metering (Cebim, 2008).

Conveyor belt rotation speed and its metering uniformity are crucial because when changing the planting speed, the conveyor belt rotation speed must be adjusted to maintain the same billet and bud metering. Thus, the relationship between planting speed and conveyor belt rotation speed must be adjusted because the metering mechanism is the main determinant of the success of the billet planter operation (Cebim, 2008; Pauli, 2009; Barros and Milan, 2010).

Thus, this study aimed to evaluate the billet metering uniformity of a sugarcane planter and the damage to buds as a function of planting speed and conveyor belt rotation speed.

MATERIALS AND METHODS

The experiment was conducted in January 2013 in the municipality of Nova Europa, in the state of São Paulo (SP), Brazil, in an area located near 21°46'3.69" S latitude and 48°36'47.83" W longitude, with an average altitude of 545 m. The soil of the experimental area was classified as Eutrophic Red-Yellow Argisol (Ultisol in the USDA soil classification system), with medium to medium-clayey texture, according to the method reported in Embrapa (2013). The sugarcane variety used was CTC4.

Mechanised sugarcane planting was conducted using a two-row sugarcane billet planter, Santal PDM 2 (Figure 1), which performs the planting operations (ploughing, fertiliser application, billet metering, insecticide application and furrow covering and compaction) in two rows simultaneously, operating without the furrow covering and compaction mechanism at the time of planting to facilitate measurement of variables. The planter was coupled to a Valtra BT 210 4x2 TDA tractor with a 2200 rpm engine with 154.4 kW horsepower (210 cv).

The sugarcane billet planter models available in the domestic market contain metering mechanisms consisting of one or two cleated conveyor belts equipped with a central deflector flap to direct the billet when metering. The model used has a new billet metering system that consists of a removable bottom, rotating metering container and single infeed conveyor belt of the outlet nozzle.

The experimental design used was completely randomised (completely randomised design, CRD), in a 2 x 2 factorial arrangement, totalling four treatments, which consisted of two planting speeds (5.0 and 6.5 km h^{-1}) and two rotation speeds of the billet planter conveyor belt (50 and 100%, which corresponds to 45 and 85 rpm, respectively, in the conveyor belt pulley) with 20 replicates per treatment. Each replicate consisted of samples from both planting rows (right and left), separated by 30 m in row length.

The billets used were characterised by collecting 30 units inside the transport truck and planter; the billets averaged 433 mm in length, 25 mm in diameter, 212 g, and 2.3 buds per billet, with 89% viability. Bud damage was caused by the mechanised harvesting operation (10.9%), and the damage resulting from the transport to the planter was virtually zero (< 0.1%).

The number of billets was determined after planting, through direct count, in four meters of the evaluation furrows. Only one evaluator performed the count for each treatment (20 replicates) for increased experimental control. The number of total buds was determined by direct count in the same billets previously obtained, in the four meters of both evaluation furrows (left and right). The number of viable buds was determined by direct count in the same billets used to assess the total number of buds, in the four meters of both evaluation furrows. Buds were considered viable when they exhibited no damage caused by pests and diseases or any cuts, from harvest until the evaluation of the planting furrows. The number of unviable buds was obtained by the difference between total buds and viable buds, thereby calculating the percentage of damaged buds over total buds.

When operating the planter, the soil water content was measured according to the method reported by Embrapa (1997) at the tillage depth range reached by the furrowers; the soil water content was 9.9% in the 0-15-cm layer, 12.5% in the 15-30-cm layer, and 10.1% in the 30-45-cm layer.

Data were processed using Minitab® 16 software. A descriptive analysis was conducted to determine the measures of central tendency (mean and median) and the coefficients of variation, skewness and kurtosis to characterise the study variables. Dispersion was classified according to the magnitude of the coefficient of variation (CV), as per Pimentel-Gomes and Garcia (2002): "low" for a CV value lower than 10%; "medium" if it is between 10 and 20%; "high" if it is between 20 and 30%, and "very high" if it is higher than 30%. The data were subjected to the Anderson-Darling normality test, and the variables with skewed distribution were transformed. The following data fit models were used: $Y' = \ln Y$ for the number of billets and $Y' = \frac{1}{\sqrt{Y}}$ for the total buds and viable buds. The transformed data were subjected to analysis of variance using the F test at 5% probability. When the F test was significant, the means were compared according to Tukey's test at 5% probability using Sisvar 4.3 software.

The variability analysis was performed by statistical process control using Minitab® 16 software. The tools used were variable control charts using the variables previously described as indicators, with non-normalised data. The mean values and the upper (UCL) and lower (LCL) control limits were defined on the charts using the overall mean of the variable ± three times the standard deviation. The LCL was considered null when its value was negative because negative values have no physical significance for the study variables.

RESULTS AND DISCUSSION

The number of damaged buds m^{-1} (Table 1) exhibited a

Figure 1. Sugarcane billet planter (a and b); billet metering system (c and d).

Table 1. Descriptive statistics parameters of the variables analysed.

Mean	Median	IQR	σ	CV	Cs	Ck	AD
Billets m^{-1}							
10.4	9.5	19.3	4.13	39.7	1.10	0.99	1.978^{A}
Total buds m^{-1}							
23.8	20.0	49.5	10.22	43.0	1.30	1.55	3.162^{A}
Viable buds m^{-1}							
19.2	16.7	40.8	8.00	41.7	1.31	1.76	2.811^{A}
Damaged buds (%)							
6.0	5.5	12.0	2.39	40.0	0.59	0.25	0.606^{N}

IQR: Interquartile range; σ: standard deviation; CV: coefficient of variation (%); Cs: skewness coefficient; Ck: coefficient of kurtosis; AD: value of the Anderson-Darling normality test; ^{A}: skewed distribution; ^{N}: normal distribution.

normal distribution. The other variables exhibited a skewed probability distribution. The mean and median values of the variables number of billets m^{-1}, total and viable buds m^{-1}, and damaged buds (%) were different from each other, indicating high data dispersion. The values of interquartile range, standard deviation and coefficient of variation, with the latter being classified as very high (Pimentel-Gomes and Garcia, 2002), also

Table 2. Analysis of variance and test of means for the variables analysed.

Factors	Billets per meter	Buds per meter		Damaged buds (%)
		Total	Viable	
Planting speed (km h^{-1}) (V)				
5.0	11.2a	24.6	20.0	5.1
6.5	9.7b	22.9	18.5	6.8
Conveyor belt speed (R)				
50%	8.2b	17.5	14.5	4.8
100%	12.6a	30.0	23.9	7.1
F Test				
V	5.189*	0.267^{ns}	0.361^{ns}	18.430*
R	29.883*	55.153*	46.389*	32.885*
V x R	1.643^{ns}	5.901*	5.838*	16.425*
CV (%)	13.91	14.67	14.85	29.66

For each factor, means followed by the same letters in each column do not differ from each other according to Tukey's test at 5% probability. ^{ns}non-significant; *significant at 5% probability, according to the F test. CV (%): coefficient of variation.

differed from each other.

The skewness coefficients indicated that the data distributions were skewed to the right with a high degree of skewness, except for the variable damaged buds, which exhibited a moderate degree of skewness. That information is confirmed by the kurtosis coefficients, which indicate that the data follow a leptokurtic distribution, that is, a more elongated curve than normal. There is an association between the kurtosis and skewness coefficients to predict the behaviour of data over time. They may affect the variability and/or distribution of logical results of a particular process or operation (Bai and Ng, 2005).

Although, the coefficient of variation of damaged buds is very high, such skewness was insufficient to render the data distribution non-normal, which may be confirmed by the Anderson-Darling test. Similar results were found by Voltarelli et al. (2013), thus characterising quality indicators of mechanised agricultural operations in which the data variation is high. The analysis of variance indicated that a higher billet-metering rate is obtained with increased conveyor belt rotation speed. However, this relationship is not directly proportional; that is, doubling the conveyor belt rotation speed does not double the number of billets m^{-1}, which increases by 53% (Table 2). When the conveyor belt operates at maximum rotation speed, the number of billets m^{-1} decreases with increasing planting speed, which underscores the need for conveyor belt speed adjustments to be dynamic; that is, as the operator changes the planting speed, the conveyor belt rotation speed must be set to compensate for this variation and maintain the desired billet metering rate. Interaction between working speed and conveyor belt rotation speed was observed for the following variables: total buds, viable buds and damaged buds (Table 3).

The number of total buds increases when doubling the metering speed of the conveyor belt, both at 5 (96% increase) and 6.5 km h^{-1} working speeds (48% increase; Table 3). The number of buds decreases when maintaining a constant conveyor belt rotation speed (100%) and increasing the working speed, which again highlights the need to adjust the conveyor belt speed. The same trend is observed for the number of viable buds (Table 3) because it is related to the number of total buds.

The mean bud density currently adopted in mechanised sugarcane plantations is 12 buds m^{-1} furrow, depending on the sugarcane variety and vegetative growth (Beauclair and Scarpari, 2006). The means of the present study were higher, reaching the closest value to the mean (16.6 buds m^{-1}) at 5.0 km h^{-1} working speed with the conveyor belt at 50% rotation speed (Table 3). The number of damaged buds (Table 3) was higher when the planting speed was increased at 100% conveyor belt rotation speed because the high rotation speed damaged a higher number of buds. No increase in bud damage occurred when increasing the conveyor belt rotation speed at 5 km h^{-1} planting speed. Conversely, the increase in rotation speed at 6.5 km h^{-1} planting speed caused a 3.8% increase in damaged buds. However, the percentage of damaged buds resulting from the planting operation is low and thus will not hinder sugarcane sprouting because seedling quality is essential in this process (Noronha, 2011). This low damage is attributed to the variety used (CTC4), which is suitable for mechanical planting, and has a good quantity of straw in the stalks, thus protecting the buds from damage,

Table 3. Analysis of the interaction between the factors working speed and conveyor belt rotation speed for the variables total buds m^{-1}, viable buds m^{-1} and damaged buds (%).

Conveyor belt rotation speed	Planting speed (km h^{-1})	
	5.0	6.5
Total buds m^{-1}		
50%	16.6^{Ab}	18.4^{Ab}
100%	32.6^{Aa}	27.4^{Ba}
Viable buds m^{-1}		
50%	13.8^{Ab}	15.2^{Ab}
100%	26.1^{Aa}	21.7^{Ba}
Damaged buds (%)		
50%	4.8^{Aa}	4.9^{Ab}
100%	5.4^{Ba}	8.7^{Aa}

For each variable, means followed by the same uppercase letters in rows and lowercase letters in columns are not different from each other according to the Tukey's test at 5% probability.

particularly from billet metering mechanisms.

Mechanical harvesting is the main cause of decreased rates of viable buds intended for sugarcane planting operations (Lai et al., 2011). Through virtual simulation analysis, the authors found that a new design of the basecutter support mechanism (vehicle and field system) that provides higher quality to the operation must be used to ensure lower damage rates in mechanical harvesting.

Uniformity analysis (Figures 2 and 3) of billet metering exhibits uniformity between the right and left furrows in all treatments. Process variation was significantly changed with the increase in the conveyor belt rotation speed of the planter, as indicated by the increase in interquartile range upper and lower limits (100% conveyor belt rotation speed). However, such variation did not affect operational uniformity because the results only extrapolated the control limits at one point, thereby indicating that the planter maintains a consistent billet metering pattern (Figure 2a).

Metering uniformity was maintained throughout the planting time. Therefore, no type of metering-hampering trend was observed with the decrease in seedling load inside the planter, which highlights the ability of the billet bin to meet the metering rate of the conveyor belt satisfactorily.

The data distribution interquartile range, although within the control limits, exhibits deficient metering mechanisms, which is the greatest challenge to sugarcane planters given the billet size variability (Ripoli and Ripoli, 2010). The authors evaluated five sugarcane planters and observed the same limitation in all machines. A wider interquartile range of total buds (Figure 2b) was observed when changing the conveyor belt rotation speed to 100%, with points occurring above and below the control limits. This exemplifies the effect of externalities on this variable, which may result from factors such as the lack of pressure in the tractor hydraulic systems or the lack of uniformity in bud number per billet. However, those observations are isolated instances within a large number of samples, and the focus should stay on the increased vulnerability of the operation as long as the metering uniformity achieved by the planter is not hindered. Similar results were also observed for the variable viable buds (Figure 3a).

The quality of sugarcane planting using seedlings derived from mechanical harvesting can be reduced given the number of buds planted in the planting furrows (Orgeron et al., 2007). This situation can occur if continuous planting monitoring is not performed thoroughly. The data for the variable damaged buds (Figure 3b) contain points outside the control limits for the 5 km h^{-1} working speed in the left planting furrow. These points are subjected to the action of natural causes only. These results indicate the need for greater control of sugarcane planting speed, which may be achieved by controlling the following factors: (raw) materials, method, measurement and machine. These factors are included in the "6 M's", as they are known in statistical process control studies.

Seedling quality is essential for sugarcane sprouting. Thus, the low average rate of damaged (or unviable) buds contributed to maintaining the planting operation under control, as also observed by Noronha (2011).

Conclusions

The planter metering mechanism exhibited uniform billet metering with low bud damage. The increase in working speed reduced the number of billets and total and viable buds. In turn, the increase in conveyor belt speed increased these parameters.

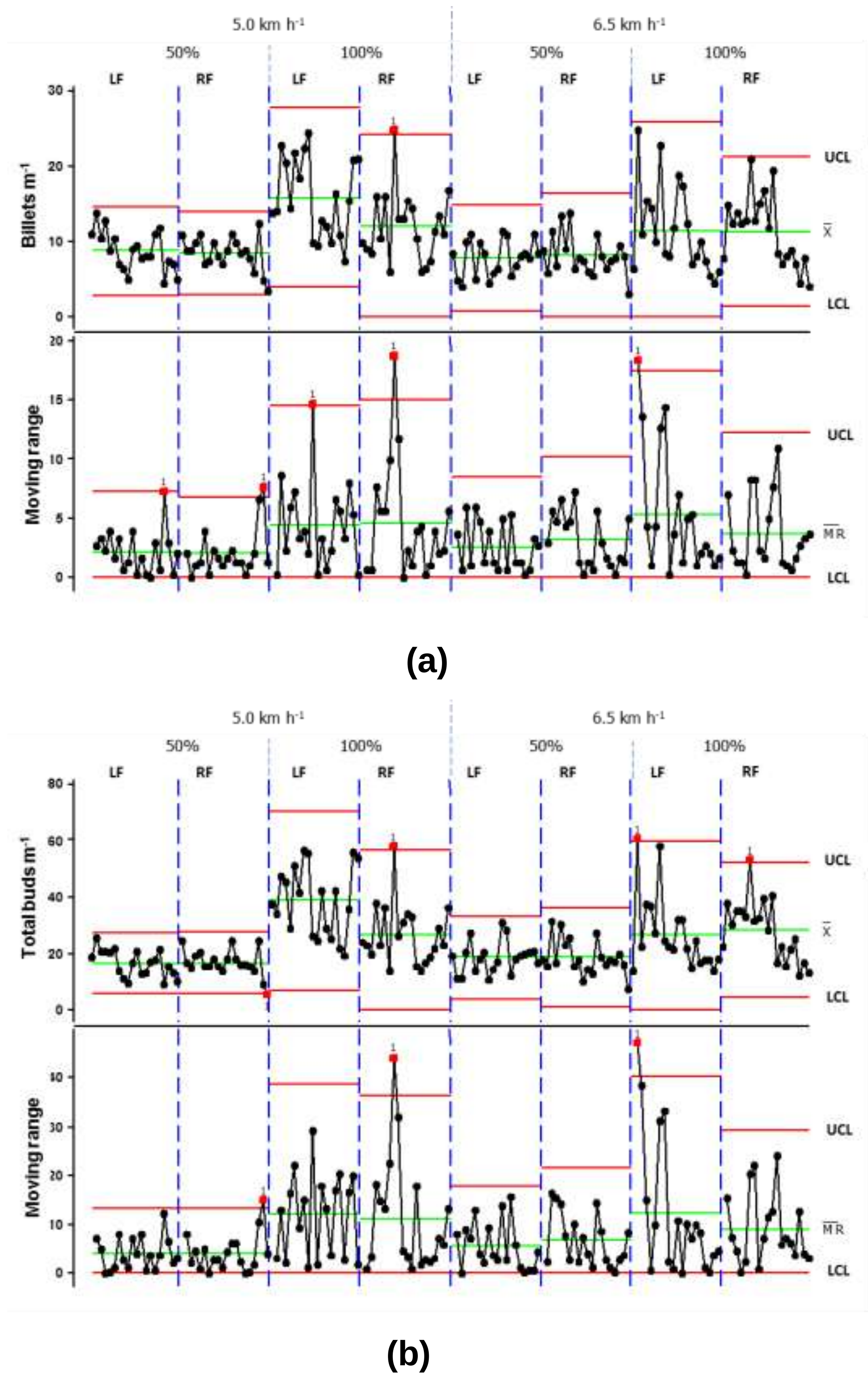
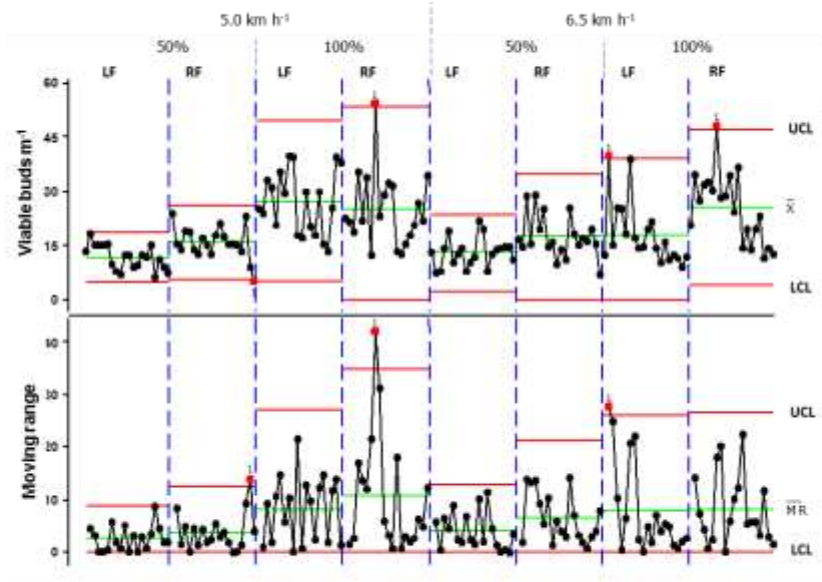
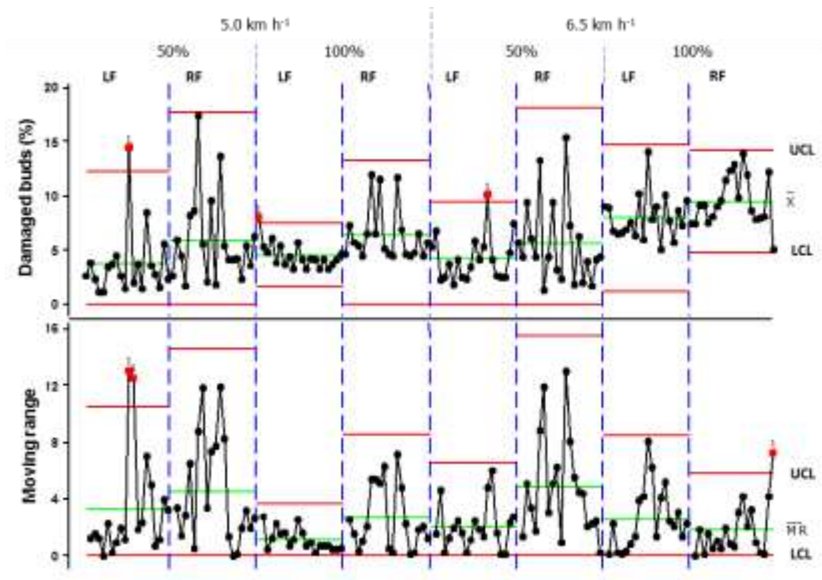


Figure 2. Control charts for the variables: billets m^{-1} (a); and total buds m^{-1} (b). LF: left furrow; RF: right furrow; UCL: upper control limit; LCL: lower control limit; X: mean of individual values; MR: moving range.



(a)



(b)

Figure 3. Control charts for the variables: viable buds m^{-1} (a) and damaged buds (%) (b). LF: left furrow; RF: right furrow; UCL: upper control limit; LCL: lower control limit; X: mean of individual values; MR: moving range.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Thermal of the Campina Grande - PB, Brazil and its environmental impacts

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With the incorporation of new behavioral habits for the most demanding customers, respect for the environment, the industrialized countries and in industrialization have adopted quality standards for air and water, and emissions standards for industrial liquid effluents and gaseous and licensing systems of polluting activities, in line with current environmental legislation. Thus, this study aimed to evaluate the environmental impacts generated by thermal factory Borborema Energy S/A, located between the municipalities of Campina Grande - PB and Queimadas - PB, describing the social and environmental impacts local, arising from the operation of the thermal factory and also proposing mitigation measures in order to mitigate the negative impacts. The observation in loco was used for data collection, using digital images, characterization and qualitative diagnosis of the impacts from the use of the identification matrix of potential impacts in thermals. Thus, it qualified that the Thermoelectric has negative nature on the impacts and medium significance (significant) on the item noise pollution and, as very significant on the items emissions of SO_2, CO_2, NOx, CO and hydrocarbons. As for the interference with flora and fauna, it was found that it exerts great magnitude and high importance (very important). Although there is an Environmental Impact Study (EIS) and Environmental Impact Report (EIR) presented and approved, this EIS and EIR disregarded some important items and does not reflect the reality in the study area and its surroundings.

Key words: Mitigating measures, soil pollution, flora and fauna.

INTRODUCTION

With the advent of new technology and global competitiveness, there is a restructuring of organizations where there is a new design in the model of social and cultural relations within and outside the enterprise environment, involving multiple agents involved in the value chain, with internal and external customers, governments, suppliers, competitors, non-governmental organizations, among others (Mattos, 2002). The respect for environment and human sets a new standard for negotiation and power between the parties, which should not be ignored by companies that want to stay on the competition (Burmann, 2010). On the other hand, the fact

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that the consumer base is known worldwide, they are increasingly aware of their rights.

Due to various disasters of industrial pollution in the 1960's, more specifically, with the implementation of the Stockholm Conference in 1972, the industrialized countries adopted quality standards for air and for water, emissions standards for liquid and gaseous industrial waste, licensing systems of polluting activities and the use of environmental impact reports (Passos, 2009).

In globalized world, industries have adopted voluntary codes of conduct as a responsible action and international standards aiming at the acceptance of the products according to international quality standards, operational safety and less harm to the natural environment (Sant'ana et al., 2010).

The environmental policy and legislation, aimed at ensuring the well-being of today's society and future generations, as well as implement a recovery increasingly effective and a more constant vigilance in order to meet the law. Therefore, enterprise characteristics and the proposed site for installation of a factory, require the assessment of the possible environmental impacts caused by the factory, under the Environmental Impact Study and Environmental Impact Report - EIS/EIR (Terra, 2007).

In this context, it brings up a question on the implementation of a Thermal Factory in Campina Grande - PB, Brazil, with the main focus to the Environmental Impact Study (EIS) in the works planned for the deployment and operation of its Thermoelectric. The scope of this study includes characterization of the project, environmental assessment, integrated environmental analysis, identification and evaluation of environmental impacts, environmental control measures and monitoring actions in the implementation of Thermoelectric of Campina Grande - PB.

The study aims to evaluate the environmental impacts generated by thermal power plant Borborema Energy S/A, located between Campina Grande - PB and Queimadas - PB, describing the social and environmental impacts local, arising from the operation of the thermal power plant and also proposing mitigation measures in order to mitigate the negative impacts.

MATERIALS AND METHODS

The study was conducted from March to December 2015 and the study area comprised of the surroundings of thermal Borborema Energy S/A, as well as its area of influence. The study is a fieldwork, exploratory, with qualitative and quantitative approach. The thermal factory is located between the city of Campina Grande - PB and Queimadas - PB, occupying a building area of approximately 84,630 m², located in the geographical coordinates 7°17'47.44"S and 35°54'30.29"W, whose altitude is 483 meters (Google Earth, 2015; IBGE, 2010). The constructed area of the thermal is considered rural, but in its surroundings there are big population, with about 20.000 inhabitants, living in the surrounding neighborhoods, which has schools, residences, and a Private Natural Heritage Reserve (PNHR) in the process of approval, of the University Support Foundation Education and Research (Furne), with 100 hectares of extension.

Embrapa (1999) classify the soil of the area as Eutrophic Litholic Neosol with horizon "A" weak, sandy texture, stony and rocky phase according to studies carried out. The relief is strong undulating and hilly, with low agricultural potential soils with moderate to strong natural fertility deficiency, strong to very strong deficiency of water and strong susceptibility to erosion (Santos et al., 2006). Among the municipalities of Campina Grande and Queimadas in the conflict area, the class of the soil is Halomórficos soil, SS1 class, Sollonetz Solodizado Ta, with the horizon "A" weak, medium texture, caatinga hypoxerophytic phase, flat relief and undulated (Embrapa, 1972). By Köppen classification, the climate is the type As' (hot and humid) with average annual temperatures between 22 to 26°C. The average rainfall is 700 mm per year, with April to September rains. The average relative humidity is about 80% (Francisco et al., 2010).

The vegetation area is an arboreal size, not too dense, with branched trees, highlighting, shrubby strata dominated by quince, the most frequent tree species are *Schinopesis brasiliensis*, *Zizyphus joazeiro*) and *Petophorum dubium*. As for fauna, different types of birds are present such as, reptiles and rodents, which are all small (Francisco et al., 2010; Ribeiro and Teotia, 2005).

As data collection instrument was used, Arcgis software 2012, GPS Garmin Extrex Vista HCx, digital camera FUJIFILM Fine PIX S2800HD, observations in loco to conduct the survey of environmental impact indicators, and also analysis of the study of impact environmental conducted by Environmental Consulting Ltda, for Borborema Energy S/A and identify potential impacts matrix adapted. The identification, characterization, diagnosis and qualitative impacts were carried out from the parameters of environmental impact assessment adapted (Table 1). Matrices allow easy understanding of the results which can be qualitative, when using the qualitative classification criteria of environmental impacts, or quantitatively, when criteria are used on the magnitude of the impact environment under the positive and negative causes known (Brito et al., 2002; Lelles et al., 2005).

In the present study, we chose to use the identification matrix because of the flexibility to qualitatively measure the impacts. In this study, we chose to use an adaptation of the original Leopold matrix identification because of the flexibility to qualitatively measure the impacts whose measurement was by the method trial specialist and experts that is based on the ability of these issue which estimates the probability of occurrence, spatial and temporal extent, and the magnitude of certain environmental impacts. The diagnoses are expressed based on the experience and knowledge of the judges, having the following classes based on:

1. Formal forecasts
2. Forecasts based on their professional experience
3. Extrapolations from known cases and
4. Pure assumptions (Sánchez, 2013).

Regarding the method of "check - list", it is observed that this is the evaluation of environmental impacts when considering the processing capacity of the physical, biotic and anthropic

RESULTS AND DISCUSSION

Taking into account the parameters of environmental impact assessment contained in Table 1, the potential impacts have been identified and diagnosed in thermal, as shown in Table 2, taking into account reviews of images and observation in loco.

In relation to the ambient air, on the issue of air emissions of particulate matter (Table 2), it was

Table 1. Parameters of environmental impact assessment, adapted.

Aspects	Classes	Note
Nature	Positive (P), Negative (N)	It indicates when the impact has effects on the environment
Form	Direct (D), Indirect (I)	As is obvious the impact resulting from a project action, or it is an impact due from the other, or other impacts generated directly or indirectly by it
Comprehensiveness	Local (L), Regional (R)	Indicates the impacts whose effects are felt in or that they may affect broader geographic areas, characterized as impacts. It was considered as a local effect to that which is limited to the area directly affected the enterprise, and regional, one that is reflected in the area of direct influence
Phase occurrence	Implantation (I), Operation (O)	It indicates the phase of the enterprise the impact can be seen
Temporality	Short term (ST), Mid-term (MT), Long Term (LT)	Differentiates the impacts as manifestation immediately after striking action
Duration	Permanent (P), Temporary (T), Cyclic (C)	Criterion which indicates the impact time duration
Reversibility	Reversible (R), Irreversible (I)	Sorts the second impact manifestation of its effects
Probability	High (H), Mean (M), Low (L)	The probability or frequency which an impact may occur almost certainly and constantly throughout the activity
Magnitude and importance	Big (B), Medium (M), Little (L)	It refers to the degree of incidence and interference of an impact on the environmental factor in relation to the universe of this environmental factor. The magnitude of an impact is therefore treated exclusively in relation to the environmental factor in question, regardless of its importance by affecting other environmental factors. 1 to 3 - Little Important; 4 to 6 - Average Important; 7 to 10 - Very Important
Meaningfulness	Little Significant (LS), Significant (S), Very Significant (VS).	It is classified into three grades according to the combination of magnitude levels

Source: Sanchez, 2013.

diagnosed that: on the aspect of nature, the four items were negative (N); on the aspect form, was presented direct (D) in all items; the comprehensiveness qualified as local (L); and the phase occurrence identified as operating phase (O) for all items. As for the temporality, we found short term (ST), to the issues of respiratory problems, irritating smell, and bad aesthetic effect; and medium term (MT), on interference with flora. On the duration, was evaluated as permanent (P), for interference with flora and bad aesthetic effect, and cyclic duration (C) for respiratory problems and irritating smell.

Reversibility was proved to be reversible (R) for respiratory problems and irritating smell, and irreversible (I) for two other potential impacts. According to the probability, only the item bad aesthetic effect was high (H), the other items were classified as mean (M). According to the magnitude and importance, only the potential impact of bad aesthetic effect was evaluated as big important for level 10 (B10), the other three are considered as the medium importance of level 6 (M6). And yet, the significance was computed as very significant (VS) and significant (S) to other potential impacts.

The pollution of the atmospheric air, vegetation, soil, surface water and groundwater affects the degree of high vulnerability of human health, with increasing risks of quality of life, social and economically of residents surrounding the thermoelectric Borborema Energy S/A (Moraes Neto et al., 2002).

During the implementation of thermal, the fauna is generally affected by human actions, considering the removal of vegetation, which changes the environment and reduces the resources available for food and shelter animals. Complementing this, the movement of people, machinery, equipment and trucks cause noise and vibrations that disturb and scare away the wildlife in

Table 2. Identification of potential impacts matrix in thermal adapted to Leopold

Potential impact					Yes	No	Nature	Form	Comprehen-siveness	Phase occurrence	Temporality	Duration	Reversi-bility	Probability	Magnitude and importance	Meaningful-ness
Environmental elements	Environment	Air	Air Emissions of Particulate Matter	**Respiratory problems:** probability of pollution particles in the human population in the surrounding.	X	-	N	D	L	O	ST	C	R	M	M6	S
				Interference with fauna and flora: habit changes, losses and population reduction.	X	-	N	D	L	O	MT	P	I	M	M6	S
				Irritating smell: present in the emission of gases.	X	-	N	D	L	O	ST	C	R	M	M6	S
				Bad aesthetic effect: the presence of smoke.	X	-	N	D	L	IO	ST	P	I	H	B10	VS
			Emission of sulfur oxides, CO_2, nitrogen oxides, hydrocarbons and carbon monoxide	**Respiratory and cardiopulmonary problems:** coming from the burning of fuel oil.	X	-	N	D	L	O	MT	P	R	H	B9	VS
				Interference with fauna and flora: coming from the burning of fuel oil.	X	-	N	D	L	O	MT	P	I	H	B9	VS
				Rain acidification: gas concentration in the atmosphere.	X	-	N	D	L	O	ST	P	I	H	B8	VS
				Contribution to the heater effect: concentration of CO_2 in the atmosphere.	X	-	N	D	L	O	LT	P	R	H	B9	VS
				Noise pollution: coming from its operation.	X	-	N	D	L	O	ST	P	R	M	M6	S
Environmental elements	Environment	Water	Percolation of rainwater in storage areas	**Contamination of groundwater and watercourse**	X	-	N	D	L	O	LT	P	R	M	M5	S
				Raising the pH	X	-	N	D	L	O	ST	P	R	M	M6	S
				Heavy metals	X	-	N	D	L	O	ST	P	I	M	M6	S
				Dissolved solids	X	-	N	D	L	O	ST	P	I	M	M5	S
			Cooling water systems	**Interference in aquatic fauna and flora**	X	-	N	D	L	O	ST	P	I	H	B10	VS
		Soil		**Removal system of the heavy ashes**	X	-	N	D	L	O	MT	C	I	M	M6	S
				Soil quality	X	-	N	D	L	O	MT	P	R	M	M6	S
	Human Environment		Spaces	**Urban**	X	-	N	D	L	IO	ST	P	I	H	B8	VS
				Agricultural	X	-	N	D	L	IO	ST	P	I	H	B8	VS
				Forestry	X	-	N	D	L	IO	ST	P	I	H	B9	VS
	Landscape		Modifications	**Visual field**	X	-	N	D	L	IO	ST	P	I	H	B10	VS
				Specific elements	X	-	N	D	L	IO	ST	P	I	H	B10	VS

N – negative; D – direct; L – local; O – Operation; IO – implantation and operation; ST – short term; MT – mid-term; LT – long term; C – cyclic; P – Permanent; R – reversible; I – irreversible; M – mean; H – high; M5 – medium importance of level 5; M6 – medium importance of level 6; B8 – big importance of level 8; B9 – big importance of level 9; B10 – big importance of level 10; S – significant; VS – very significant.

general, damaging them, so there is a tendency to disappear locally during the execution of the works, which will alter their population (Cepemar , 2001).

The emissions of sulfur dioxide by burning fossil fuels are considered as environmental impact source on the environment. In order to minimize this impact, it is suggested to increase the generation of hydroelectricity, seek to use coal with lower sulfur content, install filters and extend the weather control. Still, there is increase in the control programs of human action, using the mitigation measures, such as clean energy production from biomass, which will minimize environmental risks and increase disaster management level (Epstein, 2010).

According to Stamm (2003), work on the study of environmental impact assessment in thermal, noise, pollution and vibrations cause the local changes, both in the construction phase and the operational phase, thus bringing remote and discomfort fauna. Also, the thermo-electric bring negative impacts to the socioeconomic environment in relation to tailings deposits, waste gases, suspended dust generation, heavy traffic roads and sewers.

It is noted that the construction of a thermal or other large enterprise, mainly modifies the aesthetics of the site, providing a change or visual pollution of the site where it was built in the enterprise. In addition to causing other irreversible damage to the environment, such as the removal of vegetation, which provides a radical and abrupt change, contribute the a possible change in the microclimate of the region.

The volatile organic compounds are gases and vapors that are generally associated with unpleasant odors, which irritate the eyes, nose, skin and upper respiratory tract. Some of these compounds are understood as carcinogens (benzene and HPA are associated with leukemia) or mutagens, such as toluene and xylenes, when on prolonged exposure. High concentrations may cause nausea, headaches, tiredness, lethargy and dizziness. The NOx increases the susceptibility to respiratory infections, such as asthma and bronchitis (Alvarez et al., 2002; Schirmer, 2007).

As the emission of SO_2, CO_2, CO, NOx and hydro-carbons (Table 2), it was found that it interferes directly or indirectly on the potential impacts. In relation to nature, the five items were negative (N). On the aspect form, all potential impacts were classified as direct (D). Subsequently, the five impacts were diagnosed as a local (L) to comprehensiveness, and how operation (O) the occurrence of phase.

Regarding temporality, the items, respiratory and cardiopulmonary problems and interference with flora and fauna were assessed as mid-term (MT); the items acidification of rain and noise pollution as short term (ST), followed by long term (LT) for the item contribution to the heater effect. The duration is qualified as permanent (P) for all potential impacts. However, for the aspect of reversibility, the items respiratory and cardiopulmonary problems, contribution to the heater effect and noise pollution were considered reversible (R), while the other two items were considered irreversible (I).

About the aspect, probability was considered mean (M) only to the issue of noise pollution and high (H) for other potential impacts. Regarding the magnitude and importance, noise pollution was estimated to be medium level 6 (M6), the others impacts were evaluated as big importance of level 8 (B8) and level 9 (B9). Still in relation to the assigned, significance was inferred as significant (S) to the item noise pollution, and as very significant (VS) to the other four items.

According to Schirmer (2004), organic compounds derived oil directly which contributes to the problems of air pollution. Among them, BTEX (benzene, toluene, ethylbenzene and xylenes), and other less abundant and highly reactive compounds such as ethylene oxide, formaldehyde, phenol, carbon tetrachloride, the clorofluo-carbonados (CFC) and polychlorinated biphenyls (PCBs). The excess of emissions of CO, CO_2, NOx, SO_2, volatile organic compounds and particulate matter, not only causes health problems of human, but also impacts on the environment, both local character, such as "smog", as global, such as the heater effect (Schirmer and Rudniak, 2009).

The impacts on the physical environment originating from the thermoelectric in operation phase, can cause changes in air quality, being the main air pollutants emitted by smokestacks, the gas motor generators: nitrogen oxides (NOx), basically composed of NO (nitric oxide) and NO_2 (nitrogen dioxide). It is observed even in smaller amounts the carbon monoxide (CO), hydrocarbons and particulates matter. The gases that contribute to the heater effect are CO_2 (carbon dioxide), N_2O (nitrous oxide) and CH_4 (methane). These results corroborate to the studies of Wärtsilä (2010) held in Thermoelectric Santa Julia I in the city of Anchieta, State of São Paulo - Brazil.

In addition to impacts of mining, coal burning industries and thermals cause environmental impacts, due to the emission of particulate matter and gases. Besides being harmful to human health, these gases are mainly responsible for the formation of acid rain. Therefore, it is suggested that the use of natural gas as an alternative is less aggressive to the environment (Gonçalves, 2010).

According Hama (2001) in the study dealing on acid rain and aluminum concentration in soil near a thermal coal, the study inferred that rainwater samples analyzed around a thermal coal in northern Paraná State was reported to be pH below 5,6, characteristic of a weakly acid rain, and the concentrations of cations and anions in the rain water had high concentrations of sulfate, followed by sodium cations, calcium and ammonium.

In Brazil, in the year 2000, the thermoelectricity was an exit as contemplated during the energy rationing, which created an emergency program of building thermals. The high cost of natural gas, quoted in dollars, and coal generation unfeasible most of thermals projects. In addition, the large amount of water required for cooling systems potentiated the environmental cost of energy generation (Monteiro et al., 2004). During the operation phase of a thermal, the gas production changes the air quality and noise generation affect mainly workers, this impact could be minimized by the use of individual protective equipment (IPE) and technology of the equipment to be purchased (LCB, 2009).

In the diagnosis of environment, water part, encompassing the percolation of rainwater in storage areas (Table 2), qualified all items as negative (N), regarding the aspect of nature; they were direct (D) in the aspect form; they were considered local (L) in relation to the comprehensiveness; as operating phase (O), on the occurrence of phase. About aspect temporality, only the impact potential contamination of groundwater and watercourse has been classified as long term (LT), the others three, was considered as short term (ST).

Regarding reversibility, the items contamination of groundwater and watercourse, and raising the pH were qualified as reversible (R) and the heavy metals and dissolved solids as irreversible (I). The probability was assessed as mean (M) over all items. The magnitude and importance of potential impacts were described as medium of importance (M5 and M6), and its meaningfulness was considered significant (S).

The air emissions of particulate matter, respiratory problems, interference in fauna and flora, the irritating smell, the bad aesthetic effect, the emission of sulfur oxides, the acidification of rain, the emissions carbon dioxides, emissions of nitrogen oxides, the percolation of rainwater in storage areas, the elevation of pH, heavy and solid metal dissolved, cooling systems of water, the removal system of the heavy ash and residues solid process are significant negative environmental impacts caused by thermals (Cardoso et al., 2010).

About the cooling system of the water, environment and water part (Table 2), this is attributed to significant interference on the potential impacts observed. As the aspects highlighted on the item interference in aquatic fauna and flora, the aspect nature was assigned as negative (N); the form was direct (D); the comprehensiveness was evaluated as local (L); the occurrence of phase as operation (O); temporality as short term (ST); the duration as permanent (P); reversibility as irreversible (I); the probability as high (H); the magnitude and importance as big importance of level 10; and the meaningfulness was presented as very significant (VS).

The most important impacts of a thermoelectric are considered, as the emissions of gaseous pollutants in the atmosphere and the use of cooling water for condensation of steam. Also noteworthy is the wastewater generated in the thermals that are from the cooling process, which causes thermal pollution of water reservoirs, of water treatment systems, water contaminated with oil residues, among others (Salomon, 2003). The main environmental problems are human exposure to pollutants in air, water, soil and food which is a major contributor to mortality and morbidity (WHO, 1996). Already the fishes, require a minimum salt content to develop and the acid removes these water salts, killing them (Richard, 1998).

In the environment, soil part (Table 2), the potential impacts, removal of heavy ashes and soil quality, was diagnosed that the two items were considered negative (N) as its nature and direct (D) in relation to its form; local (L), as its comprehensiveness; operation (O) to the occurrence of phase; and mid-term (MT), to the temporality. The duration was stimulated cyclic (C) for the removal system for the heavy ashes, and permanent (P) to soil quality. According to reversibility, the only item that was reversible (R) was the soil quality. Regarding the probability, mean was considered for both items, and finally the magnitude and importance aspects, followed by meaningfulness were successively qualified as medium importance of level 6 (M6) and significant (S).

As for the volume, the coal ash is one of the largest waste generation in Brazil. However, only a small percentage is reused in the construction industry, the rest is disposed of improperly, causing serious damage to human health and the environment (Izidoro and Fungaro, 2007). The thermal effluent of interest to the environment may be classified as air, liquids and solids. In particular, the solid, such as particulate matter in combustion, in which part of the formed ash are entrained in the gas flow receiving the name of fly ash. A portion of this ash is retained by electrostatic precipitators and a small portion is discharged through the chimney. Not all fly ash is volatile: part of it is in the boiler bottom, being called residual or heavy ash, and thus are not part of air emissions (Araújo, 2002).

According to the Human Environment (Table 2), urban, agricultural and forestry were evaluated, where the potential impacts are proved with influence on the environment and negative nature (N). As regards the other aspects, the form was evaluated as direct (D); the comprehensiveness as local (L); the occurrence of phase both in installation and in operation (IO); temporality as short term (ST); the duration as permanent (P); reversibility as irreversible (I); probability as high (H); the magnitude and importance as big (B8 and B9); and meaningfulness as very significant (VS).

According to Carvalho and Cidade (2011), the implementation of the Thermal Porto do Itaqui, Sao Luis, MA, caused negative impacts, such as deforestation of the area for the construction of the thermal, leading to loss of biodiversity and the pollution of rivers by the waste; promotion of social impacts, by the dismantling of the livelihood of local people, affecting the massive rural society, thus contributing to the disorderly occupation of urban space.

Regarding the landscape, the visual field changes and the specific elements (Table 2) have been described as influential and its aspects presented are present; as negative (N) in relation to nature; as direct (D) to the form; as local (L) for comprehensiveness; with its occurrence in the implementation and operation phase (IO); with temporality of short term (ST); with duration permanent (P); the reversibility was considered irreversible (I); the probability was high (H); as big (B10) to the magnitude and importance aspect; and as very significant (VS) to the meaningfulness.

Changes in landscapes are significant in studies of environmental impacts, such as when the improper land use occur in the destruction of green areas, the burial of rivers and mangroves, and the pollution of atmospheric air and water. The landscape is not simply adding disparate geographic elements and certain portion of space. The result of the dynamic combination being unstable on physical, biological and human elements, reacting dialectically about each other, make the landscape a single and indivisible whole in constant "evolution" (Silva et al., 2007).

The biggest problem of this "evolution" is that the landscapes are modified according to the need of some individuals to meet the interests of local leaders, which takes place in the case of thermal of Campina Grande - PB, through various activities (industries, leisure, education and others), which caused a very rapid population growth and consequently caused many environmental problems.

According to the study of Tagliani (2003), environmental vulnerability means greater or lesser susceptibility of an environment impact potential caused by an anthropic use, there is no way to implement projects without promoting the disorganization of social and cultural life of the town.

CONCLUSIONS

According to qualified impacts on potential impacts of identification matrix in thermal, adapted, it appears that the sulfur dioxide emissions from burning fossil fuels and OCB1 oil, are pollutants that cause health damage to the surrounding population enterprise and suffers injury, when there is change of seasonal winds. As for mitigating and compensatory measures proposed by the project, these do not include effective measures, and are very superficial and does not address compensatory measures such as afforestation, and monitoring by trained technicians, taken from the growth of native trees to its growth and the amount of trees removal at the site to be built the thermal, in order to compensate in another area, as required by law. Although there is an EIS/EIR presented and approved, this EIS/EIR disregarded some important items and does not reflect the reality in the study area and its surroundings. Regarding the operation of the thermal, as well as being harmful to the health of the population, the noise of its operation also disturbs the sleep of people, scares off the animals and brings harm to farmers. In order to minimize the impacts, it is proposed to replace the OCB1 oil, for a less polluting fuel, control and monitoring by state agencies and planting native trees in order to compensate for the removal of these species for the construction of the enterprise.

RECOMMENDATIONS

1. According to Epstein (2010), a way to minimize impacts of thermals, would be the installation of filters and weather control and the implementation of human action control programs, as well as the production of clean energy, which can minimize environmental risks and enhance disaster management level.
2. In order to mitigate the impacts of air pollution in developing cities, coming from thermoelectric Philippi Jr.et al. (1998) proposes to carry out an environmental surveillance program.
3. In order to minimize the negative impacts caused by the implementation and operation of thermoelectric Borborema Energy S/A, in Campina Grande - PB, the use of less aggressive fuels such as natural gas and biomass; planting native trees; monitoring of fauna and flora, by environmentalists and researchers entities; treatment and control of solid and liquid waste; adequate storage of waste; investments in schools for local people, including promoting the environmental education; job creation; educational campaigns; establishment of forest nurseries; compensation to local farmers if necessary is proposed.
4. Also, to promote the training of all employees and contractors working in the region in the Awareness Programme for the Environment Areas, Quality, Safety and Health.
5. Environmental education to communities report on the impact of materials such as plastics, glass and metals and presenting techniques to transform disposable waste in handmade objects and utilities.
6. Promote the transformation of social reality and the recovery of citizenship of the local population through vocational training courses (in mechanical activities, computers, carpentry, electrician, cook, etc.), carried out in partnership with technical training courses companies by example for labor-work training site, including any jobs meeting the aspirations and needs of local populations.
7. Also, contribute to the dissemination of economic, cultural and tourist potential of the region, providing technical support for the development of projects and participating in fairs and exhibitions to promote the city of Campina Grande -PB.
8. Reaffirming the commitment of Thermoelectric of Campina Grande, which is important in producing energy to protect the environment, investing heavily in the Environmental Management Program, showing that it is possible to generate wealth and preserve our greatest

wealth being human and the environment.

Conflict of Interests

The authors have not declared any conflict of interests.

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Proximate composition, amino acid profile and some anti-nutrients of *Tithonia diversifolia* cut at two different times

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The experiment assessed the proximate and amino acid compositions, metabolizable energy and anti-nutrient constituents of *Tithonia diversifolia* leaf meal (TDLM) harvested separately in the months of May and September, respectively using standard laboratory methods. The crude protein (20.5±0.1%), crude fibre (18.8±0.01%) and ash (14±0.1%) significantly ($p<0.05$) increased in TDLM for September cutting than that of May (18.05±0.1%, 11.17±0.3% and 13.01±0.1%, respectively). All the anti-nutrient factors (ANFs), namely, oxalate, phenols, phytin, phytin P, saponins and tannins except alkaloids and flavonoids were significantly ($p<0.05$) affected by the cutting time of the plant leaf. The ANFs contents in May cutting were oxalate (1.88±0.1 mg/100 g), phenols (0.31±0.01 mg/100 g), phytin (77.3±0.1 mg/100 g), phytin P (21.8±0.1 mg/100 g), saponins (21.8±0.1 mg/100 g) and tannins (0.5±0.01 mg/100 g). The metabolisable energy (ME) for May cutting of TDLM was 2908.27 kcal/kg while that of September was 2565.18 kcal/kg. Glutamic acid had the highest value of 10.94±0.02 mg crude protein among the determined amino acids in TDLM whereas cystine was the lowest (0.94±0.01 mg crude protein for both May and September cuttings. TDLM is limiting in methionine with a chemical score of 30 in both cuttings. Proximate composition of TDLM was higher for September cutting than May and could be used as vegetable protein source.

Key words: *Tithonia diversifolia* leaf, proximate composition, amino acid profile, anti-nutrients, May cutting, September cutting.

INTRODUCTION

Plants' leaves contain nutrients that are of importance in nutrition for growth, development and production in man and animals. They are sources of protein, carbohydrates, pigments and minerals in human and animal food/feed. However, the presence of anti-nutritional factors such as oxalate, saponins and tannins limit the effective utilization

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of the nutrients in some of these leaves as sole ingredients or components of animal feeds by binding to digestive enzymes and some dietary proteins particularly in monogastrates (Aletor, 1999; Fasuyi et al., 2007). *Tithonia diversifolia,* Mexican sunflower plant, grows as weeds in many part of Southwest Nigeria acting as fallows on farmland, component of compost for organic manure and as aphrodisiac where the extracts are used as medicaments in human and animal (Ayeni et al., 1997; Adoyo et al., 1997; Inoti, 2000; Olabode et al., 2007; Akinola, 2014). *T. diversifolia* leaves have been documented to contain 18 to 21% crude protein (CP), 15 to 18% crude fibre (CF), 13 to 15% ash, 2.81 to 5.6% ether extract (EE) and 44.38 to 52% nitrogen free extract (NFE) by various workers (Olayeni, 2005; Fasuyi et al., 2007; Ekeocha, 2012). The proximate compositions of leaves have been reported to vary with cutting interval or age. Tannin content in cassava foliage was reported to increase at longer cutting intervals as high as 3.5% of the dry matter for 45 days cutting intervals when compared with the root harvest of 4.3% dry matter (Khang et al., 2005). Hiep et al. (2008) also documented the differences in the yield and chemical compositions of leaves of tropical kudzu grass (*Pueraria phaseoloides*). The study showed a decrease in the crude protein content and increasing dry matter and acid detergent fibre (ADF) in the leaves as the cutting interval progressed. However, there is dearth of information on the proximate composition at the different cutting times of the year, particular at young and maturity stages for *T. diversifolia*. This study therefore investigated the different compositions at this various times.

MATERIALS AND METHODS

Collection and preparation of *T. diversifolia* foliage

T. diversifolia leaves were harvested within the Ekiti State University, Ado-Ekiti, Nigeria. It is located on longitude 7° 40ˡ North and latitude 5° 15ˡ East of the Greenwich Meridian in the rainforest zone with average rainfall of 1500 mm, ambient temperature between 22 and 38°C and relative humidity of 70%. The leaves of the plant were harvested in May (at the onset of growth of the leaves after the dry spell) and September (before the flowering stage) which are the two significant periods in the year that could influence the nutrient composition of the leaves of the plant. The leaves were chopped and sun dried to a crispy touch, milled in a commercial feed milling machine (Artec Model 20) to *T. diversifolia* leaf meal (TDLM). The dried samples were stored in transparent polythene bags and kept in the feed store house.

Determination of proximate composition

About 2 g of the dried *T. diversifolia* leaf meal (TDLM) of the two different cuttings (that is, May and September) were analyzed for proximate composition. The dry matter (DM) was determined by drying in a Gallenkamp draught oven at 105°C for 24 h to a constant weight. The crude protein (CP) of each of the samples was determined by Kjedahl method, ether extract (EE) by using petroleum ether with a boiling point range of 40 to 60°C for extraction in a Soxhlet apparatus for 6 h, all as described by AOAC (2005). The crude fibre (CF) was analyzed using the method of van Soest and Rorbertson (1985). The ash was determined by burning the defatted sample in a muffle furnace at 600°C and percent ash of the TDLM calculated. Nitrogen free extract (NFE) was obtained by difference while the metabolisable energy (ME) was calculated using prediction equation of Pauzenga (1985), that is, ME = 35 × %CP + 81.8 × %EE + 35.5 × %NFE.

Amino acid analysis

Dried 2 g samples of TDLM was hydrolyzed at 150°C for about 90 min and the solution used for the determination by the modification of waters 'pictotag system' (Bidlingmeyer et al*.,* 1984; Gardner et al., 1991). The amino acid score was calculated as shown according to Crampton and Harris (1969) and WHO (1973).

Amino acid score = mg of amino acid per gram of test diet / mg of amino acid per gram of reference protein

Determination of anti-nutrients

Tannin

The tannin in the two samples were quantified by macerating 200 g of each of the TDLMs in 10 cm³ Folin Ciocalteu reagent (sigma) and 2.5 cm³ $NaCO_3$ (Sodium carbonate). The absorbent of the solution was measured at 725 nm after 40 min using the method of Makkar and Goodchild (1976). Tannin equivalent was calculated from the standard curve.

Phytin, phytin P and oxalate

Phytin contents were determined using 8 g of each of the samples of TDLM soaked in 200 cm³ of 2% HCl and allowed to stand for 3 h. The extract of each of the samples was filtered through a double layer filter paper, 50 cm³ of the duplicate samples of each of the filtrates were pipette into 400 cm³ beakers. 10 cm³ of 0.3% NH_4SCN (Ammonium thiocyanate) was used as an indicator and 107 cm³ of distilled water to obtain acidity of pH 4.5. Ferrous chloride ($FeCl_2$) solution containing 0.00195 g/cm³ of Fe^{2+} was then titrated against each of the solutions of the test samples until brownish yellow colouration persisted for 5 min. Phytin content was calculated by multiplying the titre value with a factor of 3.55 as described by Young and Greaves (1940). The oxalate contents were also analyzed as described by Huang and Tanudjaja (1992).

Statistical analysis

All the data collected except the amino acid composition were analyzed for mean values, standard deviation, coefficient of variation and t-test using the General linear model computer package as described by SAS (1987).

RESULTS

The proximate compositions of *T. diversifolia* harvested in May and September are shown in Table 1. Crude protein (CP), crude fibre (CF) and ash contents (20.50±0.1 g/100 g, 18.80±0.1 g/100 g and 14.10±0.1 g/100 g, respectively) were significantly higher (P<0.05) for

Table 1. May and September cuttings' proximate composition of *T. diversifolia* leaf meal.

Parameter (%)	May cutting	September cutting	t-test	Coefficient of variation (CV)
Dry matter	91.03	88.72	0.7[NS]	4.15
Crude protein	18.05± 0.1[b]	20.5± 0.1[a]	0.01*	6.98
Crude fibre	11.17± 0.3[b]	18.8± 0.01[a]	0.01*	27.93
Ash	13.01± 0.01[b]	14.1±0.1[a]	0.01*	4.42
Ether extract	5.5± 0.1[a]	4.1± 0.1[b]	.0001**	16.08
Nitrogen free extract (NFE)	51.2± 0.1[a]	42.6± 0.1[b]	0.01*	10.15
†ME (Kcal/kg) (Calculated)	2908.27[a]	2565.18[b]	0.01*	15.02

[a,b]Means with different alphabets on the same row differ significantly (P<0.05). *Significant at (P<0.05); NS= Not significant (P>0.05). †ME: Metabolisable energy.

Table 2. Anti-nutrient content of *T. diversifolia* (mg/100 g).

Parameter	May cutting	September cutting	t-Test	Coefficient of variation (%)
Alkaloids	1.24± 0.01	1.24± 0.01	100[NS]	0.72
Flavonoids	0.87± 0.01	0.87± 0.01	100 [NS]	1.02
Oxalate	1.88± 0.01[a]	1.75± 0.01[b]	0.01*	3.95
Phenols	0.31± 0.01[b]	0.51± 0.01[a]	0.01*	26.8
Phytin	77.3± 0.01[b]	79.2± 0.01[a]	0.01*	1.33
Phytin P	21.8± 0.1[b]	23.3± 0.01[a]	0.01*	3.65
Saponins	2.82± 0.01[a]	2.37± 0.01[b]	0.01*	9.50
Tannins	0.51± 0.01[a]	0.38± 0.01[b]	0.01*	16.12

[a,b]Means with different alphabets differ significantly (p<0.05). *Significant at (p<0.05); NS= Not significant (p>0.05).

September compared to May cuttings (18.05±0.13 g/100 g CP, 11.17±0.3 g/100 g CF, 13.01±0.01 g/100 g ash).

The NFE value was significantly lower (P>0.05) for September cutting (42.60±0.1 g/100 g) than that of May (51.20±0.1 g/100 g). The dry matter was not affected (P>0.05). The determined ANFs in *T. diversifolia* for May and September cuttings are shown in Table 2. The alkaloids and flavonoids were not significantly affected (P>0.05) as they recorded same values for May and September cuttings (1.24±0.01 mg/100 g and 0.87±0.01 mg/100 g, respectively). Oxalate, saponins and tannin had significantly higher values (p<0.05) in the May cutting (1.88±0.01 mg/100 g, 2.82±0.01 mg/100 g and 0.51±0.01 mg/100 g, respectively) than that of September (1.75± 0.01 mg/100 g, 2.37± 0.01 mg/100 g and 0.38± 0.01 mg/100 g, respectively. However, phenols, phytin and phytin P exhibited higher values (P<0.05) in September cutting (0.51±0.01 mg/100 g, 79.2±0.01 mg/100 g and 23±0.01 mg/100 g, respectively) compared to those of May (0.51± 0.01 mg/100 g, 79.2± 0.01 mg/100 g, 23.3± 0.01 mg/100 g, respectively. The coefficient of variation varied between 1.33 for phytin and 26.8 for phenol.

The amino acids in May and September are shown in Table 3. There were no significant differences (P>0.05%) in the values of amino acids and chemical scores. Generally, the order of ranking of the amino acids in TDLM for May and September cuttings is glutamic acid>leucine>aspartic acid> alanine> arginine> lysine>isdeucine> phenylanine> proline> glycine> serine> tyrosine>threonine>histidine>methionine. Methionine had the lowest score of 30 in both cuttings.

DISCUSSION

CP, CF and ash of *T. diversifolia* as shown in Table 1, showed significant increase in the September cutting. This indicated that these nutrients increased correspondingly as the plant aged. The result is comparable with reports of previous workers on other leaf meals such as those of *gliricidia*, cassava and amaranthus that were adjudged to be good source of protein in broiler or monogastric diets (Agbede and Aletor, 2003; Fasuyi et al., 2008). It also agrees with the findings of Kitaba (2003) and Bayble et al. (2007) that noted higher crude protein yield as Napier grass increases in age. The observed increase in the proximate values of the September cuttings may not be unconnected with the fertility of the soil which could be influenced by the pH, organic matter content and the soil type (Muya et al., 2011).

The presence of ANFs in TDLM agreed with the result of other workers (Fasuyi et al., 2010; Ekeocha, 2012). However, the ANFs content appeared to be within the

Table 3. Amino acid composition of *T. diversifolia* Leaf meal (TDLM) cut in May and September.

Amino acids	Mean value ±SD (mg/100 g) May	Mean value ±SD (mg/100 g) September	Coefficient of variation (%)	Chemical score May	Chemical score September	t-test
Alanine	6.90± 0.01	6.80± 0.2	0.22	-	-	12.3^{NS}
Arginine	5.70± 0.01	5.75± 0.03	0.27	89	89	10.7^{NS}
Aspartic acid	7.05± 0.03	6.95± 0.1	0.75	-	-	8.75^{NS}
Glutamic acid	10.94±0.02	11.04±0.01	0.36	-	-	14.1^{NS}
Histidine	2.55± 0.03	2.45± 0.01	1.96	121	121	7.89^{NS}
Iso-leucine	4.21± 0.02	3.81± 0.05	0.76	53	53	9.45^{NS}
Lysine	5.39± 0.07	4.91± 0.01	2.14	75	75	7.84^{NS}
Phenylalanine	4.08±0.01	4.11±0.04	0.49	65	65	6.67^{NS}
Proline	3.96± 0.01	5.96± 0.03	0.38	-	-	8.61^{NS}
Serine	3.55± 0.02	4.15± 0.02	1.41	-	-	7.02^{NS}
Threonine	3.06± 0.01	3.06± 0.01	0.49	63	63	4.68^{NS}
Tyrosine	3.43± 0.06	4.03± 0.01	2.91	76	76	5.99^{NS}
Glycine	3.93± 0.02	3.53± 0.03	0.96	-	-	5.10^{NS}
Leucine	8.02± 0.04	7.92± 0.01	0.87	87	87	10.44^{NS}
Methionine	1.25± 0.02	1.17± 0.02	2.01	30	30	2.93 ^{NS}
Cystine	0.93± 0.01	0.73± 0.01	1.61	39	39	3.01 ^{NS}
Valine	4.05± 0.01	3.95± 0.01	0.62	55	55	4.78 ^{NS}

NS: Not significant (p>0.05).

tolerable dietary level for monogastric animals as the data obtained in this study were within the range 0.36 to 0.38 mg/100 g for tannin, 1.5 to 2.93 mg/100 g for oxalate (Dairo, 2008; Fasuyi and Ibitayo, 2011). In addition, TDLM has been investigated as components of diets in poultry, swine and ruminant to give reasonable results (Odunsi et al., 1996; Farinu et al., 1999; Togun et al., 2006; Fasuyi and Ibitayo, 2011; Ekeocha, 2012). TDLM as shown in Table 3, has high content of amino acids notably glutamic acid (10.94+0.1 g/100 g) which is similar to the findings of Fasuyi and Ibitayo (2011). The order of occurrence of amino acid in TDLM has been noted under the aforementioned result. The limiting amino acid is methionine for both cuttings as indicated by the chemical score of 30. This shows that TDLM must be supplemented with good quality animal protein and/or vegetable sources if it must be used as vegetable protein source farm animal feeds. TDLM is analyzed to contain reasonable content of lysine which has been reportedly required in breast muscle development for a good carcass cut and general body growth in broiler chicken (Tang et al., 2007). The low values of some of the essential amino acids; however, could be improved upon through processing to make TDLM a veritable vegetable protein source in farm animal feeding.

Conclusion

T. diversifolia leaf meal has been reportedly investigated as component of animal feeds. The nutrient compositions at the start of foliation at about May were lower than for the month of September even though the crude fibre was higher. *T. diversifolia* cut in September could be a good vegetable protein source and if adequately supplemented with methionine, it could be effectively used in animal feeding.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Residues from urban vegetable pruning in the production of the medicinal mushroom *Ganoderma lucidum*

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Every month, about 40 tons of waste prunings of trees and grasses are wasted in Bauru, São Paulo, Brazil, occupying unimaginable volumes in the municipal landfill. This rich material has been tested for cultivation of medicinal mushroom *Ganoderma lucidum*, rather unknown in Brazil, compared to commonly use eucalyptus sawdust. Thus, the objective of this study was to evaluate the use of wasted urban vegetable pruning for the production of the *G. lucidum* mushroom. For so, these wastes were collected and tested with five different substrates with mixtures of tree pruning and pruning of grasses, changing the percentage content, plus a control of eucalyptus sawdust base (T) in two strains of *G. lucidum*, a total of 12 experimental treatments (substrates × strains) with 7 replicates each totaling 84 packages with 600 g the unit. The experimental cultivation was from August to December with 21 days of incubation over 67 days of production. Through the statistics of the Carbon/Nitrogen relation, biological efficiency, loss of organic matter and mass of fresh and dried mushroom, only the control treatment maintained a good performance. Pruning substrates trees and grasses have varied low to medium fungal biomass conversion potential.

Key words: Fungi, sustainable development, solid waste.

INTRODUCTION

A great amount of organic residues is monthly generated in the city of Bauru, São Paulo, Brazil, which compromises the capacity and the life span of the city sanitary landfill. Most of those residues does not have an appropriate reuse, generating a significant amount in the environment.

Due to such environmental situation and the careless behavior of the human beings by discarding residues in an irrational way, the present work is based on one of the topics registered in the Agenda 21 document signed by

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world leaders during the ECO-92 conference in Rio de Janeiro.

Once the way these residues are discarded in the environment is harmful for the sustainable development, the efficient use of the resources rejected and the proposal of new practices are urgent. Science must strengthen the correct use of those residues, in answer to the emerging necessities (Furlan de Jesus et al., 2015; Carvalho et al., 2015). Besides the environmental aspect, the use of residues for the formulation of substrates aims to minimize the production costs of the mushrooms.

Among the mushrooms with a pharmacological value, the *Ganoderma lucidum,* (known as *orelha-de-pau*; *Reishi* by the Japanese and *Ling Zhi* by the Chinese) arouses much interest on such potential, as they are reported mainly by their medicinal power among their numerous properties, and they can also be used for preventing and treating various diseases (Russell and Paterson, 2006).

The substrates used in the mushrooms cultivation are usually formulated with straw and sawdust, which allows the use of many agricultural and agroindustrial raw materials and are considered of low or none aggregate value. This practice is also an income option for growers who generate a great amount of waste, as it represents an efficient alternative to enable the use of organic material for bioconversion into products with a high added value: Mushrooms (Carvalho et al., 2015).

One of the main factors of mushrooms cultivation is the selection of substrates for the production. Biologically and economically appropriate materials are essential for a successful cultivation (Roy et al., 2015, Thakur and Sharma, 2015).

Due to its nutritional and medicinal benefits, the production of mushrooms in Brazil is growing, but its conditions lack appropriate cultivation technologies, once the technology used for years was adapted from the ones practiced in developed countries, with different weather conditions and raw material (Dias, 2010, Sales-Campos et al., 2012). Therefore, the objective of this study was to study the reuse of pruning residues in urban areas for the cultivation of *G. lucidum* as a viable option for the agricultural producer.

MATERIALS AND METHODS

The "Jun-cao" cultivation technology was used for the production of *G. lucidum*. "Jun" means "mushroom" and "Cao" means "grass". So, this process is characterized by using grass and other agricultural residues as substrates for the cultivation of mushrooms, which are packed in appropriate and sterilized bags inoculated with the mycelium of the edible or medicinal fungus. Afterwards, the fruiting bodies are produced (Rolin et al., 2014; Carvalho et al., 2015).

The preparation of the substrate and the experiment was initially carried out at the Mushrooms Module of the School of Agronomic Sciences of the São Paulo State University – UNESP, Botucatu, São Paulo, Brazil. Next, the experiment was transported to the Universidade do Sagrado Coração (USC), Bauru, São Paulo, Brazil, for the cultivation stage.

Greenhouse mounting

A greenhouse was built at the experimental bed of the Agronomic Engineering course of the Universidade do Sagrado Coração, in order to propitiate a system similar to the natural one.

The place suffers many squalls of wind that increased the difficulties and is open to sky, that is, without natural or artificial ceiling during the hours of the day when sun rays strike directly, with full exposition to the sun.

A 30 cm deep, 4 m long and 1 m wide trench was made. The internal structure was made with bamboo arches, 150 µ wide transparent plastic cover and two layers of 70% shade cloth were placed 40 cm away from each other in order to decrease temperature.

Preparation of the substrates

After collection, the pruning residues were taken to the USC and dried in a sheltered environment, with natural ventilation, and afterwards sent to the Mushrooms Module of the São Paulo State University (UNESP), Botucatu, São Paulo, where they were submitted to a conventional forage grinder.

The experimental design was totally randomized, in 6×2 factorial scheme, corresponding to six types of cultivation substrates based on organic residues and two *G. lucidum* strains (Table 1), with 7 repetitions each (block with 600 g of substrate), adding up 84 experimental units.

G. lucidum strains used were GLM-09/01 and GLM-10/02, which are kept at the Mushrooms Module, School of Agronomic Sciences, UNESP, Botucatu, São Paulo. The preparation of the inoculum followed the methodology proposed by Minhoni et al. (2005).

After being homogenized and moistened, the substrates were placed in special HDPE (high density polyethylene) packs, capable to resist the sterilization process.

Each pack was compressed and a PVC segment and a piece of cotton cloth were added to the top of the pack, after involving their openings with aluminum foil. Next, the packs were submitted to the sterilization process at 121°C for 4 h.

Then, samples of the cultivation substrates were obtained soon after sterilization. Three samples of the different kinds of substrates were collected and sent to chemical characterization analysis (N, organic matter, C, C/N, humidity and pH), according to the Lanarv (1988) methodology.

The inoculation of packs with strains GLM-09/01 and GLM-10/02 of *G. lucidum* was performed after they were cooled until the environment temperature in the lab by using a laminar flow chamber in appropriate aseptic conditions, in order to avoid the contamination by other microorganisms.

Next, the packs were taken to incubation in an acclimatized room and kept at 25°C for three weeks, corresponding to the colonization period of the substrate by *G. lucidum* strains.

After the colonization period, the packs were transferred to the rough greenhouse built in the experimental area of mushrooms cultivation of the Sagrado Coração University, Bauru, São Paulo. The average temperature was kept at 25 ± 5°C and relative humidity of 60 to 85%. The packs were arranged totally at random.

Harvesting and data collection

Finally, mushrooms were harvested after their development, weighed, dehydrated and weighed again for obtaining the dry weight. The cultivation substrates were also weighed and samples were removed for chemical characterization of the substrates, loss of organic matter and biological efficiency.

A total of four harvestings were performed, beginning in October and ending in December, 2014. Data were registered in Microsoft

Table 1. Experimental treatments used in this research.

Treatments	*G. lucidum* strains	Formulation of the substrates[1]
1	GLM 09/01	100% tree pruning (TP)
2		100% grass pruning (GP)
3		75% TP + 25% GP
4		50% TP + 50% GP
5		25% TP + 75% GP
6		Witness (100% eucalyptus sawdust)
7	GLM 10/02	100% tree pruning (TP)
8		100% grass pruning (GP)
9		75% TP + 25% GP
10		50% TP + 50% GP
11		25% TP + 75% GP
12		Witness (100% eucalyptus sawdust)

[1]All substrates were added with 18% of wheat bran and 2% of limestone (dry weight). Humidity was adjusted to 65%.

Excel® spreadsheet to provide the statistical calculations.

Loss of organic matter

This evaluation was performed according to Rajarathman and Bano (1989). The loss of organic matter (LOM) is the index that evaluates the decomposition of the substrate by the fungus during cultivation. It is determined by the difference between the dry mass of the initial substrate and the dry mass of the residual substrate (post-harvest):

$$LOM\ (\%) = \frac{Dry\ mass\ of\ the\ initial\ substrate\ (g) - Dry\ mass\ of\ the\ residual\ substrate\ (g)}{Dry\ mass\ of\ the\ initial\ substrate\ (g)}\ X\ 100$$

Biological efficiency

Yield was expressed by means of the biological efficiency (BE), which represents the conversion percentage of the substrate into fungic biomass (mushrooms).

$$BE\ (\%) = \frac{Total\ fresh\ mass\ of\ mushrooms\ (g)}{Dry\ mass\ of\ the\ initial\ substrate\ (g)}\ X\ 100$$

Statistical analysis

The data analyses were submitted to variance analysis. Averages were compared by Tukey's test (5%) by using the SISVAR 4.2 software developed by the Departmento of Exact Sciences of the Federal University of Lavras, Minas Gerais, Brazil (UFLA).

RESULTS AND DISCUSSION

According to the variation factors obtained in the Tukey's test observed in Table 2, we noticed the outstanding relations that influenced statistically the results of this research.

Contaminations with other microorganisms occurred in all the substrates, especially in the ones almost without production, such as 100% grass pruning and 25% tree pruning + 75% grass pruning, due to the low colonization of the substrate after the transference to the rough greenhouse, when the cotton plugs were removed from all the treatments.

C/N ratio

Regarding C/N ratio, Philippoussis et al. (2007) reported that carbon and nitrogen contents of the substrate influence on fruiting precociousness and yield. Boyle (1998) reports that lignin degradation is also important for growth, as long as fungi may have access to the nitrogen contained in the wood components.

According to Hsieh and Yang (2004), the *G. lucidum* species requires an optimal C/N ratio of 70/1 and 80/1 for efficient growth and low production cost. For Gurung et al. (2012), most of medicinal fungi require optimal pH of 5.5 to 6.5.

The average contents obtained by analyses in initial and final C/N ratio are described in Table 3.

We observed that all the initial substrates obtained optimal pH (between 5.7 and 6.3) for the production and development of *G. lucidum* (Table 3).

The substrates had average C/N ratio varying from 35/1 (100% tree pruning) to 70/1 (Control - eucalyptus sawdust) in the initial substrate (Table 3). All pruning results obtained unfavorable C/N ratio compared to the ones obtained by Hsieh and Yang (2004) because all the substrates were formulated considering the proportions of the materials used, that is, 80% of prunings (residue), 18% of bran and 2% of limestone for all the substrates, different from other works in literature.

Many mushroom producers in Brazil use the proportion-based formulation. If they chose the

Table 2. *ƒ* values expressed in percentages obtained in the variance analysis of LOM, FMB and BE provided by different substrates during the cultivation of strains of GLM 10/01 and GLM 09/01 of *G. lucidum.*

Variation factors	LOM	FMB	BE
Substrate (S)	5.8**	69.0**	77.7**
Strain (L)	10.3**	0.2^{ns}	0.7^{ns}
S × L	9.4**	1.5^{ns}	1.2^{ns}

LOM = Loss of organic matter; FMB = Fresh mass of Basidioma; BE = Biological Efficiency; ** significant at 1% level; ns = not significant.

Table 3. C/N ratio and pH of the initial and final substrates, according to strains, used during the cultivation cycle of *G. lucidum.*

Treatments	Initial substrate			
	N%	C%	C/N	pH
T - ES				
1 - 100% TP	0.5	19	35/1	6.3
2 - 100% GP	0.5	20	36/1	5.8
3 - 75% TP 25% GP	0.6	22	37/1	6.1
4 - 50% TP 50% GP	0.6	22	37/1	6
5 - 25% TP 75% GP	0.5	20	41/1	5.9
	Final substrate			
Strain GLM-10/02				
T - ES	0.4	15.5	35/1	4.2
1 - 100% TP	0.8	20	23/1	5.5
2 - 100% GP	0.5	12.5	25/1	8.7
3 - 75% TP 25% GP	0.7	18.5	25/1	4.3
4 - 50% TP 50% GP	0.8	18.5	23/1	4.3
5 - 25% TP 75% GP	0.9	18.5	19/1	4.3
Strain GLM- 09/01				
T - ES	0.5	16	29/1	4.2
1 - 100% TP	0.8	14.5	18/1	4.3
2 - 100% GP	0.4	10	22/1	8.3
3 - 75% TP 25% GP	0.8	19	24/1	4.5
4 - 50% TP 50% GP	0.6	11.5	19/1	4.7
5 - 25% TP 75% GP	0.6	12.5	19/1	4.5

ES = Eucalyptus sawdust; TP = tree pruning; GP = grass pruning. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

recommended C/N-based formulation, a previous analysis of the formulation would be necessary, followed by C/N calculation.

Once these analyses represent a cost, many of the producers use proportions-based formulations. Thus, the idea of this research was to simulate what most of producers do.

Biological efficiency

The biological efficiency of each treatment corresponds to the transformation of the substrate matter into mushroom biomass. This index is the most used by the researchers, what makes the comparison of the results with literature easier (Tisdale et al., 2006). Figure 1 characterizes the percentage of biological efficiency reached for each treatment. For the tested residues mixed, the best results for biological efficiency were for substrates 100% tree pruning, 75% tree pruning + 25% grass pruning and 50% tree pruning + 50% grass pruning.

The average of the biological efficiency varied from 0% for the substrate containing 100% of grass pruning (lowest) to 36% for the control substrate containing eucalyptus sawdust (Figure 1). Similar results to the highest values obtained in this experiment were found by Erkel (2009) when he used poplar sawdust (a European

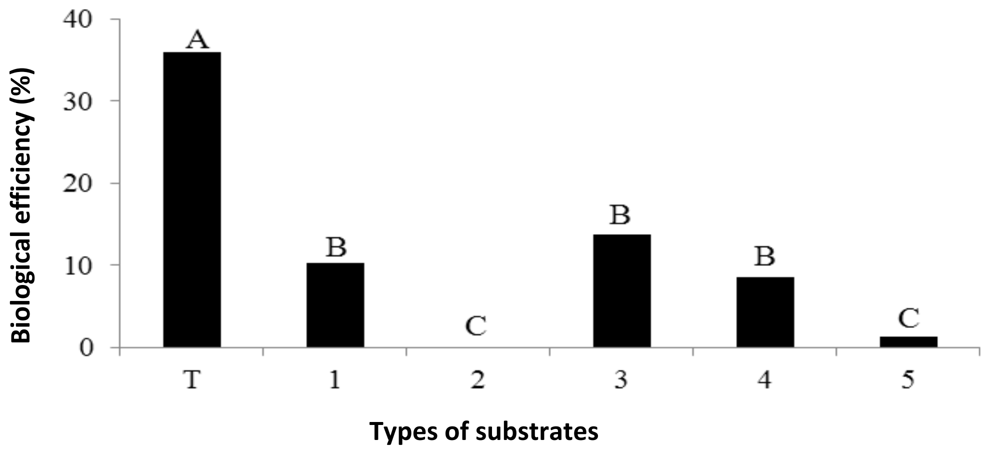

Figure 1. Biological efficiency (%) in the treatments of *G. lucidum* cultivated in substrates based in organic residues of prunings in urban areas. Averages followed by the same letters are not statistically different among each other, according to the Tukey's test (5%). Coefficient of variation: 77.7%. Values are average of 7 repetitions. T = Witness - eucalyptus sawdust; 1 = 100% tree pruning (TP); 2 = 100% grass pruning (GP); 3 = 75% TP + 25% GP; 4 = 50% TP + 50% GP; 5 = 25% TP + 75% GP. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

tree used in the paper industry) supplemented with gluten and sugar cane molasses in the proportions of 1, 2 and 3% as substrate in the cultivation of *G. lucidum*. Higher results were obtained and the most satisfactory were verified in the treatment, sawdust + sugar cane molasses 1% (BE 20.3%), followed by the treatment, sawdust + gluten 1% (BE 19%). The treatments with the highest supplementation levels (2 and 3%) obtained lower values of BE (%), showing that the ideal supplementation was 1% for both supplements. As well as Aysun and Gokcen (2009), who used substrates based on sawdust supplemented with residues of green tea in the proportions of 75:25, 80:20, 85:15 and 90:10, the highest results were obtained in the proportions 80:20 (BE 34.90%) and 75:25 (BE 31%).

Rolim et al. (2014) obtained higher results than the others (BE - 72%) by cultivating *G. lucidum* in substrate based on elephant grass + mango tree sawdust, supplemented with 10% of wheat bran and 10% of crushed sugar cane. Percentages close to the lowest result obtained in the experiment were reached by Gurung et al. (2012), who cultivated this experiment fungus in substrate based on Sal sawdust (*Shorea robusta*) supplemented with 10% of wheat bran and Sal sawdust + 10 % of rice bran, and obtained BE of 0.0 and 0.81%, respectively.

The authors also obtained 0% of BE when they used mango tree sawdust supplemented with 20% of wheat bran as substrate for the cultivation.

The biological efficiency obtained in the experiment was not satisfactory regarding most data observed in literature. Such fact might probably be associated with the nitrogen sources present in the initial substrate (Table 3). According to Hsieh and Yang (2004), the species *G. lucidum* requires a 70:1 to 80:1 C/N ratio for a satisfactory growth and the average C/N ratio in this experiment was of 35:1 to 41:1 to the treatments utilizing tree pruning and/ or grass pruning.

Therefore, his substrates containing grass pruning mostly were not biologically efficient as an alternative for the cultivation of *G. lucidum* in Bauru, São Paulo, resulting in higher preference for tree pruning.

Fresh mass of basidiomata

The results of total fresh mass of the basidiomata (Figure 2) were obtained by adding up the production of each treatment in the four harvestings carried out during the experiment, in a total of 67 days of production in greenhouse, preceded by 21 days of incubation, totalizing 88 days of experiment.

It was observed that the Control treatment obtained mushrooms with the highest mass and the best combination tested was the substrate with 50% of tree pruning + 50% of grass pruning, with some statistical differences from the others.

During the cultivation cycle, the sum of the basidiomata fresh mass obtained the lowest average of 0.0 g for 100% grass pruning and the highest one of 88.5 g for the Control treatment containing eucalyptus sawdust.

With an yield of 51.6 g, the substrate made up with 50% of tree pruning + 50% of grass pruning was statistically the best residue mix tested. This result shows that this substrate produced a little more than half of the mass yield of the Control substrate.

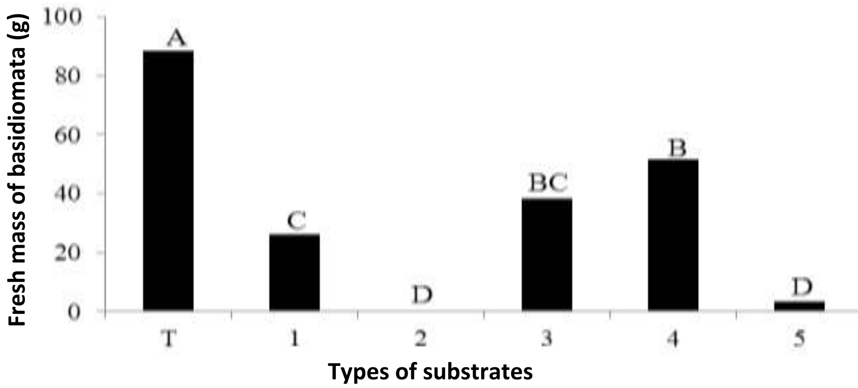

Figure 2. Total fresh mass of basidiomata of the substrates used in the cultivation of *G. lucidum*, based on organic residues of prunings in urban areas. Averages followed by the same letters are not statistically different among each other, according to the Tukey's test (5%). Coefficient of variation: 69.0%. Values are average of 7 repetitions. T = Witness - eucalyptus sawdust; 1 = 100% tree pruning (TP); 2 = 100% grass pruning (GP); 3 = 75% TP + 25% GP; 4 = 50% TP + 50% GP; 5 = 25% TP + 75% GP. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

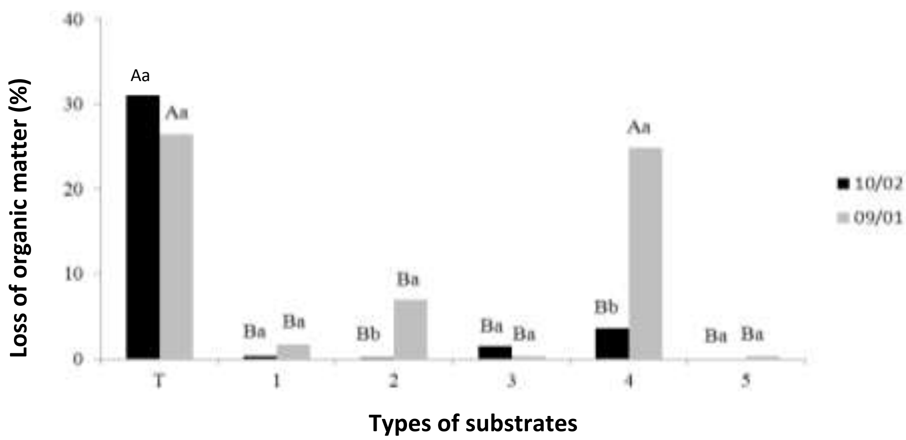

Figure 3. Loss of organic matter of strains GLM-10/02 and GLM-09/01 of *G. lucidum*, cultivated in substrates based on organic residues of pruning in urban areas. Averages followed by equal letters are not statistically different among each other, according to the 5% Tukey's test. Uppercase letters compare the substrates among each other and lowercase letters compare the strains inside the same substrate. Coefficient of variation is 9.4%. Values are average of 7 repetitions.T = Witness – eucalyptus sawdust; 1 = 100% tree pruning (TP); 2 = 100% grass pruning (GP); 3 = 75% TP + 25% GP; 4 = 50% TP + 50% GP; 5 = 25% TP + 75% GP. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

Loss of organic matter

The loss of organic matter (LOM) evaluates the reduction of the decomposed matter by the action of the fungus, as a consequence of the production process.

The best result was obtained by the Control treatment with eucalyptus sawdust, with 31.1% (strain GLM-10/02) and 26.4% (strain GLM-09/01). The substrate with 50% of tree pruning + 50% of grass pruning distinguished itself with strain GLM-09/01, reaching 24.6% (Figure 3).

By observing the data of the fresh mass of basidiomata produced (Figure 2) and the loss of organic matter (Figure 3), we notice that the Control treatment, eucalyptus sawdust, obtained the best yields. Moreover, the Control treatment obtained the best performance: 31.3% (strain GLM-10/02) and 26.4% (strain GLM-09/01) in relation to the loss of organic matter.

The substrates and the strains of the treatments 4 (50% tree pruning + 50% grass pruning) and 2 (100% grass pruning) distinguished themselves in loss of organic matter, with 24.9 and 7.0%, respectively, especially for strain GLM-09/01, expressed in the statistic comparison (lowercase letters in Figure 3).

The curious fact in the analysis of the fresh mass of basidiomata (Figure 2) and the loss of organic matter (Figure 3) of Treatment 2 is the absence of basidiomata production (0 g). However, there was 7% of loss of organic matter. According to Zadrazil (1978), it is justified by the loss of CO_2 and H_2O during the metabolism of the microorganisms and not only by removing materials for the formation of the basidiomata.

Conclusions

1. The alternative use of the urban organic residues tree and grass pruning is little viable for the cultivation of *G. lucidum*.
2. The strains GLM-09/01 and GLM-10/02 of *G. lucidum* have good results with eucalyptus sawdust. Only strain GLM-09/01 stands out with the residues substrate, in the ratio of 50% tree pruning + 50% grass pruning.
3. This research shows another possibility to produce mushrooms and improves the studies in the area of medicinal mushrooms production, with hypothesis for new tests for edible mushrooms also.
4. New proposals of solutions appear to reduce organic residues discharge by using them to produce food, generate income and improve their use instead of sending them to a sanitary landfill.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Increased phenylpropanoid accumulation in essential oils of *Petroselinum crispum* at different *Sulphur* dilutions

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This study aimed to evaluate the influence of different doses of *Sulphur* on yield and chemical composition of parsley essential oil. *Sulphur* was applied at different dilutions in the centesimal scale ranging from 0c (control), 6, 12, 18, 24 to 30c. The experiment was carried out in the field, and for each treatment there were five replications, that is, five vases with one plant each. The evaluated parameters were: plant height (cm), fresh biomass of the aerial parts and root (g), yield and chemical composition of the essential oil. The oil was obtained by hydrodistillation and analyzed by gas chromatography and mass spectrometry (GC/MS). The results indicated that 12c dilution caused aerial part inhibition; there was an inhibitory effect on roots at 12 and 30c dilutions, and 6, 12 and 30 c dilutions inhibited fresh biomass when compared to the control (0c) and the other dilutions. Regarding EO yield, an increase in yield (%) at 18c (0.150 ± 0.01) and 30c (0.180 ± 0.01) dilutions occurred when compared to control (0c) (0.017 ± 0.01). The essential oil presented phenylpropanoids as the main class in its composition and apiole and myristicin as major compounds in all evaluated treatments. The 12c dilution allowed an increase in apiole (96.24%) and decrease in myristicin (3.76%). However, myristicin has an increase in treatments 6c (14.65%), 18c (10.46%), 24c (13.66%) and 30c (14.80 when compared to the control 0c (5.99%). In conclusion, a stimulating or inhibitory response occurred in the evaluated parameters depending on the utilized dilutions. The increase in apiole and myristicin is considered an economically important factor because they are substances utilized by the pharmaceutical, food and agricultural industries.

Key words: Parsley, apiole, myristicin, phenylpropanoids, yield, dilutions.

INTRODUCTION

Petroselinum crispum (Mill.), (Apiaceae) popularly known as parsley, garden parsley, chopped greens or rock parsley, stands out as one of the most consumed herbs worldwide. In Brazil, parsley is cultivated mainly by small rural producers to be sold as herbs, it can be used fresh or dehydrated, and its most consumed parts are leaves, petioles and seeds (Petropoulos et al., 2009). The plant reproduces better in sandy-clayey soil with high content of organic matter, good fertility and pH between 5.8 and 6.8 (Heredia et al., 2003). Some cultures are highly perishable, have short cycle (three months), and are susceptible to several pathogens (Rodrigues et al., 2010). Besides, its *in natura* utilization, it is used to obtain essential oil (EO) which has two main phenylpropanoids: apiole and myristicin in its composition. Almost 90% of the EO may consist of apiole; however, EO´s chemical composition can be altered due to several factors like plant genotype, location of plant cultivation, type of utilized soil, harvesting time, luminosity, altitude, temperature and water management (Kurowska and Galaska, 2004; Morais, 2009; Borges et al., 2016**)**. Parsley EO is highly valued in the international market and broadly used in the food industry to aromatize meats, canned foods and processed vegetables. In agriculture, studies have shown its potential to control bovine tick (Camilotti et al., 2015).

It is estimated that 3% of the world production of essential oil is utilized by the pharmaceutical industry, 34% by the beverage industry and 63% by food and cosmetic industries. Brazil is one of the four greatest world producers of EO alongside India, China and Indonesia, but it suffers from chronic problems such as the absence of maintenance of oil quality standard (Bizzo et al., 2009). In the production of EO, cultivation type is one of the factors that directly interfere in the quality since the industries in the market of aromatic and culinary plants have greater interest in acquiring a product that has a standard in its chemical composition, which is the case of the cosmetic industry where the alterations in the oil may interfere in the perfume aroma (Craveiro and Queiroz, 1993). According to Bastos (2007), the utilization of agrochemicals (chemical fertilizers and agrotoxic products) directly affects the chemical composition of essential oils, and then alters their quality, making their utilization unviable. Therefore, organic fertilization has been recommended for medicinal, aromatic and culinary plants. According to Corrêa Junior and Scheffer (2009), medicinal plants from organic cultivation are more resistant to pests and diseases, reducing the need of phytosanitary control.

In agriculture, the indiscriminate utilization of chemical products has increased the resistance of insects, pests, phytopathogenic fungi, weeds and environmental contamination. Natural products (EO, plant vegetal extracts and substances obtained from high dilutions) have been used as an alternate method within organic agriculture, and can be utilized as bioherbicides (Isman, 2006; Mapeli et al., 2010; Ootoni et al., 2013). Thus, the Brazilian Ministry of Agriculture and Supply recommends the utilization of high dilutions in the production of organic foods (Brazil, 1999). These substances have been utilized by several segments in agriculture, including germination (Hamman et al., 2003), seedling production (Bonfim et al., 2008), pest control (Almeida et al., 2003), and has been promoting general improvement of the plant, production of more vigorous seeds, production variation, yield of active principles (phytochemicals), adaptation to adverse conditions and productivity, reducing the use of fertilizers and chemical pesticides, and resulting in greater safety to the product and the producer (Andrade et al., 2001; Shah-Rossi et al., 2009). Among these substances, *Sulphur* stands out because it presents a broad action spectrum to improve the general aspect of a plant in order to strengthen natural defenses, increasing resistance under nutritional, unfavorable climatic conditions to its development; it also shows development of essential oil and aerial parts (Bonato and Silva, 2003; Oliveira et al., 2014).

Sulphur is an essential element for plant development and is found in the macronutrient group like nitrogen, potassium, calcium and magnesium (Bonato and Silva, 2003). This element is a key nutrient for plant development because it participates in the synthesis of amino acids such as cysteine, cysteine and methionine which are needed for protein formation and are also fundamental in the development of certain vitamins, glutathione and co-enzyme (Coleman, 1966). In soils, 90% of sulfur is found as organic form but most of the cultivable soils present deficiency of this element, main the soils with little organic matter and those submitted to burning, which causes the loss of this element by volatilization. Throughout time, this deficiency also occurred due to the substitution of sulfur as fungicide and insecticide (Coleman, 1966; Oliveira et al., 2014). The objective of several producers and the aim of researchers (Toledo et al., 2015) has been to search for alternatives in the production of healthy foods without agrochemical residues and smallest possible impact on the environment, produced in an economically and socially sustainable manner. Thus, the goal of this study was to evaluate the utilization of different *Sulphur* dilutions in the development of parsley and its EO yield and chemical

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Table 1. Analysis of pH, macro and micronutrients of soil utilized in parsley cultivation.

pH and Macronutrients								
pH	**$cmoldm^{-3}$**						**$mg\ dm^{-3}$**	
($CaCl_2$)	Al^{3+}	$H^+ + Al^{3+}$	Ca^{2+} Mg^{2+}	K^+	SB	CTC	P	C
5.57	0	2.63	5.00	0.32	13.46	15.35	199.70	13.25
Micronutrients								
		(%)		**$mg\ /dm^3$**				
V	Ca	Mg	K	Ca/Mg	Ca/K	Mg/K		
87.68	57.02	21.94	1.34	1.94	42.66	21.94		

Ca, Mg, Al: extracted with KCl 1 mol L -1; **P, K:** extracted with Mehlic 1; **H + Al:** SMP method; **C:** Walkley and balcknethod; **SB**: Sum of Bases. **Source**: Laboratório Solo Fértil (Umuarama-PR).

composition.

MATERIALS AND METHODS

Botanical Identification

The experiment was carried out in the Medicinal Garden of Paranaense University UNIPAR Campus II, in the city of Umuarama, northwestern region of Paraná State, Brazil S23° 46,225’ and WO 53° 16,730’, 391 m of altitude, from October 2012 to February 2013. The plant was identified by Professor Ezilda Jacomasi of the Department of Pharmacy of Paranaense Univeresity (UNIPAR), Paraná State, Brazil. A voucher specimen is deposited at the UNIPAR Herbarium (code number 192).

Culture implementation

The soil utilized in the experiment was collected at 20 cm of depth from experimental beds and homogenized; its chemical and physical properties were determined (Table 1). The soil was poured into 30 10 L polypropylene vases (27 cm of height and 25.5 cm of diameter), without any nutritional correction or addition of fertilizers or agricultural pesticides. A 180 cell seed tray (3.5 cm²) was used with four seeds per cell. After 30 days, three cells with four seedlings each were transplanted equidistantly to each vase. After 40 days, trimming was done and one plant per vase remained.

Preparation of treatments

Each treatment consisted of a *Sulphur* dilution in the centesimal scale (c) (10^{-2} g/mL). Five dilutions were tested: 0c (control), 6c (10^{-12}), 12c (10^{-24}), 24c (10^{-48}) and 30c (10^{-60}) (Bonato et al., 2009). For the control, distilled water was used. The dilutions were obtained according to the phamacotechnique for insoluble drugs in the centesimal scale whose technique is described in the Brazilian Homeopathic Pharmacopoeia (Brasil, 2011). After dilution preparation (6, 12, 18, 24 and 30c), they were diluted in distilled water at the proportion of 0.1/100mL. The diluted treatments were applied to the seedlings in the seed tray and to the plants in the vases. 2 mL of dilutions were weekly applied to each cell of the seed tray for four weeks until transplantation to vases. 250 mL of dilutions were applied weekly to the vases until the end of the plant cultivation (Bonato and Silva, 2003). The experiment was carried out in the field, and for each treatment there were five replications, that is, five vases with one plant each. The evaluated parameters were: plant height (cm), fresh biomass of the aerial part and root (g), yield and chemical composition of the essential oil. The plant height was calculated by measuring the distance between the basis and the stem apex using a measuring tape.

Obtention and yield of parsley essential oil

At the end of the vegetative cycle, during flowering, which was characterized by the emergence of panicles (Lorenzi and Matos, 2008), the aerial parts at 2 to 3 cm above the soil were removed, fresh mass (g) was measured and submitted to the extraction of essential oil by hydrodistillation for 2 h (Petropoulus et al., 2008; Petropoulus et al., 2010). The oil was removed from the equipment using *n*-hexane, filtered in anhydrous sodium sulfate and stored under refrigeration (3°C). The essential oil was measured in (g/g) of fresh weight.

Chemical identification of parsley essential oil by GC/MS

The essential oil were analysis were carried out in a gas chromatograph (Agilent 7890 B) coupled to mass spectrometer (Agilent 5977 A), equipped with a DB-5 capillary column (30 m x 0.25 mm x 0.25 µm, Agilent, PA, USA) using the following conditions: injector temperature of 250°C, injection volume 1 µL at injector (split 2:1; 2.1 mL min^{-1}), initial column temperature of 40°C, and gradually heated to 300°C at 6°C min^{-1} rate. The carrier gas (helium) flow was set at 4.8 mL $minute^{-1}$. The temperature transfer line was held at 250 and 320°C, respectively. The mass spectra were obtained in the range of 40 to 500 (*m/z*) provided through scan mode with solvent delay time of 3 min, and the compounds were identified based on comparison of their retention indices (RI) obtained using various n-alkanes (C7-C26). Also, their EI-mass spectra were compared with the Wiley library spectra and the literature (Adams, 2007).

Chemicals and reagents

All used solvents were of analytical grade. Homologous series of C7 to C25 n-alkane and *n*-nonadecane reference chemicals used for identification were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals, all of analytical grade, that is, anhydrous sodium sulfate, *n*-hexane, used in this study were purchased from Merck (Darmstadt, Germany), unless stated

Table 2. Influence of *Sulphur* at 6c, 12c, 18c, 24c, 30c and 0c dilutions on *P.crispum* considering the development of aerial parts (cm), root development (cm), fresh mass (g) and the yield of essential oil (%) as parameters.

Treatments	Development of aerial parts(cm)	Root Development (cm)	Aerial part (fresh biomass) (g)	Yield of essential oil (%)
0c	25.58 ± 0.65[a]	44.00 ± 4.50[ab]	36.73 ± 4.04[a]	0.017 ± 0. 01[a]
6c	22.42 ± 0.65[bd]	46.00 ± 1.73[a]	21.73 ± 2.10[b]	0.035 ± 0. 01[b]
12c	18.25 ± 1.04[c]	34.16 ± 0.60[c]	24.20 ± 1.13[bc]	0.024 ± 0. 01[c]
18c	24.29 ± 0.44[ad]	38.83 ± 2.16[ac]	24.36 ± 2.33[bd]	0.150 ± 0.01[d]
24c	22.29 ± 1.52[d]	38.33 ± 2.02[bc]	30.07 ± 1.42[acd]	0.050 ± 0. 01[e]
30c	25.38 ± 0.85[a]	34.00 ± 1.52[c]	12.87 ± 0.55[b]	0.180 ± 0. 01[f]

*The values for average and standard deviation were obtained from sextuplicate. Averages followed by the same letters in the column do not differ among themselves by TLSD test , p ≤ 0.05.

otherwise.

Statistical analysis

The experimental was completely randomized. Prior analysis of variance (ANOVA) excluded outliers on plant fresh mass using the box plot method. Data were subjected to one-way ANOVA using general linear model with mixed-effects and balanced design, considering each *Sulphur* dilution as one treatment, and compared to Duncan's test ($P \leq 0.05$) using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). To comply with ANOVA assumptions, data were previously checked by Levene´s test.

RESULTS AND DISCUSSION

The analysis of the soil utilized for the parsley cultivation is found in (Table 1**)**. The soil granulometric analysis indicated that it contains a mixture of coarse sand (28.60%), fine sand (50.60%), silt (1.40%) and clay (19.40%), and it was classified as sandy soil according to the Normative Ruling 2, from October 9, 2008 of the Brazilian Ministry of Agriculture, Livestock and Supply (Brasil, 2008). The soil also presented high fertility with base saturation (V=87.68%). Therefore, the results showed soil with pH within normality, between 5.57 and 6.0, and with appropriate contents of micro and macronutrients for the development of *P. crispum*, which according to Andrade et al. (2010), better develops in soils with pH (5.8 to 6.8), high content of organic matter and good fertility. The choices of 6, 12, 18, 24 and 30c *Sulphur* dilutions are based on the fact that the physiological responses do not depend only on the utilized substance, but on the utilized dilutions. The effect of *Sulphur* treatment with different dilutions on the development of aerial parts (cm), root development, fresh mass of aerial part (g), and EO yield (%) are described in (Table 2). The results indicated that there was significant inhibition at 12c dilution on the development of aerial parts when compared to the control (0c), 6, 24 and 30c dilutions. Regarding root development, dilutions of 12 and 30c presented inhibiting effect on root when compared to the control (0c); as for fresh biomass, it was observed that dilutions of 6, 12 and 30c inhibited biomass compared to control (0c) and dilution of 24c.The different *sulphur* dilutions significantly influenced essential oil yield (Table 2). The greatest yields were provided by 30c (0.180 ± 0.01) and 18c (0.150 ± 0.01) dilutions when compared to control (0c) (0.017 ± 0.01). The influence of *Sulphur* dilutions on the yield of essential oil was also observed by Bonato et al. (2009) who carried out an experiment with *Mentha arvensis* which resulted in increase of the yield at 6c dilution and a reduction at dilutions 12, 24 and 30c.

The influence of different *Sulphur* dilutions on the chemical composition of parsley essential oil is described in (Table 3), where 48 compounds were identified; the predominant class was phenylpropanoids: (0c) (66.60%), 6c (71.20%), 12c (100.00%), 18c (63.00%), 24c (76.41%) and 30c (75.77%). Apiole was the major component in all treatments: (0c) (60.27%), 6c (56.55%), 12c (96.24%), 18c (51.91%), 24c (62.75%) and 30c (60.97%). The second main compound in quantity in EO was myristicin presented (0c) (5.99%), 6c (14.65%), 12c (3.76%), 18c (10.46%), 24c (13.66%) and 30c (14.80%). These results are in accordance to Borges et al. (2016) who found apiole, followed by myristicin as the main compound of oil from parsley cultivated in the same region of this experiment when the plant was submitted to different water stress management levels. The treatment 12c presented a different behavior from the others, by increasing apiole (96.24%) and decreasing myristicin (3.76%), showing the other compounds just as traces within the chromatograms as it can be observed in (Figure 1). However, myristicin increased in treatments 6c (14.65%), 18c (10.46%), 24c (13.66%) and 30c (14.80%) when compared to the control 0c (5.99%). Similarly, treatments 18c and control (0c) presented, besides apiole and myristicin, *p*-cymene(4.63; 3.70%) and *p*-cimenene (4.78; 5.73%), respectively, as major compounds in their composition; they were also different from treatments 6, 24 and 30c which had myristicin (14.65, 13.66 and 14.80%) and apiole (56.55, 62.75 and

Table 3. Chemical composition of *Petroselinum crispum* essential oil under different *Sulphur* dilutions (0, 6, 12, 24 and 30c).

Peak	[A]Compounds	RI[a]	0c	6c	12c	18c	24c	30c	Methods of identification
1	Sabinene	1038	t	t	t	t	t	t	a,b
2	β-pinene	1039	t	t	t	t	t	t	a,b
3	ρ-cymene	1073	4.63	2.90	t	3.70	1.94	2.66	a,b
4	ρ-cymenene	1119	4.78	2.50	t	5.73	3.07	2.56	a,b
5	Citronellal	1120	1.79	1.69	t	0.39	1.13	2.97	a,b
6	ρ-methyl-acetophenone	1124	t	t	t	t	t	t	a,b
7	ρ-cymen-8-ol	1125	0.68	1.36	t	2.30	t	2.14	a,b
8	Myrtenal	1126	t	t	t	t	t	t	a,b
9	o-cumenol	1129	0.71	0.50	t	0.50	0.43	1.00	a,b
10	Phellandral	1130	t	t	t	0.64	t	t	a,b
11	*cis*-piperitol	1137	2.40	2.50	t	1.40	1.25	2.39	a,b
12	Cinnnamaldehyde	1201	t	t	t	t	t	t	a,b
13	Carveol	1209	1.44	1.30	t	t	1.64	t	a,b
14	Cuminal	1226	0.33	t	t	0.48	t	t	a,b
15	2-bornanone-oxime	1235	t	t	t	t	t	t	a,b
16	Carvenone	1244	0.73	0.76	t	0.76	0.55	t	a,b
17	*trans*-myrtanol	1258	t	t	t	t	t	t	a,b
18	Cuminol	1260	0.47	t	t	0.75	t	t	a,b
19	Thymol	1263	0.64	0.86	t	0.81	0.53	t	a,b
20	Carvacrol	1272	0.82	0.89	t	1.14	t	t	a,b
21	n.i.	1301	0.69	0.46	t	t	t	t	a,b
22	*cis*-Citral	1333	0.77	t	t	0.60	0.52	t	a,b
23	Limonene aldehyde	1344	0.70	0.77	t	0.85	0.70	t	a,b
24	α-copaene	1378	t	t	t	t	t	t	a,b
25	β-elemene	1395	1.21	1.76	t	1.54	1.81	2.11	a,b
26	α-guaiene	1447	0.74	t	t	1.10	0.55	t	a,b
27	*cis*-β-farnesene	1460	t	2.26	t	0.37	t	t	a,b
28	Germacrene D	1472	2.06	t	t	2.01	2.22	2.61	a,b
29	*trans*-β-ionone	1488	t	t	t	t	t	t	a,b
30	*trans*-α-farnesene	1501	t	t	t	0.38	t	t	a,b
31	β-bisabolene	1504	t	t	t	0.63	t	t	a,b
32	Germacrene A	1507	0.65	0.75	t	0.76	0.76	1.41	a,b
33	Myristicin	1515	5.99	14.65	3.76	10.46	13.66	14.80	a,b
34	δ-cadinene	1518	t	t	t	t	t	t	a,b
35	*trans*-γ-bisabolene	1527	0.89	1.01	t	0.96	1.00	1.73	a,b

Table 3. Contd.

36	Elimicin	1544	t	t	t	0.34	t	t	a,b
37	5β, 7βH, 10α – Eudesm-11-en-1α-ol	1562	t	t	t	0.47	t	t	a,b
38	Caryophylene oxide	1572	0.36	0.73	t	0.55	t	t	a,b
39	Carotol	1588	t	0.85	t	0.90	t	t	a,b
40	Apiole	1680	60.27	56.55	96.24	51.91	62.75	60.97	a,b
41	Caryophylene acetate	1681	t	t	t	0.40	t	t	a,b
42	Acethophenone (2,4,6-trimethyl-3-methyl)	1682	0.34	t	t	0.85	0.69	t	a,b
43	Benzyl benzoate	1757	0.52	0.85	t	0.73	0.72	t	a,b
44	β-costol	1761	0.67	1.12	t	0.92	0.85	t	a,b
45	β-bisabolenol	1776	0.84	1.09	t	1.40	0.75	t	a,b
46	n.i.	1792	0.42	t	t	0.47	t	t	a,b
47	n.i.	1817	0.74	0.80	t	0.85	0.59	t	a,b
48	Farnesyl acetate	1828	0.93	0.75	t	1.42	1.89	2.65	a,b
49	Phytol	1922	0.32	t	t	0.39	t	t	a,b
50	Hexadecanoic acid	1937	0.67	t	t	0.65	t	t	a,b
51	4-methoxy-stilbene	1988	0.34	t	t	0.29	t	t	a,b
	Compounds groups (%)								
	Monoterpene hydrocarbons	-	9.41	5.20	0.00	9.43	5.01	6.22	
	Oxygenated monoterpenes	-	11.46	12.09	0.00	8.00	6.32	7.50	-
	Sesquiterpene hydrocarbons	-	5.55	5.78	0.00	7.75	5.34	7.86	-
	Oxygenated sesquiterpenes	-	3.03	4.59	0.00	5.96	2.19	0.00	-
	Oxygenated diterpenes	-	0.32	0.00	0.00	0.39	0.00	0.00	-
	Phenylpropanoids	-	66.60	71.20	100.00	63.00	76.41	75.77	-
	Others	-	3.86	2.06	0.00	4.15	3.73	2.65	
	Total Identified (%)	-	98.38	99.66	100.00	98.68	98.41	100.00	

[a]Identification based on retention index; [b]identification based on comparison of mass spectra; n.i: unidentified compound; t: trace; [A]Compound listed in order of elution from a DB-5 column.

60.97%), respectively, as their major compounds as shown in Figure 1. This oscillation in the chemical composition of the essential oil in function of *sulphur* dilution was also observed by Oliveira et al. (2014) who utilized the same *Sulphur* dilutions and the same method utilized in this experiment in their studies.

The authors found alterations in the chemical composition of *Ocimum basilicum* L. EO. They verified that *Sulphur* substantially increased the concentration of the major compound, linalool: 12c (33.14%), 6c (30.92%), 30c (27.13%), 24c (23.86%) and 18c (19.68%) when compared to control (0c) (7.41%). However, the treatments caused decrease of α-bergamotene: 18c (7.47%), 24c, (6.68%), 6c (5.47%), 30c (5.22%) and 12c (5.06%), when compared to control (0c) (15.45%) According to Dayenas et al. (1988) and Bonato

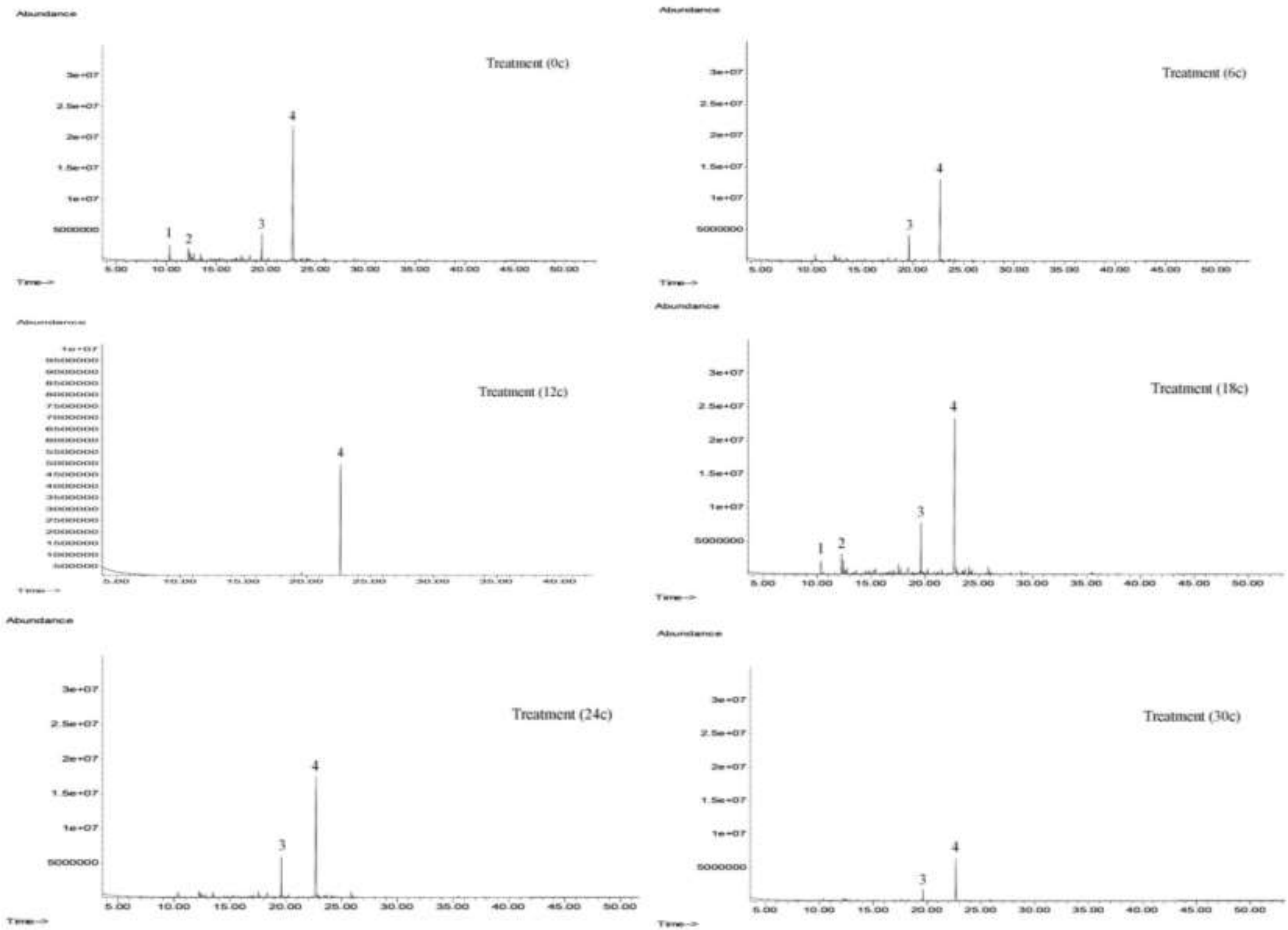

Figure 1. Chromatograms obtained by GS of major compounds of *Petroselinum crispum* essential oil extracted from leaves and submitted to treatments with *Sulphur* under dilutions (0, 6, 12, 18, 24 and 30c). 1 = ρ-cymene; 2 = ρ-cymenene; 3 = Myristicin; 4 = Apiole.

and Silva (2003), *Sulphur* frequently causes different effects, depending on the application. In certain cases, an increase may occur, whereas in others, inhibitions may be reported within a specific physiological variable. Such behavior is still not fully explained. One of the hypotheses, based on biodynamic agricultural data, is that such behavior may be related to the existing rhythmic movements in nature. Another hypothesis, based on experimental data, is that such behavior is due to similarity between the applied homeopathic drug and the organism (Bonato, 2007).

The high apiole concentration in 12c dilution and the increase in myristicin in dilutions 6, 18, 24 and 30c makes *Sulphur* utilization at high dilutions extremely important in vegetal nutrition when the objective is to isolate plant compounds. According to the studies done by Reyes-Munguía et al.(2012), apiole presented antioxidant, chemo preventive and antifungal potential. Studies done by Camilotti et al. (2015) on parsley EO and it major compound, apiole, found high activity against bovine tick and *Aedes aegypti* larvae. Regarding myristicin, studies proved its action as anti-depressing, anti-inflammatory and antioxidant (Tisserand and Balacs, 1994); chemo preventive agent (Zheng et al., 1992), larvicide against *A. aegypti* (Marston et al., 1995), insecticide against *Spilarctia obliqua* (Srivastava et al., 2001). The results found in this study demonstrated that Sulphur and ultra-dilutions represent an important tool within organic agricultural practices in order to stimulate plant to increase the concent of active principles that are interesting for several agroindustrial segments.

Conclusion

The application of different *Sulphur* dilutions to parsley provided alterations in the development of aerial parts, roots, fresh mass, yield and chemical composition of parsley essential oil. Pheynilpropanoids are a predominant class in parsley essential oil; apiole and myristicin were the major compounds in all evaluated treatments. The 12c dilution allowed an increase in apiole (96.24%) and decrease in myristicyn which increased in treatments 6, 18, 24 and 30 c. The increase in apiole and myristicin is considered an economically important factor because they are substances utilized by the pharmaceutical, food and agricultural industries.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Two approach comparison to define crop management zones (MZs)

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The use of yield-level management zones (MZs) has demonstrated high potential for site-specific management of crop inputs in traditional row crops. Two approaches were use: all variables approach (all_Var) and stable variables approach (sta_Var). In each approach, variables selected had significant correlation with yield, while all redundant and non-autocorrelated variables were discarded. Two fields were use in this study: Field 1 (17.0 ha soybean field located in Cascavel, Paraná, Brazil); and Field 2 (35.0 ha corn field located in Wiggins, Colorado, US.). Two, three, four, and five MZs were created using fuzzy c-means clustering technique. The proposed methodology for define MZs is simple and allowed create good-quality MZs. It also founded that not-stable-over-time variables are not useful to define MZs.

Key words: Precision agriculture, spatial variability, fuzzy clustering, autocorrelation, cross-correlation.

INTRODUCTION

A management zone (MZ) is a sub-region of a field that expresses a relatively homogeneous combination of yield-limiting factors for which a single rate of a specific crop input is appropriate (Doerge, 1996). Delineation of MZs and management of crop inputs have proved economical for application of variable rate inputs (Koch et al., 2004). Several researchers have successfully used one or more of these factors in combination with yield maps and sometimes use only multiple year yield maps in delineating MZs (Blackmore, 2000; Fraisse et al., 2001; Johnson et al., 2003; Schepers et al., 2004).

Numerous approaches are presented in literature for purpose of delineating MZs using yield maps, using cluster analysis, such as K-means and Fuzzy C-means (Taylor et al., 2007; Li et al., 2007). Although several techniques have been developed to delineate MZs, only few studies compared MZs in terms of their accuracy (Hornung et al., 2006). The following cluster performance indices can be used:

1. Variance reduction (VR; Ping and Dobermann, 2003; Xiang et al., 2007)

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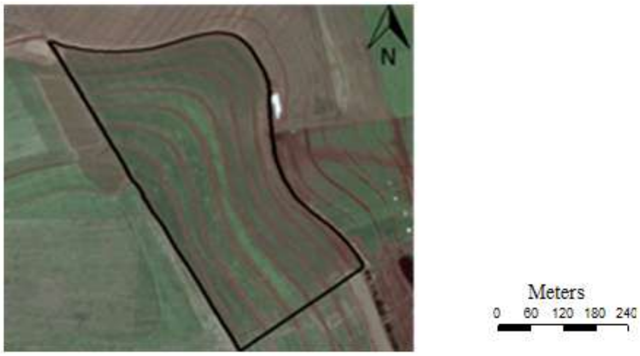

Figure 1. Experimental field 1, (Cascavel/Paraná, Brazil), cultivated with soybean crop.

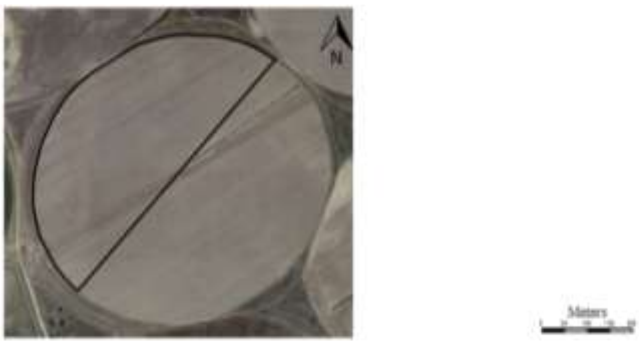

Figure 2. Experimental field 2, (Wiggins, CO, USA), cultivated with corn crop.

2. Fuzziness performance index (FPI); and
3. Modified partition entropy index (MPE; Fridgen et al., 2004). To evaluate the quality of the cluster process, two analyses could be conducted:

The average comparison test (analysis of variation, ANOVA; Bazzi et al., 2015)
Smoothness index (SI; Schenatto et al., 2016), which is a measure of the smoothness of the contour curves.

Although a careful definition of MZs requires a lot of steps and analysis about data. The goal of this study was to analyze viability of MZs definition using stable and non-stable variables and use methodology to define best number of zones for each case. This would be very interesting for producers who are only starting to use precision agriculture.

MATERIALS AND METHODS

Study site and data collection

Field 1: Data from a 16.9 ha soybean field (Figure 1) were evaluated. It is located in a rural area of municipality of Cascavel, Paraná state, Brazil (24°57'13"S and 53°34'02"W, average elevation: 650 m). The soil is classified as distroferric red latossol (Embrapa, 1999) with 70% clay. A Trimble Geo Explorer XT 2005 Geodesic Differential Global Positioning System (GDGPS) with post-processing was used for georeferencing the research area. Soil samples (n = 87) were irregularly spaced throughout field. The following soil attributes were measure:

1. Soil texture (clay, silt, and sand)
2. Cone index (0 to 10 cm, CI_0_10; 10 to 20 cm, CI_10_20; and 0 to 20 cm, CI_0_20)
3. Chemical attributes: organic C, pH, Ca, and Mg, P and K, Cu, Zn, Fe, and Mn, and H+Al; and
4. Physical attributes: bulk density. The methodology from Embrapa (2009) was used to measured these variables. These attributes were used to define management zones because they have influence with potential yield to corn and soybean crops. Numerous researches were used for these attributes to define management zones, such as, Blackmore (2000), Fraisse et al. (2001), Johnson et al. (2003), Schepers et al. (2004), Taylor et al. (2007) and Li et al. (2007).

Field 2 (Figure 2) was approximately 35 ha in size and was nearly level (0 to 2% slope), with an average elevation of 1437 m. The area was cultivated with corn and is located in the rural area of the municipality of Wiggins, Colorado, USA (40°19'59"N and 104°01'50"W). The dominant soil types were Valentine fine sand (mixed, mesic, Typic Ustipsamment) and Dwyer fine sand (mixed, mesic, Ustic Torripsamment) series (USDA, 1986). The following soil attributes were measure: soil texture (clay, silt, and sand); ammonium; cation exchange capacity (CEC); nitrate; organic matter (OM); and chemical attributes (P, pH, K, Zn).

Exploratory analysis and data interpolation

Exploratory analysis was apply to characterize variability in soil data with coefficient of variation (CV). The Anderson–Darling and Kolmogorov–Smirnov tests applied to test normality data at 0.05 significance level. Potential outliers were identify using box-plots. Inverse distance weighting (IDW) was apply to interpolate field data. IDW was choose because of its simplicity, and successful results of interpolation of field data have been reported (Jones et al., 2003). A leave-one-out cross-validation applied to identify number of neighbors and exponent distance used in interpolation of each variable. For this methodology, a routine was write using software R.

Spatial correlations

Moran's bivariate spatial autocorrelation statistics (Czaplewski and Reich, 1993) were apply to assess spatial correlation between analyzed attributes and to establish spatial correlation matrix. This matrix checks and identifies attributes that influence yield positively or negatively, and checks if a sample was correlate spatially (spatial autocorrelation). The attributes used in the generation of MZs were selected by the variable selection method proposed by Bazzi et al. (2013):

1. Elimination of variables with non-significant spatial correlation at 5% probability level
2. Elimination of variables that had no spatial correlation with yield
3. Ordering variables according to the degree of spatial correlation with yield; and
4. Elimination of redundant variables (that are spatially correlated with each other), giving preference to the maintenance of variables that have a higher correlation with yield.

Table 1. Data layers used for the delineation of management zones.

Field	Variable	No. of data points	Data density (points ha^{-1})	Stable variable approach (sta_Var)	All variables approach (all_Var)
1	Yield	68	4.02	x	x
	Elevation, slope	87	5.15	x	x
	Soil organic matter	87	5.15	x	x
	Soil texture (clay, silt, and sand)	45	2.66	x	x
	CI_0_10; CI_10_20; and CI_0_20	87	5.15	x	x
	Chemical attributes: C, Ph, H+Al, Ca, Mg, K, Cu, Zn, Fe, Mn	87	5.15		x
	Physical attributes: bulk density	87	5.15	x	x
	Selected variable			**Elevation, slope**	**CI_10_20**
2	Yield	63	1.80	x	x
	soil texture (clay, silt, and sand)	63	1.80	x	x
	Ammonium, CEC, Nitrate, OM, chemical attributes (P, pH, K, Zn)	85	2.43		x
	Selected variables	-	-	Sand	OM

CI - Cone index; CEC - cation exchange capacity; OM - Organic Matter; CI_0_10 - averaged CI from 0 – 10 cm; CI_10_20 - averaged CI from 10 – 20 cm; CI_0_20 - averaged CI from 0 – 20 cm.

Delineation of management zones

Clustering methods are appropriate for dividing data into groups with homogeneous characteristics. Of these, the Fuzzy C-Means is used most often (Li et al., 2007; Fu et al., 2010; Zhang et al., 2013; Li et al., 2013; Moral et al., 2010). This algorithm uses a weighting exponent to control the degree of sharing between classes (Bezdek, 1981), allowing individuals to exhibit partial adhesion in each of the classes, which is important when dealing with the continuous variability of natural phenomena (Burrough, 1989).

Usually only those attributes that do not vary appreciably over time as chemical attributes are used to create MZs that are intended for use for many years (Doerge, 1996). However, for special conditions, MZs are use for nutrients and lime application in the following year. Chemical attributes may be included as MZ attribute variables. Two approaches were apply to delineate MZs: all variables approach (all_Var) and stable variables approach (sta_Var). In the first approach, variables with a significant spatial cross-correlation with the yield were select to delineate MZs. The selected variables may or may not be stable over time. In sta_Var, only those variables that were stable over time (Table 1) and spatially cross-correlated with the yield (Doerge 1996) were consider for delineation MZs (Table 1).

Two to five MZs were created using interpolated data and fuzzy c-means clustering; for this purpose, Software for Defining Management Zones (SDUM; Bazzi et al., 2013). Each variable was standardize according to Eq. 2 prior to clustering:

$$VS_z = \frac{V_z - M}{R} \quad (1)$$

where: VS_z is the value of the standardized variable at the spatial location of position *z*; V_z is the value of the original variable at the spatial location of *z*; M is the median; and R is the range of the data (Mielke and Berry, 2007).

Evaluation of management zone delineation

Variance reduction (VR; Equation 2; Xiang et al., 2007):

$$RV = 1 - \frac{\sum_{i=1}^{c}\left(W_i * V_{um_i}\right)}{V_{área}} * 100 \quad (2)$$

where c is the number of MZs; W_i, the proportion of the area in each MZ; V_{um_i}, the variance of the sample data of each MZ; and $V_{área}$, the variance of the sample of the data for the entire field.

Fuzziness Performance Index (FPI; Equation 3; Fridgen et al., 2004)

$$FPI = 1 - \frac{c}{(c-1)}\left[1 - \sum_{j=1}^{n}\sum_{i=1}^{c}(u_{ij})^2 / n\right] \quad (3)$$

where c is the number of clusters; n, the number of observations; u_{ij}, the element of the fuzzy membership matrix.

Modified partition entropy (MPE; Equation 4; Boydell and Mcbratney, 2002)

$$MPE = \frac{-\sum_{j=1}\sum_{i=1} u_{ij}\log(u_{ij})/n}{\log c} \quad (4)$$

Table 2. Descriptive statistics of attributes of Field 1 – Cascavel.

Variable	Minimum	Mean	Median	Maximum	CV	Skewness[1]	Kurtosis[2]
Yield (Mg ha^{-1})	1.55	2.65	2.58	4.34	22.52	-0.40 [a]	-0.12 [A]
Elevation (m)	701	706	705	713	0.48	0.35 [a]	-1.12 [C]
*Slope (%)	0.08	1.83	1.59	5.12	63.8	0.84 [b]	0.09 [A]
P (ppm)	9.60	19.85	18.10	71.60	41.61	3.25 [b]	17.4 [B]
C	21.82	28.87	29.22	36.62	10.99	-0.08 [a]	-0.50 [A]
Ph	5.00	5.55	5.60	6.10	4.64	0.00 [a]	-0.68 [A]
H+Al	3.18	4.91	4.96	7.20	18.95	0.27 [a]	-0.45 [A]
Ca	5.52	7.56	7.48	9.86	12.95	0.11 [a]	-0.49 [A]
Mg	1.51	2.44	2.40	3.69	16.55	0.34 [a]	0.32 [A]
K	0.15	0.30	0.29	0.89	33.82	2.36 [b]	11.1 [B]
Cu	1.20	2.56	2.50	4.10	27.57	0.18 [a]	-0.80 [C]
Zn	1.00	4.15	2.60	17.90	81.67	1.48 [b]	2.31 [B]
Fe	28.00	34.55	35.00	47.00	10.13	0.60 [b]	1.03 [A]
Mn	39.00	67.06	67.00	98.00	18.09	0.03 [a]	0.00 [A]
Clay (%)	60.00	70.42	72.00	79.00	7.13	-0.74 [c]	-0.36 [A]
Silt (%)	15.00	19.69	19.00	25.00	11.40	0.57 [b]	-0.27 [A]
Sand (%)	6.00	9.89	9.00	19.00	38.58	1.26 [b]	0.48 [A]
CI_0_10 (MPa)	1099.70	1643.50	1633.60	2380.4	17.25	0.08 [a]	-0.39 [A]
CI_10_20 (MPa)	1550.80	2347.10	2220.5	3769.30	18.02	1.13 [b]	0.88 [A]
CI_0_20 (MPa)	1482.50	1995.30	1900.20	2995.30	15.55	0.91 [b]	0.50 [A]
Density (kg kg^{-1})	0.82	1.13	1.14	1.30	6.78	-1.00 [c]	2.51 [B]

[1] Skewness: symmetric (a), positive skewness (b), negative skewness (c); [2] Kurtosis: mesokurtic (A), platykurtic (B), leptokurtic (C); * is not normally distributed at the 0.05 level of significance.

where C is the number of clusters; n, the number of observations; and u_{ij}, the *ij*-th elements of the fuzzy membership matrix.

RESULTS AND DISCUSSION

Descriptive statistics

The descriptive statistics for selected attributes of Field 1 are indicated that all variables, except for slope, were it is normally distributed (Table 2). Most variables showed symmetric and mesokurtic distributions. The CV was low for elevation, pH, bulk density, and clay; medium for Fe, C, silt, Ca, cone index (0 to 10 cm, CI_0_10; 10 to 20 cm, CI_10_20; and 0 to 20 cm, CI_0_20), Mg, Mn, and H+Al; high for the yield and Cu; and very high for K, sand, P, the slope, and Zn. In case of Field 2 (Table 3), clay, silt, sand, OM, pH, and Zn were normally distributed. The CV was low for the pH and sand; medium for yield; high for Zn, OM, CEC, ammonium, and K; and very high for P, nitrate, clay, and silt.

Spatial autocorrelation and cross-correlation

The attributes analyzed values for Field 1 were show at Table 4. Variables Cu, clay, elevation, CI_10_20, CI_0_20, sand, slope, P, C, Zn, and bulk density had significant positive autocorrelations. Variables CI_10_20, elevation, CI_0_20, bulk density, slope, sand, clay, silt, pH, P, and H+Al were significantly cross-correlated with yield. Bazzi et al. (2013) found a high correlation between soybean yield with soil sand percentage, generating agricultural area MZs with this layer. Similarly, Schenatto et al. (2016), Peralta and Costa (2013) and Gavioli et al. (2016) generated MZs from elevation data and CI in experimental soybean and cornfields.

The values for Field 2 were show in Table 5. Variable yield, P, nitrate, Zn, CEC, OM, K, clay, sand, silt, and ammonium exhibited significant autocorrelations. Variables OM, CEC, sand, NH3, clay, K, silt, and Zn were significantly cross-correlated with the yield. Other studies yielded similar results: for example, Bansod and Pandey (2013) and Jaynes et al. (2003) identified high correlation between CEC and yield.

Management zones

Figure 3 shows the MZs as a function of zone number (from two to five) and approach (sta_Var and all_Var) for Field 1 (Cascavel) and Field 2 (Wiggins). In all cases, this can be see when the number of zones increased, the amount of fragments of the area is bigger.

For Field 1, data layers selected to delineate MZs using

Table 3. Descriptive statistics of the attributes of Field 2 (Wiggins).

Variable	Minimum	Mean	Median	Maximum	CV	Skewnes[1]	Kurtosis[2]
*Yield (Mg ha^{-1})	4.43	11.51	12.25	14.08	18	-1.58 [c]	2.52 [B]
*P (ppm)	3.00	11.40	9.00	32.00	58	1.16 [b]	0.84 [A]
Zn (ppm)	1.20	3.47	3.55	5.50	25	-0.18 [a]	0.10 [A]
Ph	7.30	7.71	7.70	8.10	3	0.00 [a]	-0.15 [A]
OM (%)	0.50	0.96	0.90	1.60	24	0.42 [a]	-0.54 [A]
*CEC (cmol kg^{-1})	3.60	7.21	6.55	14.60	29	0.87 [b]	0.62 [A]
*Nitrate (ppm)	1.00	3.63	3.00	12.00	53	1.55 [b]	3.68 [B]
*Ammonium (ppm)	4.72	7.23	6.99	14.59	23	1.80 [b]	4.99 [B]
*K (ppm)	89.00	154.77	147.00	272.00	26	0.67 [b]	0.07 [A]
Clay (%)	4.00	9.24	10.00	20.40	38	0.69 [b]	0.62 [A]
Silt (%)	2.00	5.29	4.40	10.40	40	0.46 [a]	-0.32 [A]
Sand (%)	71.60	85.47	85.60	93.60	6	-0.75 [c]	0.41 [A]

[2] Skewness: symmetric (a), positive skewness (b), negative skewness (c); [3] Kurtosis: mesokurtic (A), platykurtic (B), leptokurtic (C); CEC - Cation Exchange Capacity; OM - Organic Matter; * is not normally distributed at the 0.05 level of significance.

Table 4. Spatial autocorrelation from the analyzed attributes in Field 1 (Cascavel).

Yield	-0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-0.01	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NS	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	-0.04	0.06	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	*	**	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH	0.04	-0.01	-0.01	-0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	*	NS	NS	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-
H_Al	-0.03	-0.00	-0.03	0.01	-0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	*	NS	*	NS	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ca	0.00	-0.02	-0.04	0.00	0.01	-0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NS	NS	*	NS	NS	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mg	-0.02	0.01	0.04	-0.01	0.00	-0.01	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NS	NS	*	NS	NS	NS	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. Contd.

	Yield	C	P	pH	H_Al	Ca	Mg	K	Cu	Zn	Fe	Mn	CI_0_10	CI_10_20	CI_0_20	Elevation	Clay	Silt	Sand	Slope	DE
K	0.00 NS	0.01 NS	0.01 NS	-0.01 NS	0.02 NS	0.01 NS	-0.00 NS	-0.01 NS	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
Cu	0.00 NS	-0.09 **	-0.17 **	-0.01 NS	0.05 **	0.04 *	-0.02 NS	-0.00 NS	0.33 **	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
Zn	0.00 NS	-0.00 NS	0.05 **	-0.01 NS	-0.01 NS	-0.00 NS	0.02 NS	0.01 NS	-0.06 **	0.04 **	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
Fe	0.00 NS	0.00 NS	0.03 NS	0.02 NS	-0.02 NS	0.01 NS	0.01 NS	0.00 NS	-0.05 **	0.02 NS	-0.00 NS	- -	- -	- -	- -	-	- -	- -	- -	- -	- -
Mn	-0.01 NS	-0.01 NS	-0.05 **	0.00 NS	0.02 NS	0.00 NS	0.00 NS	0.01 NS	0.10 **	0.01 NS	-0.01 NS	0.03 NS	- -	- -	- -	- -	- -	- -	- -	- -	- -
CI_0_10	0.01 NS	-0.01 NS	0.02 NS	0.00 NS	-0.01 NS	0.02 NS	0.02 NS	0.01 NS	-0.01 NS	0.01 NS	-0.01 NS	0.02 NS	0.00 NS	- -	- -	- -	- -	- -	- -	- -	- -
CI_10_20	0.08 **	-0.06 **	-0.07 **	0.03 *	-0.01 NS	0.03 *	-0.04 *	-0.03 *	0.08 **	-0.05 **	-0.02 NS	0.00 NS	0.01 NS	0.18 **	- -	- -	- -	- -	- -	- -	- -
CI_0_20	0.08 **	-0.06 **	-0.07 **	0.03 *	-0.02 NS	0.03 NS	-0.03 *	-0.02 NS	0.08 **	-0.04 **	-0.02 NS	-0.00 NS	0.01 NS	0.16 **	0.14 **	- -	- -	- -	- -	- -	- -
Elevation	0.08 **	-0.09 **	-0.08 **	0.02 NS	-0.00 NS	0.03 NS	-0.05 **	-0.02 NS	0.11 **	-0.07 **	0.01 NS	0.01 NS	-0.04 **	0.17 **	0.15 **	0.19 **	- -	- -	- -	- -	- -
Clay	-0.04 **	0.09 **	0.13 **	0.01 NS	-0.04 **	-0.04 **	0.03 NS	-0.00 NS	-0.28 **	0.04 **	0.05 **	-0.08 **	0.02 NS	-0.11 **	-0.12 **	-0.13 **	0.31 **	- -	- -	- -	- -
Silt	0.04 **	-0.04 *	-0.07 **	0.00 NS	0.02 NS	0.03 *	-0.02 NS	-0.01 NS	0.10 **	-0.03 NS	-0.02 NS	0.02 NS	-0.01 NS	0.08 **	0.08 **	0.08 **	-0.12 **	-0.00 NS	- --	- -	- -
Sand	0.05 **	-0.08 **	-0.08 **	-0.00 NS	0.02 NS	0.05 **	-0.03 NS	-0.02 NS	0.17 **	-0.02 NS	-0.03 *	0.04 **	-0.01 NS	0.11 **	0.10 **	0.11 **	-0.20 **	0.06 **	0.10 **	-	- -
Slope	-0.05 **	-0.01 NS	-0.07 **	-0.02 NS	0.04 **	-0.01 NS	0.03 *	0.00 NS	0.12 **	-0.05 **	-0.01 NS	0.03 NS	-0.02 NS	-0.04 **	-0.04 *	0.01 NS	-0.05 **	0.01 NS	0.02 NS	0.07 **	- -
Density	-0.07 **	0.07 **	0.05 **	-0.01 NS	-0.01 NS	-0.03 NS	-0.01 NS	0.01 NS	-0.09 **	0.03 *	0.01 NS	-0.02 NS	0.02 NS	-0.11 **	-0.10 **	-0.13 **	0.12 **	-0.04 **	-0.07 **	0.00 NS	0.03 *

* Significant at 0.05 level; ** Significant at 0.01 level; CI - cone index; DE: Density.

Table 5. Spatial autocorrelation from analyzed attributes in Field 2 (Wiggins).

	Yield	Clay	Sand	Silt	Ammonium	CEC	Nitrate	OM	P	pH	Potassium	Zn
Yield	0.08 **	-	-	-	-	-	-	-	-	-	-	-
Clay	0.11 **	0.15 **	-	-	-	-	-	-	-	-	-	-
Sand	-0.12 **	-0.14 **	0.15 **	-	-	-	-	-	-	-	-	-
Silt	0.08 **	0.06 **	-0.08 **	0.08 **	-	-	-	-	-	-	-	-
Ammonium	-0.01 NS	0.03 *	-0.00 NS	-0.04 **	0.04 **	-	-	-	-	-	-	-
CEC	0.12 **	0.11 **	-0.15 **	0.14 **	-0.05 **	0.20 **	-	-	-	-	-	-
Nitrate	0.11 **	0.09 **	-0.13 **	0.14 **	-0.02 NS	0.18 **	0.27 **	-	-	-	-	-
OM	0.13 **	0.13 **	-0.15 **	0.13 **	-0.04 **	0.19 **	0.19 **	0.19 **	-	-	-	-
P	0.00 NS	0.02 NS	-0.03 *	0.02 NS	0.05 **	0.01 NS	0.17 **	0.04 **	0.31 **	-	-	-
pH	-0.01 NS	-0.02 NS	0.01 NS	0.01 NS	-0.03 **	0.02 NS	0.01 NS	0.01 NS	0.01 NS	0.01 NS	-	-
Potassium	0.11 **	0.09 **	-0.12 **	0.12 **	-0.05 **	0.18 **	0.18 **	0.17 **	0.04 **	0.03 *	0.15 **	-
Zn	0.07 **	0.08 **	-0.10 **	0.09 **	0.03 NS	0.11 **	0.23 **	0.14 **	0.24 **	0.02 NS	0.12 **	0.23 **

*Significant at 0.05 level; ** Significant at 0.01 level; OM - Organic Matter; CEC - Cation Exchange Capacity.

sta_Var (approach) were elevation and slope, whereas MZs based on all_Var based on CI_10_20. CI_10_20 was not selected as sta_Var because it was not stable over time. However, because it showed highest spatial correlation with yield and was cross-correlated with elevation and slope, it was included in all_Var selection. In this sense, in the lower areas, soybean yield was lower probably because rainfall was higher than average during cultivation, which may have resulted in excessive water in soil (Fausey, 1999). For Field 2, MZs based on sta_Var approach were delineated using sand, whereas in all_Var approach, OM used it. As in Field 1 case, OM was not select as sta_Var because it was not stable over time. In both approaches, selected correlated layers (sand and OM) were negatively spatially correlate with yield. In addition, sand and OM were correlate to CEC, meaning that this variable used to define MZs if sample and analysis costs were prohibitive.

The average yields differed significantly between two MZs (Table 6) in both fields. With three, four, and five zones only for Field 2, the average yields differed significantly. From these results and those reported by Zhang et al. (2013), it can be seen that the division of the area into two MZs was adequate for producing zones with different yields.

The VR of an MZ indicates whether MZ delineation

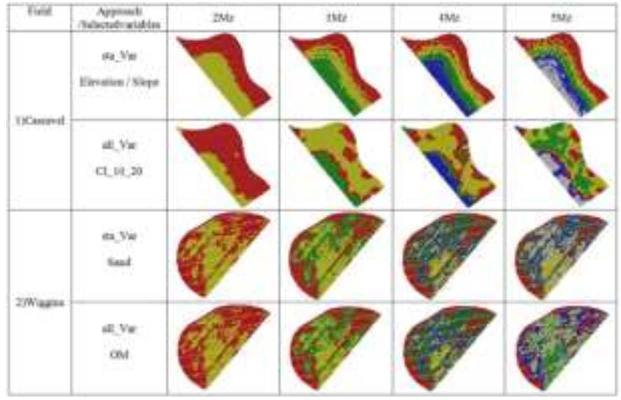

Figure 3. Management Zones (MZs) as a function number of zones (from two to five), and layers section approach (stable variables (sta_Var) and all variables (all_Var) for the Field 1, Cascavel (Brazil) and Field 2, Wiggins (USA), using Fuzzy C-means.

Table 6. Average Yield in each zone for both approaches (sta_Var – stable variables and all_Var – all variables) in Fields 1 (Cascavel) and 2 (Wiggins).

Field	No. of zones	MZ approach	Selected layers	Averaged Yield in t ha^{-1} in each Zone (% of area)					VR%	FPI	MPE	ICVI	SI
				Zone 1	Zone 2	Zone 3	Zone 4	Zone 5					
1	2	sta_var	Elevation / Slope	2.32a (56.0)	2.78 (44.0)	-	-	-	21.1	0.11	0.02	0.28	97.8
		all_var	CI_10_20	2.41a (78.1)	2.93b (21.9)	-	-	-	12.1	0.04	0.01	0.24	98.0
	3	sta_var	Elevation / Slope	2.37a (40.6)	2.44a (30.9)	2.90b (28.5)	-	-	12.0	0.17	0.03	0.56	90.2
		all_var	CI_10_20	2.46a (24.2)	2.41a (57.4)	2.92b (18.5)	-	-	13.9	0.09	0.02	0.42	93.7
	4	sta_var	Elevation / Slope	1.77a (35.9)	1.55a (22.4)	2.50a (20.8)	1.92b (20.9)	-	19.6	0.21	0.04	0.56	87.0
		all_var	CI_10_20	2.43a (28.9)	2.35a (44.6)	2.64ab (11.4)	2.97b (15.2)	-	13.5	0.11	0.02	0.55	90.4
	5	sta_var	Elevation / Slope	2.47a (30.8)	2.02a (19.4)	2.78ab (15.3)	2.91ab (16.4)	2.85b (18.1)	14.5	0.26	0.05	0.77	80.4
		all_var	CI_10_20	2.48a (15.7)	2.51a (31.9)	2.29a (31.8)	3.34bc (8.4)	2.86ac (12.2)	11.8	0.14	0.03	0.72	87.0
2	2	sta_var	Sand	10.3a (53.5)	13.0b (46.5)	-	-	-	49.9	0.07	0.01	0.52	87.2
		all_var	OM	10.5a (59.1)	13.1b (40.9)	-	-	-	45.2	0.07	0.01	0.34	87.1
	3	sta_var	Sand	9.6a (35.7)	13.2b (34.0)	12.1c (30.3)	-	-	68.2	0.09	0.02	0.36	77.6
		all_var	OM	9.7a (38.1)	13.3b (31.1)	12.2c (30.7)	-	-	66.2	0.10	0.02	0.35	77.3
	4	sta_var	Sand	9.2a (29.7)	13.5b (22.4)	11.7c (25.3)	12.6d (22.7)	-	75.1	0.11	0.02	0.15	68.6
		all_var	OM	9.0a (32.0)	13.5b (29.9)	11.7c (27.4)	12.7d (10.6)	-	77.6	0.26	0.04	0.69	68.9
	5	sta_var	Sand	8.8a (23.6)	13.5b (19.7)	11.2c (17.9)	12.1d (19.9)	12.8e (18.9)	81.1	0.12	0.02	0.13	61.9
		all_var	OM	8.4a (18.9)	12.3b (20.2)	13.6c (17.0)	11.2d (26.6)	12.9e (17.3)	84.5	0.13	0.02	0.35	62.6

Within a row, means with different letters are significantly different at $p < 0.05$; CI - Cone Index.

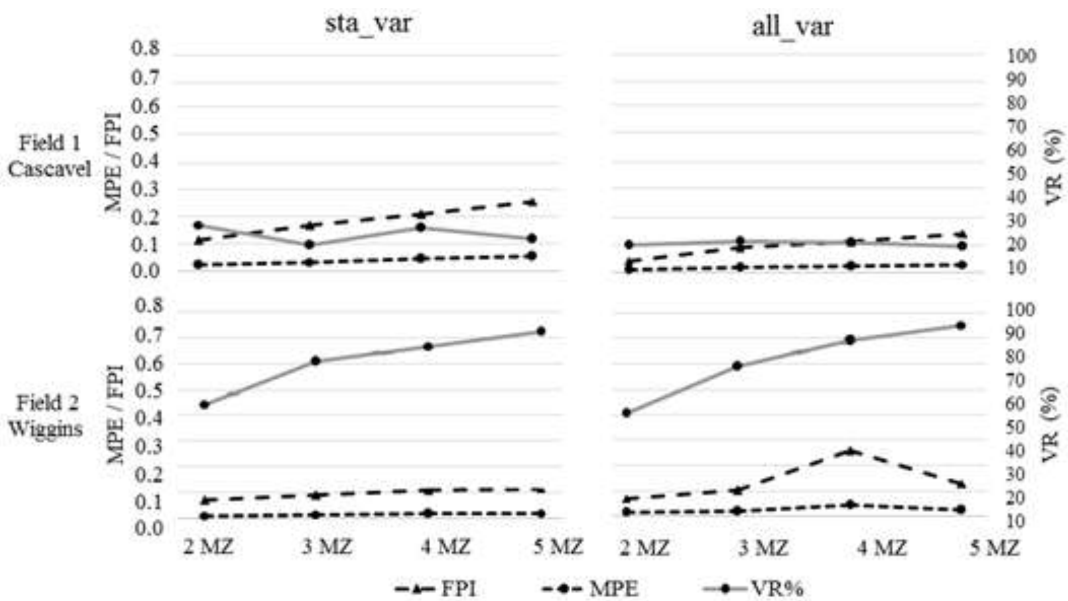

Figure 4. Evaluation indices MPE, FPI, VR, SI and ICVI used by evaluate management zones as a function of number of management zones.

is more efficient than having no MZs on field. In all cases (Table 6 and Figure 4), VR values were positive, indicating that in all cases, total variance reduced, as expected. In case of Field 1, VR remained reasonably constant with MZs number. However, in Field 2 case, VR increased with MZ number. The VR results confirm ANOVA results because it turns out that divisions within each zone did not show much variance from average, indicating regions of similar productive potential. These results corroborates those obtained by Xiang et al. (2007) and indicated that when divisions are satisfactory, VR tends to increase despite an increase in MZs number because there is smaller variation within each zone.

ANOVA shows for Field 1, that best division was two MZs (for sta_Var and all_Var) and up to five MZs for Field 2 (sta_Var and all_Var) (Table 6). The FPI, MPE, and VR indices (Figure 4) were also evaluated; smaller FPI and MPE, shows better clustering test for both fields, considering FPI and MPE. Best efficiency was obtain with two MZs, but best option with VR was two (sta_Var) and three (all_Var) MZs for Field 1 and five MZs for Field 2 (sta_Var and all_Var).

Two approaches comparison (sta_Var and all_Var) for two MZs (selected as best number of MZs) showed that sta_Var provides better VR. That means there is no meaning of using all variables (time stable or not) in defining MZs. The sta_Var MZs (with two MZs) presented a reduction in variance from 21 (Field 1) to 50% (Field 2). Because the MZ definition through Fuzzy c-means does not use variable yield only if several years' yields could find other important and not redundant variable, the MZs would be same.

Conclusions

The methodology proposed for MZs define has practical value and allowed MZs definition with good quality. This results may be confirmed on the analysis of the variance reduction and Anova. For divisions in two zones (best division by MPE and FPI indexes), the variance reduction and Anova showed have different yield potential in each zone when the fields were divided. The stable variables approach performed better than all variables in all cases because they showed better results on the analysis from variance reduction and Anova, indicating that stable variables have influence in the yield capacity and can be used to define management zones that must be used for a long time. The stable variables approach also served as the source of recommendation and soil analysis and pre-plant or in-season fertilizer recommendation for precision agriculture.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Assessment of a crambe (*Crambe abyssinica* Hochst) crop under no-tillage in different sowing dates

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Crambe (*Crambe abyssinica* Hoschst) is a winter oilseed crop with yield potential of 1500 kg ha^{-1}. It is indicated for crop rotation systems and tolerates moderate frost. However, crambe presents thermal and water limitations that influence sowing dates since it needs water at blooming and at least 200 mm rainfall until it reaches the flowering stage. This study aimed to assess the performance of a crambe crop in different sowing dates. The experiment was conducted on the experimental farm of Assis Gurgacz College (Faculdade Assis Gurgacz – FAG) Cascavel – Paraná, at an altitude of 700 m, within latitudes 24°56'25.39" S and 24°56'45.39" S and longitudes 53°30'9.89" W and 53°31'17.01" W. The experimental design consisted of randomized blocks with three sowing dates (April, June and July) and five replications. Phenometric parameters such as plant height, dry mass, plants per meter, grain yield and mass of 1000 grains were assessed and data were subjected to Tukey's test at 5% probability. Phenometric variables were influenced by sowing dates. Degree days and rainfall influenced the results. April has proven to be the best month for sowing.

Key words: Winter oilseed crop, cycle, development, production, *Crambe abyssinica* Hoschst.

INTRODUCTION

Crambe (*Crambe abyssinica* Hochst) is an oilseed native to the Mediterranean area from Ethiopia to Tanzania that is tamed and adapted to dry and cold areas of that region. It is a winter species sown after the harvest of soybean from March to May, with total oil content of 26 - 38% (Pitol, 2008). Therefore, crambe has stood out as an alternative to be used in biodiesel processing and as a provider of feedstock for insulating fluids used in high voltage electric equipment (Laghetti, 1995; Lazzeri et al., 1997; Souza et al., 2009).

Crambe also presents low incidence of pests due to the presence of glucosinolate, and reduction of diseases in dry weather (Pitol et al., 2010). Its yield potential ranges from 1,000 to 1,500 kg ha^{-1} and it is sensitive to strong

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frosts during the seedling and flowering stages. Crambe has a short growing season as it blooms at 35 days after sowing (DAS) and can be harvested at 90 DAS, depending on the maturation of plants (Pitol, 2008; Carneiro et al., 2009; Falasca et al., 2010). It can also be used in crop rotation systems as an alternative for soil cover in no-tillage, with sowing densities that ranges from 12 to 15 kg ha^{-1} (Pitol, 2008).

Despite showing good adaptation and use of no-tillage, initially expansion was not successful in mass production for ground cover. However, since its inclusion in the country, it showed good yield potential of grain and oil, stimulating the creation of a single cultivar available in Brazil, Brilhante FMS. With the creation of PNPB, National Program for Biodiesel Production and Use, intensified its cultivation, mostly in the Midwest and South of the country (Pitol et al., 2010).

In order to determine the most adequate sowing date in each region, water restrictions and temperature must be taken into account (Pitol et al., 2010). Water conditions and solar radiation levels are important factors in the development of a plant, but even when such factors are ideal, plant growth may be affected by temperatures that differ from those tolerated by it (Went, 1953). Plant development can be influenced by the interaction between photoperiod and temperature, sowing date and latitude (Wallis et al., 1981).

As reported by Falasca et al. (2010), ideal temperature for crambe development during the vegetative phase ranges from 15 to 25°C and plants can tolerate up to -6°C for a few hours without suffering significant damage (Fowler, 1991). Its thermal needs were defined with approximately 1350°C degree days accumulated between sowing and physiological maturation, considering temperatures above 2.5°C for the sum (Kmec et al., 1998).

Crambe demands good soil humidity for germination and plant establishment, as well as rainfall between 150 and 200 mm until reaching the flowering stage. After this period, absence of rain prevents the occurrence of diseases (Graser, 1996; Pitol et al., 2010).

According to Pereira et al. (1992), the interaction between species and sowing dates has been an important tool for researchers to assess and improve the agronomic capability of tropical leguminous plants. Thus, acquiring information on the climatic requirements of plants allows the execution of a crop cycle planning in order to define the best sowing dates in locations where cultivation does not take place but meets climatic requirements (Pilau et al., 2011). Therefore, this work aimed to assess the development of a crambe crop under no-tillage on three sowing dates.

MATERIALS AND METHODS

The experiment was carried out in 2012 on the experimental farm of Assis Gurgacz College – (Faculdade Assis Gurgacz – FAG), located in the city of Cascavel – Paraná, at an altitude of 700 m, within latitudes 24°56'25.39" S and 24°56'45.39" S and longitudes 53°30'9.89" W and 53°31'17.01" W. Local climate is classified as subtropical Cfa, according to Köppen's classification, with average annual rainfall over 1800 mm with no defined dry season and possibility of frosts during winter.

The experiment area was cultivated under no tillage for more than 20 years, with soybean or maize in summer crops and wheat or oat in fall/winter crops. Soil is classified as eutroferric Red Latosol – (LVef - Oxisol) with clayey texture (EMBRAPA, 2006). Soil was sampled from 0 to 0.02 m depth and taken to a soil analysis laboratory. Table 1 presents the results of the chemical features analysis. Accumulated rainfall, sum of degree days and sowing period cycles are presented in Table 2.

Figure 1 depicts levels of rainfall as well as minimum and maximum air temperature during crambe development under different sowing dates. Weeds in the area were desiccated with glyphosate herbicide in the rate of 2.5 L ha^{-1} before sowing. Sowing of crambe cultivar FMS Brilhante, developed by Fundação MS was performed on three different dates (23/04/2012, 14/06/2012 and 19/07/2012) in no-tillage system, with a tractor and a seeder/fertilizer set at a depth of 0.03 m, with inter-row spacing of 0.45 m, no base and cover fertilization, and seed density of 12 kg ha^{-1}.

The occurrence of pests and diseases was monitored during the entire crop cycle. On the eleventh day after sowing, 0.2 L.ha^{-1} of Lambda-cyhalothrin + Thiamethoxam insecticide, was applied with the aid of a 20 L knapsack sprayer to effectively control *Diabrotica speciosa* (Germar).

Others weed controls was made according to the needs and technical recommendation of culture. The experimental design consisted of randomized 4 x 5 m blocks. Seeds were sown on three different dates (April, June and July) and each treatment had five replications. The following phenometric features were assessed during crambe cycle:

Plant height

Assessment under all sowing dates was performed during flowering stage. Five plants were randomly collected from each plot and measured from ground level to their apex with a graduated ruler (Jasper, 2009).

Plant dry mass

Plants were dried up at 65°C for 72 h in oven with air recirculation and weighed in a precision balance. After determining dry mass in grams (g), data were converted into kg ha^{-1} (Freitas, 2010).

Plants per meter

The number of plants per meter was counted during harvest with the aid of a 5-m measuring tape. Measurement was randomly performed in each experimental unit.

Grain yield

Samples were collected from each unit by using a wooden frame measuring 1 m^2. All plants within the frame were collected. Grains were manually removed from plants and put in empty fertilizer bags. Later, grains were cleaned with the aid of sieves. Humidity was determined by drying grains in oven at 110°C for 24 h. The material was then weighed on an analytical balance for the obtainment of grain mass data (BRASIL, 1992).

Table 1. Chemical features of soil samples collected from 0 to 0.02 m depth in the experiment area.

Soil attributes	$cmol_c\ dm^{-3}$	Reading
Calcium (Ca^{+2})	5.39	High
Magnesium (Mg^{2+})	2.30	High
Potassium (K^+)	0.30	Medium
Aluminum (Al^{+3})	0.00	Low
H + Aluminum ($H^+ + Al^{3+}$)	5.76	High
Sum of bases (S)	7.99	High
CEC (T)	13.75	High
	$g\ dm^{-3}$	
Carbon (C)	27.12	High
Organic matter (OM)	46.65	High
	%	
Base saturation (V)	58.11	Medium
	$mg\ dm^{-3}$	
Phosphorus (P)	7.50	High
pH $CaCl_2$ (0.01 mol L^{-1})	5.20	

Source: EMBRAPA (2009).

Table 2. Sowing period cycles, sum of degree days and accumulated rainfall.

Cycle (days)	Degree days (°C)	Rainfall (mm)
125	1070.00	468.9
103	941.15	186.2
78	893.55	145.7

Mass of 1000 grains

After determining grain yield, 100 grains per sample were counted and weighed, and the results were multiplied by 10 (BRASIL, 1992). Weights were corrected to standard humidity of 13%. Data obtained were subjected to analysis of variance (ANOVA) and means comparison by Tukey's test at 5% significance in ASSISTAT software.

RESULTS AND DISCUSSION

Results of the analysis of variance and mean comparison by Tukey's test at 5% significance of plant height (H), dry mass (DM), number of plants per meter (P/M), mass of 1000 grains (MTG) and grain yield under different sowing dates are shown in Table 3.

All variables were significantly influenced by sowing dates. The coefficient of variation (CV) of all variables remained homogeneous according to classification proposed by Gomes and Garcia (2002). Plant height and number of plants per meter had homogeneous CV with low dispersion. Mass of 1000 grains and yield had coefficient of variation with medium dispersion. Dry mass presented CV with high dispersion, however, it presented the highest variability among data classified as heterogeneous CV.

Plant height

There was significant statistical difference at 1% probability. The highest increase was observed in the crop sown in July, with an average height of 111.92 cm. Similar data were observed by Barbisan et al. (2009), who studied the influence of different sowing dates on canola plant height. The results also match those obtained by Barni et al. (1995) and Capone et al. (2012), in an experiment on sunflowers.

Plant dry mass

This variable was influenced by the sowing date. The highest average dry mass yield of 14.47 t ha^{-1} was achieved when sowing was performed in April. Moreover, under the conditions in which this experiment was carried out, early sowing resulted in higher dry mass yield.

Barbosa et al. (2011) achieved higher dry mass yield when performing sowing with cover plants in two different dates (March and April). Dry mass is important because it is used as straw in no tillage, protecting the soil from the impact of raindrops that cause erosion (Rangel et al., 2003).

Plants per meter

Sowing in April and June resulted in 35.6 and 35.2 plants per meter respectively. These results differ statistically from the sowing performed in July (30.8 plants per meter). The difference in July can be because of rainfall inferior to 5 mm until the seventh day after sowing, which may have contributed to a decrease in the final plant density.

Grain yield

This variable was influenced by the sowing date. Highest average grain yield was achieved with sowing in April (1892.18 kg ha^{-1}). The lowest yield occurred with sowing in July (530.73 kg ha^{-1}). This difference represents a reduction of 71.95%.

The same happened in experiments conducted by Pitol et al. (2010) in different locations in the state of Mato Grosso do Sul, in which sowing performed in April resulted in higher yield than that performed in May. There was also a decrease in yield with late sowing in studies carried out in the states of Goiás and Mato Grosso (Pitol et al., 2010).

When choosing sowing dates, environmental factors such as temperature, soil humidity and photoperiod, which interact with plants and cause variations in their characteristics, such as productivity were taken into

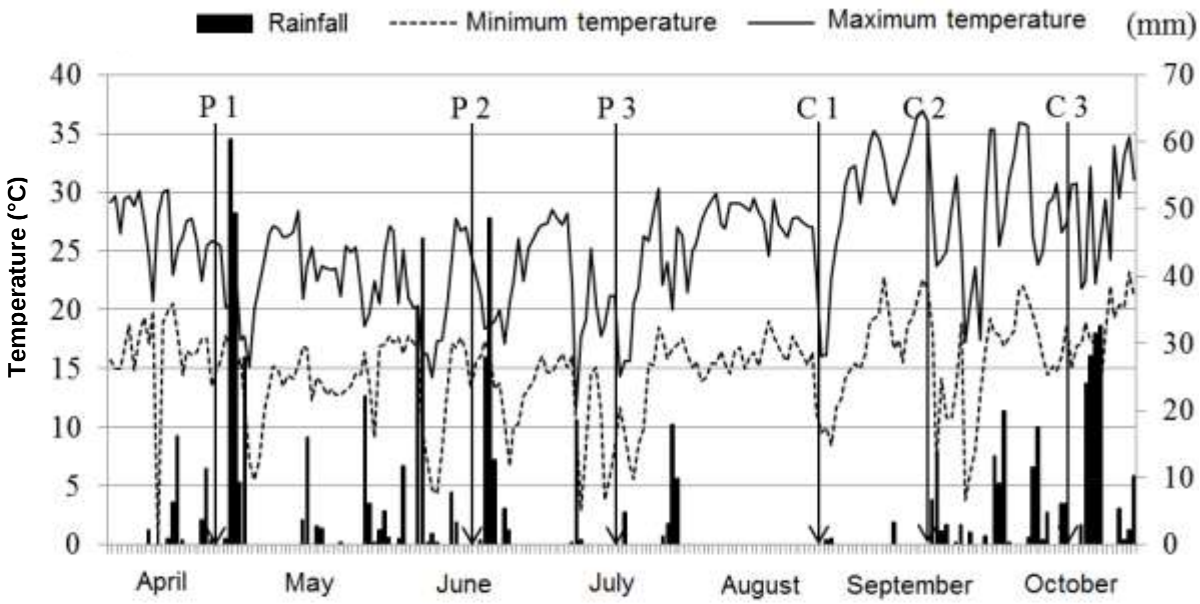

Figure 1. Minimum and maximum air temperature and rainfall during crambe development under three different sowing dates in Cascavel, PR, 2012. P1: Sowing in April; P2: Sowing in June; P3: Sowing in July; C1: Harvest in August; C2: Harvest in September; C3: Harvest in October.

Table 3. Analysis of variance of the crambe crop regarding plant height (H), dry mass (DM), number of plants per meter (P/M), mass of 1000 grains (MTG) and crambe yield under different sowing dates.

Sowing dates	Variables				
	H (cm)	DM (t ha^{-1})	P/M	MTG (g)	Yield (kg ha^{-1})
April	97.94^{b}	14.47^{a}	35.6^{a}	7.82^{a}	1892.18^{a}
June	111.92^{a}	6.61^{b}	35.2^{a}	7.64^{ab}	1350.72^{b}
July	65.04^{c}	4.09^{b}	30.8^{b}	6.33^{b}	530.73^{c}
F	278.01**	30.00**	6.00*	6.05*	42.99**
CV (%)	3.52	26.35	7.90	10.22	18.97
MSD	5.83	3.99	4.88	1.34	436.83

**Significant at 1% probability ($p < 0.01$); *significant at 5% probability ($0.01 =< p < 0.05$). ns: non-significant ($p >= 0.05$). CV: Coefficient of variation. MSD: minimum significant difference.

account (Peixoto et al., 2000).

Mass of 1000 grains

The sowing date influenced this variable. Sowing performed in April resulted in the highest average among treatments (7.82 g). As stated by Guarienti et al. (2004), temperature favors a higher number of days with green plants, helps in photosynthesis and grain filling and subsequently increases their weight. Thermal sum presented higher value of degree days when sowing was performed in April, with 1070°C. Table 2 shows the relationship between sowing dates and degree days. Toebe et al. (2010) considered the minimum temperature of 2.5°C and obtained results from 1165.3 to 1175.8°C between crambe emergence and senescence. In an experiment on crambe carried out in Rio Grande do Sul, the authors obtained an average of 690.64°C between plant emergence and maturation, based on the minimum basal temperature of 9.5°C (Pilau et al., 2011).

Gilmore Jr and Rogers (1958) reported that the thermal sum is obtained by the sum of temperature during plant cycle, considering minimum basal temperature. Such parameter is relevant once basal temperature controls plant growth and development. It is important to highlight the influence of the degree days level on crambe productivity and development, mainly when sown in July, which present the lowest sum of temperature and lower values as compared to other sowings regarding all variables analyzed.

Throughout the study, there was large rainfall variability. With sowing in April, there were 468.9 mm of rainfall during crambe development; in other months, rainfall was much lower. In June, rainfall was 186.2 and 145.7 mm in July (Figure 1). As rainfall in April was the most elevated, there was a considerable incidence of diseases during that period. During the month of June, there was a total rainfall of 186.2 mm concentrated between sowing and flowering. According to Pitol et al. (2010), crambe demands 150 to 200 mm of water until its complete flowering.

During the third month of sowing, July, total rainfall was inferior to the minimum demanded by crambe. The concentration of rains occurred only during flowering and graining. Pitol et al. (2010) also state that after sowing, crambe needs at least 50 mm of water in order to develop and in this study, the rainfall that occurred during germination was lower than 10 mm, it might have contributed to the low results of the variables analyzed.

CONAB (2012) reports that Brazilian regions that obtained the best yields of vegetal species were the ones in which climatic conditions were favorable, however, regions that presented low rainfall levels had inferior yield in comparison with previous harvests. Another relevant factor presented in Table 2 shows the cycle difference with different sowing dates. The cycle started in April had 125 days, whereas the ones started in June and July had 103 and 78 days, respectively. Such variation must be highlighted to affect the planning of agricultural harvests.

In the area of Maracaju-MS, where temperatures are elevated, the plant presents a cycle of 90 days, according to Pitol et al. (2010). Pilau et al. (2011) in a study conducted in 2009 and 2010 observed different cycles ranging from 77 to 136 days. According to the authors, air temperature was responsible for the variation in the development cycle. Thus, cycles under higher average temperature showed decreased plant development time, whereas inferior average temperature values extended the cycle.

Sowing performed in April had the lowest average temperature (18.8°C) during the 125 days of development cycle. Sowing in July presented the highest average temperature (22.0°C) during the 78 days between crambe planting and harvest. These data match those obtained by Pilau et al. (2011).

In general, the assessed variables in the three sowing dates were affected by rainfall and degree days. In the first period, rainfall contributed to the high incidence of diseases. As for the sowing performed in July, the low sum of degree days and rainfall affected the results negatively. Elevated average temperatures contributed to earlier cycles.

Conclusion

Different sowing dates affected the phenometric variables assessed. Rainfall and sum of degree days were the factors that most influenced the results of different sowing dates. April is proven to be the best period for sowing.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Impact of the pressure plates of picker cotton platforms on the efficiency and quality of cotton fibers

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An experiment was performed in a cotton-producing property located in Primavera do Leste - MT - (Brazil). the quantitative losses and the presence of bark and stems in the cotton fiber quality measuring instrument as analyzed by High Volume Instrument (HVI) was noted . The study was conducted to investigate a combined speed of work with a mechanical adjustment of the distance of the pressure plates and the screws on the platform. Another objective of this study was to determine reducing quantitative and qualitative losses of cotton fiber. The platform was set at three different velocities (1.02, 1.38 and 1.60 m s^{-1}). Two different distances (plate with pressure – 3 mm and plate without pressure 6 mm were set as the distance between the spindles and the pressure plates. At these distances, the plants are pressed onto the spindle exerting pressure on the spindles. The collected fiber content of impurities and the efficiency of the machine were analyzed as a function of the total productivity and quantitative losses. Factorial statistical analysis was performed on the data using the Tukey test at 5% probability. A greater presence of impurities and lowest cotton crop losses was observed at the smallest distance between the spindles and the pressure plates.

Key words: Picker, cotton, fiber quality, harvest efficiency, losses.

INTRODUCTION

In the State of Mato Grosso, all cotton is harvested mechanically. In the 2013/2014 season, 61,310 ha from the 613,100 ha planted included systems with narrow lines less than 0.50 m (AMPA, 2015). With the exception of dense crops that are generally harvested with machine stripper type comb platforms, most of the State Mato Grosso cotton is harvested with a machine picker.

The main collection system used by cotton growers

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involves the use of rotary spindle platforms. This is also known as a picker and it functions in a manner similar to manual picking. The performance of the cotton picker with a picker platform can affect the quantity and quality of harvested cotton. Movement speed, variety, line spacing, plant conditions, defoliation, the plume humidity, and adjustments associated with harvesting units are among the factors that affect the harvest efficiency.

Baker et al. (2003) studied the effects of harvester pickers with respect to qualitative and quantitative losses. They found that the interaction between the machine speed and the cultivated variety was significant. They also noted that small adjustments in the collection of units and speed were highly effective in reducing losses and waste in the presence of cotton fibers.

Baker and Hughes (2008) evaluated the impact of changes in the length and diameter of the rotation axes and spindles on cotton losses, trash content, and quality of cotton fibers. They concluded that the rotation of the spindle should be at least 2000 rpm. They also indicated that the changes in length and shaft diameter did not significantly affect the amount of waste present in the samples.

During the harvest, losses should be monitored in order to detect and fix possible errors that may occur during the process. Significant investments in the agricultural production and its importance on the world stage require the reduction of losses in quantity and quality in order to contribute to increased farmer profitability (Mion et al., 2015).

The cotton harvest is a crucial moment for reducing quantitative losses. Hence, losses can be partially avoided or substantially minimized by taking certain precautions. These precautions include closer monitoring of working speeds, settings, plates adjustments; proper cleaning and maintenance of knowledge embedded electronics; regular maintenance intervals and the refilling of grease, water and detergent humidification systems.

The quality of cotton commercially available is influenced by several physical factors. These physical factors can be affected at each stage of production. This underscores the importance of the variety, environment, harvesting processes and processing of cotton plumes.

The cotton is classified based on the length and uniformity of fiber micronaire, color, and trash. The amount of waste present in the crop and its subsequent removal from the cotton may affect some properties such as fiber, degree of color, and trash. Additionally, it may heavily impact the spinning and textile manufacturing industry.

The cotton is then separated from other plant materials in the field or cotton with cleaning devices. When compared with manual harvesting, mechanical cotton harvesting reduces costs and harvesting time. However, mechanical harvesting generally decreases the cotton fiber quality, particularly in terms of increases in the level of NEP content and foreign matter in the fiber (Calhoun et al., 1996; Hughs et al., 2000; Baker and Brashears, 2000; Willcutt et al., 2002; Baker and Hughes, 2008; Faulkner et al., 2012). As noted by Funk et al. (2005), mechanically harvested cotton contained approximately 10 to 35% of foreign matter, including leaves, bark, sticks, stem, seeds and other debris.

The objective of this study was to determine the effects of the travel speed of the cotton picker and the pressure plate of the units on the qualitative and quantitative performance of the cotton fiber harvested in the State of Mato Grosso - Brazil.

MATERIALS AND METHODS

The study was conducted in an area of 0.81 ha, on a property in Primavera do Leste municipality – MT, Brazil. The variety of cotton sown was FM 944GL, with spacing of 0.90 m.

Cultural planting practices defoliation followed agronomic recommendations. The peeling occurred 20 days before the harvest. The machine used was a cotton blue 2805 model cotton harvester with picker platform brand Montana (Figure 1) with 4 units, and 16 bars with 20 spindles each, totaling 320 spindles per drum.

The experimental area consisted of 24 plots with an area of 108 m^2 (3.6 × 30 m). Prior to the passage of the harvester, productivity and the pre-harvest loss (action on climatic conditions and cultural practices) were estimated. For this, an area demarcated as 9.0 m^2 (5.0 × 1.9 m) within the sampling area was used. All the cotton present in plants and on the soil surface was manually collected.

Following the passage of the harvester in the plot, post-harvest losses were obtained by using a marquee on 9 m^2. These losses were related to the cotton that is retained in the stem of the culture after the passage of the machine and knocked to the ground by the action of the machine.

During the harvest, cotton lint was collected in the harvester basket in order to determine the impurities present in the cotton fiber. Approximately 3 kg of seed cotton was randomly collected within each portion and packed in raffia bags. All samples were identified and tied to prevent contamination at the time of storage.

Before sending the samples to the laboratory, a ginner with 20 saws was used to separate the fiber from the seeds. The middle portions of the samples were collected. Both the tops and the ends of the ginned cotton were discarded to avoid the increase in long and short fibers.

The experimental design was a randomized block design with four replications in a 2x3 factorial. Two distances of 6 mm and 3 mm were set between the pressure plates and the screws on the platform. Three harvest velocities of 1.02, 1.38, and 1.60 m s^{-1} were employed. The moisture of the fiber was instantly determined during the harvest period and was monitored with the aid of a portable measuring apparatus of Hygron mark. During the experiment, the moisture level was close to 7%, and it reduced during the experiment.

The collected materials were properly stored, identified and later sent to the laboratory to be analyzed and measured during the HVI analysis. Impurities constitute the materials that are not considered as production losses. These were manually separated from the fiber. The total losses were determined from the sum of the results obtained for the soil loss and the plant loss.

These impurities were separated into two categories (stem and bark) and the weights were noted to measure the amount of contaminants present on the fiber. Prior to manual separation of the impurities, the total sample was weighed. The weight of impurities in the fiber, and the weight of the total sample were obtained as a

(A) (B) (C)

Figure 1. Harvester (A) harvesting unit (B) and adjustment of the nip pressure plate (C).

percentage of branches and bark determined in relation to the total weight of the sample (Figure 1).

In determining the travel speed, a distance of 50 m was demarcated. The time taken to walk the distance was monitored. Three repetitions were performed resulting in the average length of machine traveled.

The efficiency of the harvester was determined in accordance with Rodriguez (1977) as per the following Equation (1):

$$\text{Picker efficiency} = \frac{100 \times \text{harvested cotton}}{\text{harvested cotton} + \text{post harvest losses}} \quad (1)$$

All results obtained in the experiment were subjected to analysis of variance by the F test. When significant, the Tukey test at 5% probability was performed using ASSISTAT software (Silva and Azevedo, 2014).

RESULTS AND DISCUSSION

The results presented in Table 1 demonstrate that irrespective of the working speed, the cotton harvested by a harvester without a pressure plate had lesser amount of cargo hulls. The shell contents without the pressure plates ranged from 2.01 to 2.29% and the shell contents with pressure plates ranged from 3.28 to 2.93%. These results were in agreement with the increased presence of impurities due to clamping plates noted by Belot and Vilela (2006) and Willcutt et al. (2006).

These results also indicated the presence of bark similar to that obtained by Faulkner et al. (2011) and Sui et al. (2010) with values ranging between 1 and 4% for harvesters using a picker system. Sui et al. (2010) state that the separation and the cleaning process of the cotton fibers needs to be more aggressive as increasing the peel can contribute to increasing the amount of short fibers in the samples.

The low pressure on the board and the increased speed decreased the amount of bark in the cotton fiber by approximately 29.39, 38.71 and 21.81% for the three velocities, respectively when compared to the values with the pressure plates. According to Bragg et al. (1995) increased bark concentrations did not significantly reduce the quality, but the number of yarn breaks during spinning increased by about 66% for each percent increase in bark content.

Table 1. Average barks (%) based on the pressure plates and the travel speed (m s^{-1}).

Pressure plates	Velocity (m.s^{-1})		
	1.02	**1.38**	**1.60**
Plates without pressure	2.21^{aA}	2.01^{aA}	2.29^{aA}
Plates with pressure	3.13^{bA}	3.28^{bA}	2.93^{bA}

The averages followed by the same letter did not show statistically significant differences for the Tukey test at 5% probability.

Table 2. Average stem (%) present in the basket of the combined samples with and without pressure plates as a function of the velocities (m s^{-1}).

Treatments	Average
Plates without pressure	0.51^{b}
Plates with pressure	0.65^{a}
Velocity (m.s^{-1})	**Average**
1.02	0.56
1.38	0.60
1.60	0.59

The averages followed by the same letter did not show statistically significant differences for the Tukey test at 5% probability.

Table 2 shows the percentage of stems present in the cotton fiber. This percentage demonstrates that the interaction between the pressure plates and travel speeds was not significant. The interaction demonstrated that the unfolding of the pressure plates contributed to increasing the amount of stems present in the cotton samples. An important function of the pressure plate is to

Table 3. The efficiency of the cotton harvester (%) with and without pressure on the plate and as a function of the velocities ($m.s^{-1}$).

Treatments	Average
Plates without pressure	89.18[b]
Plates with pressure	92.96[a]

Velocity ($m.s^{-1}$)	Average
1.02	90.90
1.38	91.41
1.60	90.89

The averages followed by the same letter do not show statistically significant differences for the Tukey test at 5% probability.

press the capsule cotton from the spindles. This contributes to the increased presence of branches in the cotton load.

The values ranged from 0.51 to 0.65% for the boards with pressure and without pressure, respectively. The plates with pressure contributed to a 21.5% increase in the amount of stalks in the harvested fiber. Table 3 shows the associated travel speed data for the presence of stem variable. It was observed that regardless of the displacement speed, there were no differences in the amount of cotton in the stem load.

The average values of the cotton picker efficiency shown in Table 3, demonstrate that the tightening of the pressure plates increased the harvest efficiency (92.96%) compared to the values with no pressure plates (89.18%). In this case, the increase in harvest efficiency was approximately 4.06%. These results were consistent with Belot and Vilela (2006) who concluded that the tightening of the pressure plates of the bodies of procurement units increased the harvesting efficiency.

It is important to note that the values found relating to the performance of the harvester are above those accepted by manufacturers that specify a 95% limit for the efficiency of platforms with rotating spindle units. Corroborating this, Öz et al. (2011) evaluated different spacing and varieties. They observed that losses in cotton harvesting should not exceed the 5% limit. Bassini (2014) reported that the collection efficiency varied with plant height, seed and lint moisture, density, and plant productivity. The author also commented that regardless of these variables, the harvesting efficiency should exceed 95%, in order to justify the high costs of cotton harvesting borne by farmers.

The results of cotton losses due to the variation in the speeds are shown in Table 3. The harvesting efficiency values are below those recommended by the machine manufacturers (98%). This demonstrates the need for the farmer to pay attention to the management of cultures that can influence the efficiency. Regardless of the speed, these values are close to those specified by Vieira et al. (2001) where ideal losses are cited as between 6 and 8%. However, we have to ensure that these losses do not exceed 5%.

Table 4. Average results of HVI elongation, strength, short fiber index, trash, uniformity, micronaire, reflectance, color and length.

Pressure plates	Velocity (ms^{-1})		
	1.02	1.38	1.60
Elongation (%)			
Plates without pressure	7.63	7.25	7.48
Plates with pressure	7.43	7.23	7.53
Strength (gf/tex)			
Plates without pressure	30.93	30.45	30.35
Plates with pressure	29.15	30.18	30.75
Short fiber index (%)			
Plates without pressure	8.65	7.53	7.83
Plates with pressure	9.03	8.45	8.30
Trash (%)			
Plates without pressure	6.75	7.50	7.00
Plates with pressure	6.50	5.75	7.00
Uniformity (%)			
Plates without pressure	81.28	82.35	81.78
Plates with pressure	81.03	81.56	81.75
Micron air (µg/in)			
Plates without pressure	4.04	4.05	3.91
Plates with pressure	4.07	4.06	4.09
Reflectance			
Plates without pressure	73.08	72.30	73.60
Plates with pressure	71.68	72.83	71.93
+b			
Plates without pressure	8.35	7.95	8.38
Plates with pressure	8.33	8.53	8.28
Length (mm)			
Plates without pressure	29.08	29.27	28.26
Plates with pressure	28.45	29.08	29.45

The averages followed by the same letter do not show statistically significant differences for the Tukey test at 5% probability. *plates without pressure – (distance 6 mm). **plates with pressure – (distance 3 mm).

For the analysis of fiber quality for each treatment, the average of HVI results are shown in Table 4. It can be observed that the variation of the pressure plate velocities and the interaction between them was not statistically significant for all the variables. However, as this factor can vary based on the year, varieties, harvest season, defoliation, type harvester, and environment, it should be noted that these differences may be noticed in years with adverse conditions. This was noted by Kerby et al. (1986) by using different harvesting systems.

Willcutt et al. (2002) also analyzed several factors inherent in the presence of trash and samples. In a similar vein, Faulkner et al. (2008) compared three different crop systems.

Bragg et al. (1995) found that elevated concentrations peels did not significantly reduce the quality of the yarn produced with an early spinning rotor structure. However, they find that the number of yarn breaks during spinning increased to approximately 66% for each percent increase in bark content.

Conclusion

The increased distance between the spindles and the pressure plates reduced the quantity of the shells during independent cotton harvest picking speed. Thus, it was important to reduce the aggression fibers at the time of ginning, and to reduce the use of electricity to remove the impurities in the cotton. The distance between the spindles and lower plates reduced the amount of stems in the cotton. The shortest distance between the spindles and the plates exerted a higher pressure. This is because there was a reduction in the area that the cotton mass must flow to. Hence, the losses due to the contact between the spindle and the boll were lower. The quality of the fibers was not affected by the change in velocities and the distance between the spindles and the plates.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Biomass and nutrition stock of grassland and accumulated litter in a silvopastoral system with Cerrado species

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The crop-livestock-forest integration systems are a form of sustainable production, creating a consortium between the cultivation of crops and forest production and the creation of pastures for livestock breeding, seeking a synergy between the system components. Therefore, integrating agricultural crops with tree species provides countless benefits to the components, such as the maintenance and increase in nutrient cycling. Therefore, the objective of this study was to evaluate the biomass and nutritional stock of Brachiaria pasture and accumulated litter in a silvopastoral system with tree species of the Cerrado. Three silvopastoral systems, formed with the forage species *Urochloa decumbens* (Brachiaria) integrated with three tree species, namely *Dipteryx alata* (baru tree), *Caryocar brasiliense* (pequi) and *Eugenia dysenterica* (cagaita), were evaluated. Litter and pasture were collected in each system at different sample distances from the tree. The amount of biomass and the nutritional contents of both components were evaluated by chemical analysis. The largest amount Brachiaria pasture biomass and largest amount of litter was found in the silvopastoral system with baru trees. The pasture differed nutritionally. The pastures associated with baru trees and to pequis have higher amounts of macronutrients. There are nutritional differences with respect to the sampling positions.

Key words: Brachiaria, Baru, Pequi, Cagaita, CLFi, Cerrado, Brazil.

INTRODUCTION

The expansion of agriculture has contributed to the growth of Brazil as a supplier of agricultural products (Townsend et al., 2009). However, the unrestrained expansion of agriculture has caused profound changes in natural

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resources, in addition to changes in natural vegetation and soil properties (Lago et al., 2012; Santana et al., 2016). The aggressiveness of agriculture can be controlled in the way the activities are practiced and in how the environment is managed. Within this context, the crop-livestock-forest integration (CLFi) or agro-sylvopastoral system, is a sustainable production strategy that integrates forestry, agricultural and livestock activities, in the same area, either in intercropping, succession or rotation (Machado et al., 2011; Lana et al., 2016).

Integrated systems can be classified into four types (Balbino et al., 2011): crop-livestock-forest integration (CLFi/agrosilvopastoral), crop-livestock integration (CLi/agropastoral), livestock-forest integration (LFi/silvopastoral) and crop-forest integration (CFi). Soil fertility improvement, increased carbon sequestration and the supply of forage and more favorable environmental conditions for animals are highlighted amongst the benefits (Costa et al., 2002; Abreu et al., 2016). The tree component in agroforestry systems helps maintain nutrient cycling (Montagnini, 1992; Upson et al., 2016) by decomposing litter. Decomposition of litter is considered the primary means of transfer of nutrients to the soil, enabling its reabsorption by the living plant (Schumacher et al., 2004; Santana et al., 2016). The shade of trees and the presence of litter reduce the high soil temperatures and the trees also attenuate wind speed (Monteith et al., 1991; Ong et al., 1991; Assis et al., 2015; Brito et al., 2015). These two factors affect evaporation rate, soil water balance and evapotranspiration, as it increases the humidity available to plants, influencing the yield of agricultural crops and pastures (Duboc, 2007; Lemos-Junior et al., 2016).

Some LFi studies have shown native or exotic species being used in association with animals, as well as reviews of agronomic crops (Lemos-Junior et al., 2016). Duboc (2005) stated that to which extent, there is greater disclosure and encouragement for using native Cerrado species in an interaction system, an increase in the interest in the rational cultivation of these species may occur. At this juncture and with the need for a production that is increasingly sustainable and compatible with livestock and forestry production, the objective of this study was to evaluate biomass and nutritional stock of Brachiaria pasture and litter in silvopastoral system with tree native species of Cerrado, in Brazil.

MATERIALS AND METHODS

Study area

This study was carried out at the experimental area of the School of Agronomy, of the Federal University of Goias (Universidade Federal de Goiás; SA/UFG), at Goiânia, Goiás. In the area, the soil is a red oxisol and the plantations near one another. According to Köppen, the climate is predominantly tropical (Aw), with the marked division of two well defined seasons during the year: humid summer, from December to March, and dry prevailing winter from June to August. The average temperature varies between 18 and 26°C, as the average annual rainfall is about 1300 -1700 mm, concentrating in the months from October to March (in the spring and summer seasons). Between May and September is the dry season, a period when the rains are rare and may occur drought. Between the months of July to August, the humidity drops too (dry weather) and may be between 15 and 30%. Three silvopastoral systems formed with the forage species *Urochloa decumbens* (Stapf) R.D.Webster (Brachiaria) integrated with three tree species, *Dipteryx alata* Vog. (Baru tree), *Caryocar brasiliense* Camb. (Pequi) and *Eugenia dysenterica* D.C (Cagaita) were evaluated. Each system has a total area of 7590, 5725 and 15525 m^2, respectively. The plantations were carried out in January 1998, spaced 6 x 6 m, and the plants come from a germplasm collection of the SA/UFG (Access to the map of the Germplasm bank).

Samples processing

Litter and above-ground biomass were collected following an experimental design of randomized blocks with six replicates, for each system and the sampling distances from the tree were recorded. A total of five above-ground biomass and three litter samples were collected, where each block was located 50 m from each other. All the material was stored in properly labeled Kraft paper bags.

The methodology adapted from Freitas et al. (2013) was used to evaluate the productivity of pasture and litter, relative to the distance from the tree. The Brachiaria data was collected in the center of a sampling quadrant (Figure 1), a meter on the right and left of the trees, and between right and left plants, using a template of 0.50 x 0.50 m (0.25 m^2), removing the superficial biomass with pruning shears. The litter data was collected in the center, and between right and left plants, after the pasture was collected (Figure 1).

Litter was collected following the methodology determined by Lima et al. (2015), using a template of 0.25 x 0.25 cm (0.0625 m^2), in the same place in which the above-ground was harvested, removing all the material until the soil was exposed. Roots still attached to the soil were avoided. Each sample was packed into labeled paper bags and taken to the Forest Ecology Laboratory.

The above-ground and litter of Brachiaria samples were dried in the laboratory, in a circulating and air renewal chamber at 70°C for approximately 72 h until reaching a constant weight. The final weighing of the Brachiaria samples were carried out individually. In addition, the total weight of each Brachiaria sample was obtained, and then each sample was divided into leaves and mixture (root, flowers and seeds). Each fraction was weighed once again.

The nutrition stock was evaluated using two samples for each tree per component (above-ground biomass and litter) in each different position. Three of the samples collected were mixed, and two sub-samples were made from the mixture, that is, two composite samples. At the end, a total of six samples of pasture and four samples of litter were analyzed per system of tree species, adding up to 30 samples.

The material of the composite samples was milled (Lippel crusher and in a Wiley type mill), and finally sieved in a sieve with mesh opening of 1.0 mm (20 mesh). Then, the material was directed to a laboratory for chemical analyses to determine micronutrient and macronutrient quantities in the plant tissue, following the methodology described by Tedesco et al. (1995) and Miyazawa et al. (1999).

Statistical analyses

A t-test was used to compare the three silvopastoral systems with

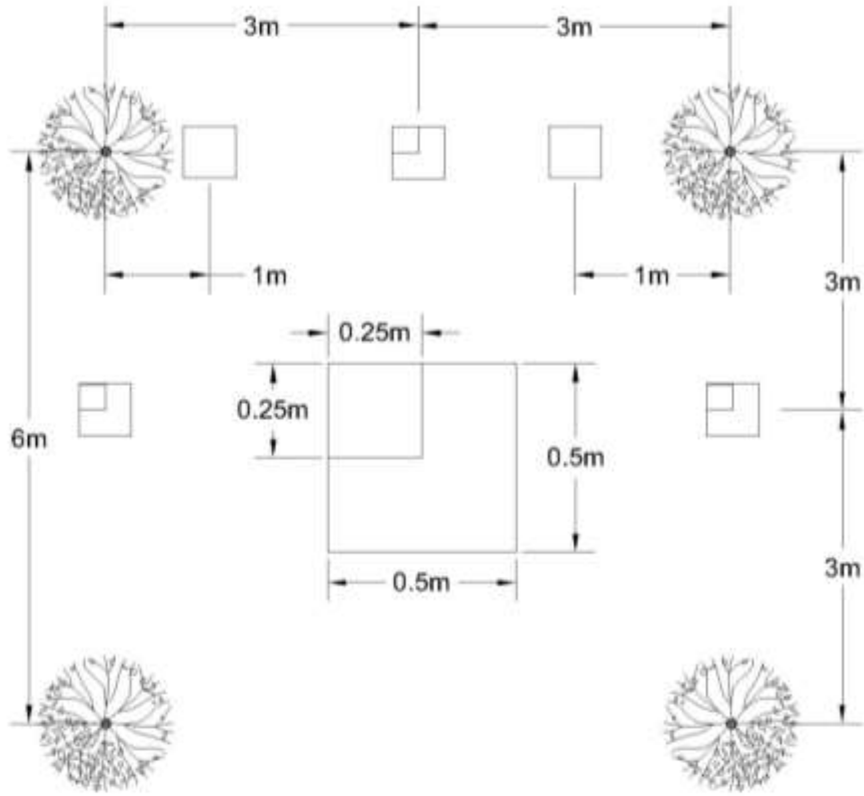

Figure 1. Sketch of the methodology used to sample above-ground biomass and litter in the experimental area of the School of Agronomy, of the Federal university of Goiás (Universidade Federal de Goiás, SA/UFG), Goiânia, Goiás. In the figure are represented trees with spacing and sampling locations.

regards to above-ground biomass and litter of Brachiaria. A Tukey test was used for a multiple comparison among treatments. A regression analysis was carried out between the variables to assess the relationship between distance and biomass.

A principal components analysis (PCA) was carried out with a covariance matrix to assess the nutritional contents in pasture and litter, and for the following parameters nutrition stock with regards to the different tree species: nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), cobalt (Co) and molybdenum (Mo) (Monteiro et al., 2016). The different sampling locations (between lines, between plants and one meter from the plant) were also considered in the analyses (ANOVA two way). All analyses were conducted considering a significance level of 95%.

RESULTS

The highest Brachiaria above-ground biomass was observed in the silvopastoral system with Baru trees (2.23 ton/ha; F=8.51; p=0.001) (Figure 2A). Above-ground biomass in this system was approximately 23% higher than in Cagaita system (1.81 ton/ha) and 32% higher than in Pequi system (1.69 ton/ha) (Figure 2A). Above-ground Brachiaria biomass within the systems with Cagaita and Pequi trees did not differ statistically.
The Baru system had a higher above-ground biomass in the center of the sampling area, between the tree lines (F=2.68; p=0.03). Still, above-ground biomass of Brachiaria in the Baru system was similar to that observed for the other systems in the position within the same row and 1 m from the tree (Figure 2B). Above-ground of Brachiaria was similar in all sampling distances for the Cagaita and Pequi systems.

The Baru system had a higher amount of litter biomass (7.67 ton/ha), of about 66% more litter than the area with Cagaita trees (4.63 ton/ha) and 88% more than the area with Pequi trees (4.07 ton/ha) (F= 675.46; p=0.000; Figure 3). There were no significant differences between the system with Cagaita and Pequi trees (Figure 3). In addition, there was no difference in the production of accumulated litter between areas at the center, between tree lines or between plants for either system with the different tree species.

The pasture showed significant differences in its nutritional balance in the presence of the tree species, as well as among sampling positions (Table 1). The stocks of N had a significant difference in the central sampling

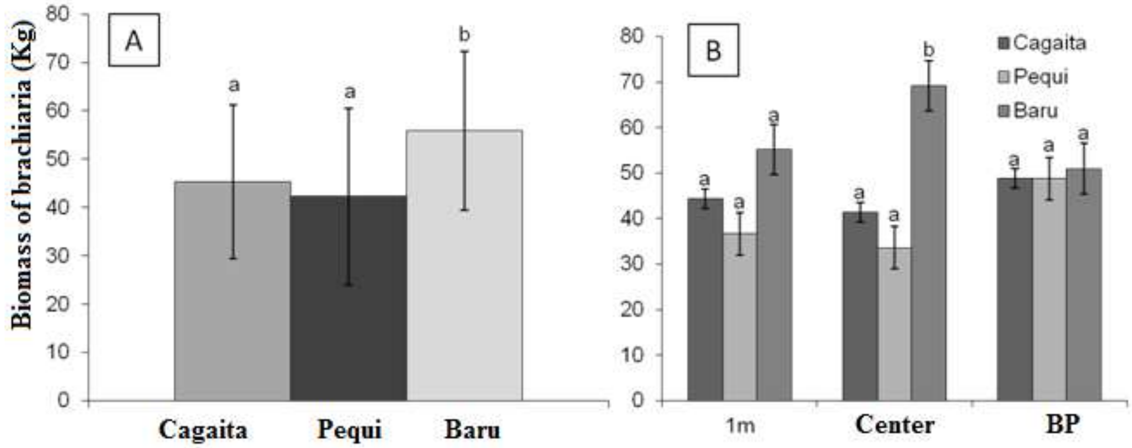

Figure 2. A. Average biomass of Brachiaria (*U. decumbens*) in the silvopastoral systems cultivated with three different tree species (Cagaita, Pequi and Baru); B. Biomass of *Brachiaria* at different distances in the system (1 m from the tree, at the center between the tree lines and between plants - BP), measured in kg according to the sampling square.

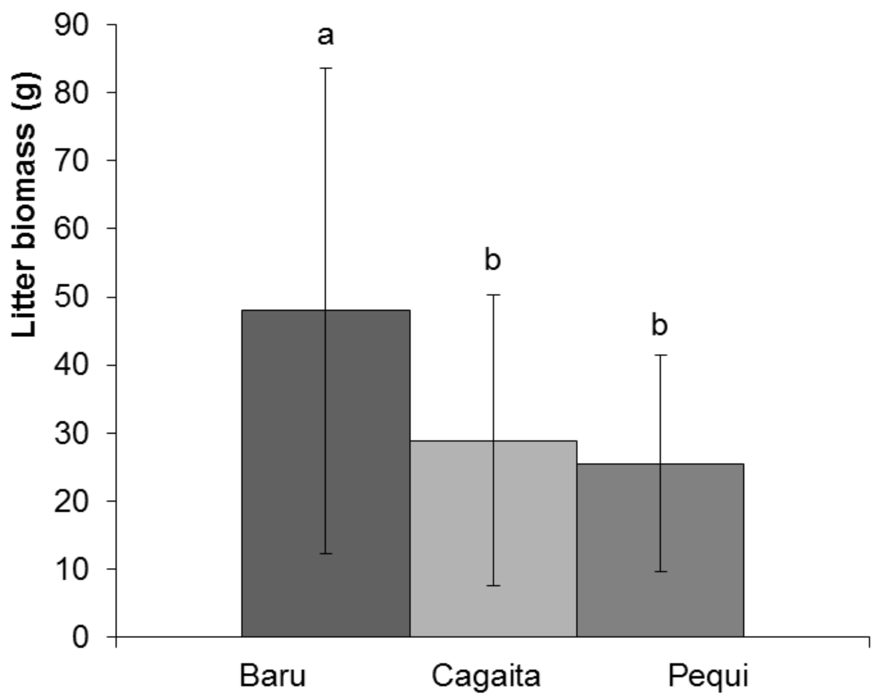

Figure 3. Litter biomass accumulated between silvopastoral systems using three different tree species (Cagaita, Pequi and Baru) (measure in grams regarding sampling).

position associated with the cagaita tree. The Brachiaria had 12% more N when associated with the Baru tree and 8% when associated with the pequi tree. Nitrogen stocks were also significantly affected when the Brachiaria was collected between plants in the system integrated with the baru tree. Therefore, pasture integrated with Baru trees exhibited a 13% increase in N when analyzed with regards to the same sampling position for Cagaita trees.

The stocks of K in the Brachiaria were statistically different when samples were taken from the position of 1 m in the cagaita system. Despite being significant, K stocks were 10% smaller in a same sampling position for

Table 1. Nutritional stock in *Brachiaria* grass (*U. decumbens*) and litter in integrated pasture systems at different distances in the system (1 m from the tree, at the center between the tree lines and between plants - BP) with native trees in Goiânia, Goiás. Different letters indicate statistical difference in a multiple comparison Tukey test with 95% significance between different positions within each species.

	Species	Distance	N	P	K	Ca	Mg	S	Na	Cu	Fe	Mn	Zn	Co	Mo
			$g.kg^{-1}$						$mg.kg^{-1}$						
Pasture	Pequi	1m	14.10^{a}	1.60^{a}	20.30^{a}	3.45^{a}	3.15^{a}	1.20^{a}	92.00^{a}	8.50^{a}	217.50^{a}	137.00^{a}	27.50^{a}	0.12^{a}	0.53^{a}
		BP	14.20^{a}	1.55^{a}	20.50^{a}	3.10^{a}	2.95^{a}	1.05^{a}	92.50^{a}	10.50^{a}	140.50^{b}	139.00^{a}	25.50^{a}	0.13^{a}	0.51^{a}
		Center	13.85^{a}	1.45^{a}	22.60^{a}	2.70^{b}	2.25^{b}	1.10^{a}	98.50^{a}	12.00^{a}	143.00^{b}	113.00^{a}	24.50^{a}	0.11^{a}	0.48^{a}
	Cagaita	Center	15.00^{a}	1.45^{a}	21.85^{a}	2.80^{a}	2.65^{a}	1.30^{a}	112.50^{a}	10.00^{a}	179.50^{a}	94.50^{a}	26.50^{a}	0.13^{a}	0.52^{a}
		BP	12.50^{b}	1.45^{a}	20.80^{a}	3.35^{a}	3.25^{a}	1.35^{a}	106.00^{b}	12.50^{a}	122.50^{a}	98.00^{a}	30.00^{a}	0.13^{a}	0.59^{a}
		1m	13.25^{b}	1.35^{a}	18.40^{b}	3.75^{a}	3.30^{a}	1.20^{a}	97.50^{a}	23.00^{b}	251.50^{b}	56.50^{b}	27.50^{a}	0.13^{a}	0.58^{a}
	Baru	Center	13.35^{a}	1.55^{a}	21.60^{a}	2.70^{a}	1.85^{a}	1.10^{a}	96.50^{a}	9.00^{a}	250.00^{a}	86.00^{a}	27.00^{a}	0.11^{a}	0.48^{a}
		1m	13.10^{a}	1.60^{a}	19.20^{a}	2.90^{a}	2.30^{a}	1.05^{a}	104.00^{b}	12.00^{a}	273.00^{a}	101.50^{a}	29.50^{a}	0.10^{a}	0.44^{a}
		BP	14.20^{b}	1.40^{a}	20.20^{a}	3.00^{a}	2.10^{a}	1.00^{a}	98.00^{a}	8.00^{a}	290.00^{a}	90.00^{a}	27.00^{a}	0.10^{a}	0.45^{a}
Litter	Pequi	Center	6.60^{a}	0.95^{a}	3.00^{a}	4.45^{a}	1.20^{a}	0.95^{a}	87.50^{a}	11.50^{a}	2480.00^{a}	126.50^{a}	33.50^{a}	0.10^{a}	0.55^{a}
		BP	8.60^{b}	1.05^{a}	2.60^{a}	6.30^{a}	1.25^{a}	1.15^{a}	90.50^{a}	10.50^{a}	2225.00^{a}	153.50^{a}	28.50^{a}	0.11^{a}	0.53^{a}
	Cagaita	Center	14.10^{a}	1.05^{a}	2.60^{a}	15.60^{a}	1.55^{a}	0.95^{a}	89.00^{a}	8.50^{a}	2800.00^{a}	138.00^{a}	22.00^{a}	0.09^{a}	0.50^{a}
		BP	12.45^{b}	1.10^{a}	2.30^{a}	15.95^{a}	1.70^{a}	0.90^{a}	92.00^{a}	7.50^{a}	2315.00^{b}	157.00^{a}	23.00^{a}	0.09^{a}	0.48^{a}
	Baru	Center	13.60^{a}	1.15^{a}	3.60^{a}	9.90^{a}	1.45^{a}	0.95^{a}	92.00^{a}	8.00^{a}	1523.50^{a}	118.00^{a}	26.50^{a}	0.09^{a}	0.43^{a}
		BP	15.50^{b}	1.15^{a}	3.75^{a}	10.85^{a}	1.90^{a}	1.30^{b}	110.50^{b}	9.50^{a}	5360.00^{b}	162.50^{a}	31.50^{a}	0.14^{a}	0.56^{a}

systems with the pequi and 4% smaller when for systems with Baru trees. Ca stocks in the pasture also exhibited significant differences in the central sampling position associated with the Pequi, and were 3% smaller in the Cagaita system. The amount of the element Mg in Brachiaria differed among systems in the central position of sampling, was 21% higher for the pequi than for the Baru tree and 17% lower for the Brachiaria in the Cagaita system, for the same sampling position. The Na stock in the pasture had significant differences for the “1 m” collection point, where it was 6% higher for Baru systems than for the cagaita system and 13% higher than the Pequi system. Differences in Na stock were also observed for the position “between plants”, where the pasture from the cagaita system had 14% more Na than the pequi and 8% more than the baru system.

The amount of Cu differed significantly for the “1 m” collection point, where 170% more Cu was recorded in the pasture for the Cagaita than in the pequi system and 91% more than in the Baru. The amount of Fe in the pasture differed between sampling positions, being greater at 1 m away from the plant for the Pequi, Baru and Cagaita systems. However, the Cagaita system had 15% more Fe than the Pequi system. The element Mn was also recorded in higher amounts for samples collected 1 m from the Cagaita trees, and was 142% lower for the Pequi trees and 79% lower for the Baru trees.

The nutrition stocks of the litter also showed some distinctions. The element N had a significant difference with regards to the collection position for the three native species. The litter of Baru trees had 3% more N at the central sampling position and 136% more N than the Pequi. The litter of Baru trees had 44% more S than when associated with Cagaita trees and 13% more than when associated with Pequi, for samples collected between plants.

The Na stocks in the litter of Baru systems was 20% higher than the recorded for systems with Cagaita and 22% more than for pequi, for samples collected between plants. The litter of the system integrated with Baru trees had 3% more Na in the central sampling area than the Cagaita

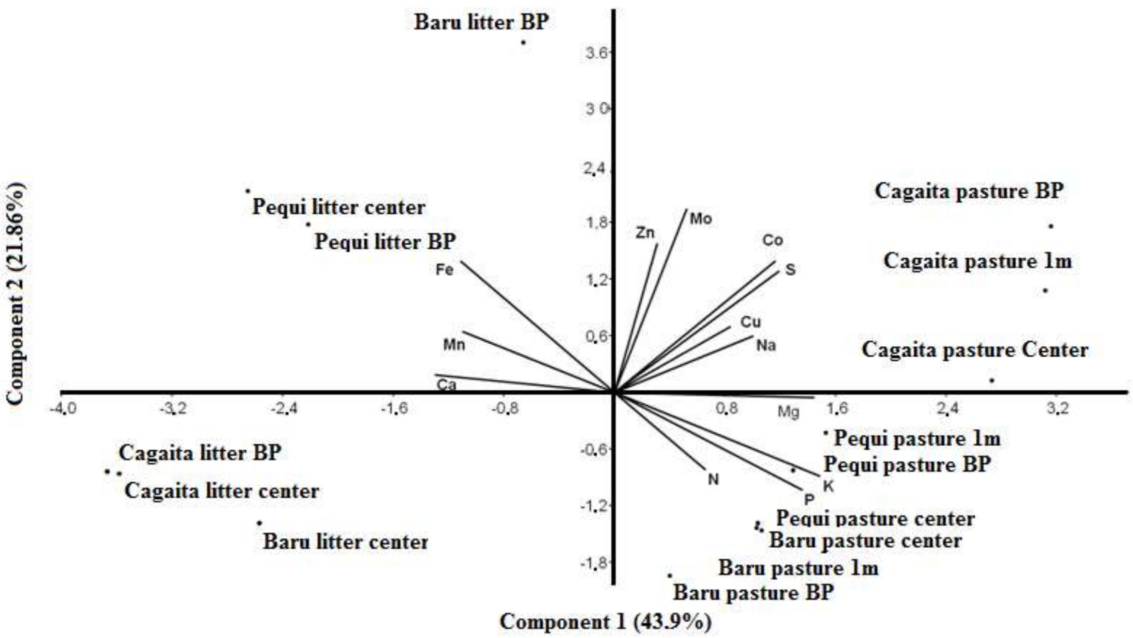

Figure 4. Principal component analysis of the integration systems with different tree species (Pequi, Baru and Cagaita) evaluating the nutritional stocks of *Brachiaria* grass (*U. decunbens*) and litter (Component 1: nitrogen comprising 43.9% of the variance and Component 2: phosphorous comprising 21.86% of the variance).

system and 5% more than the system integrated with Pequi. The litter of Baru systems had 83% more Fe than the Cagaita system, and 12% more than the litter of the Pequi system, in the central sampling area. The stock of Fe in the litter of the baru system was 131% higher than the stock recorded for the litter of the Cagaita system and 140% more than the observed for the Pequi system in the sampling position “between plants”.

The elements recorded in larger quantities in the litter were Ca, Fe and Mn. In addition, these elements were directly and positively related, where the higher the calcium, the higher the iron and manganese contents. On the other hand, the relationship observed for these three elements opposes that observed for N, P and K. The elements N, P and K occur in lower quantities in the litter and larger amounts were observed in the pastures integrated with the three native Cerrado species, especially in those integrated with baru and pequi trees (Figure 4). The pastures integrated with Baru and Pequi trees have higher amounts of macronutrients with regards to the nutritional characteristic of the grass. Other mineral elements such as Zn, Mo, Co, S, Cu and Na were recorded in higher concentrations in pastures associated with Cagaita trees, and were all positively related with each other.

DISCUSSION

The largest amount of Brachiaria pasture biomass and largest amount of litter was found in the silvopastoral system with Baru trees. The pasture differed nutritionally, being that pastures associated with Baru trees and Pequi have higher amounts of macronutrients. The livestock farming forest integration system seems to be an efficient system in agroforestry production and in improving the quality of pastures of the Cerrado. The use of trees native to the biome in the pastures make it more similar to natural areas of Cerrado *sensu strictu* and “campo sujo”, in addition to the benefits also inherent in the natural areas such as increasing organic matter deposition, nutrient cycling and improving soil cycling and microclimate characteristics.

The tree canopies break down wind and change the microclimate of the understory, consequently reducing the evaporation rate of herbaceous plants (Castro et al., 2008). The areas beneath the canopy and litter production retain soil humidity in dry periods, improving the production of grass. Shading caused by trees may impair the productivity of grasses, due to competition for light, in addition to the natural competition for water and nutrients. Therefore, spacing is essential for a good development of the tree-grass system, reducing the competition imposed by the shadows of the canopies (Galzerano and Morgado, 2008; Venturoli et al., 2015). The spacing for planting vary depending on the species and are important for a good soil cover (Gonçalves et al., 2015). The most commonly used spacing range from 4 to 16 m between lines and 50 cm to 2 m between plants (within the lines) (Gonçalves et al., 2015).

Sousa et al (2007) studied the productivity of *Brachiaria brizantha* cv. Marandu in a silvopastoral system and noted that despite dry matter (DM) reduced due to shadowing, this reduction is associated with larger Brachiaria leaves instead of stems. They also reported an increase in crude protein content in relation to the Brachiaria grown under full sun.

Andrade et al. (2003), assessed the development of six forage grasses (*B. brizantha* cv. Marandu, *B. brizantha* cv. MG-4, *B. decumbens* cv. Basilisk, *Panicum maximum* cv. Mombaça, *Melinis minutiflora* and *Hyparrhenia rufa*) in a silvopastoral system in Paracatu, Minas Gerais, a Cerrado region, associated or not with the leguminous plants *Stylosanthes guianensis* cv. Mineirão and *Eucalyptus* sp. Grasses were established between Eucalyptus lines, in plots of 12 x 10 m, and the highest biomass production capacity was recorded for the species *B. brizantha* cv. Marandu and *B. decumbens.*

The production litter by falling leaves, twigs, flowers and fruits contribute to the transfer of nutrients from the canopy to the ground, and the leaves account for approximately 60% of this transfer (Poggiani, 2012). The tree species used in this forest and livestock integration system are semi-deciduous, and produce leaves from July to September (Silva-Júnior, 2005). The plants partially lose their leaves at the same time of year, increasing the deposit of nutrients in litter and soil (Caldeira et al., 2007). The low concentration of potassium (K) in the litter may be related to the high mobility of the K within plants, which transfer the element from senescent leaves for younger parts. Potassium is also movable in the soil, so much of it is lost by leaching, especially in the rainy season (Caldeira et al., 2007).

Calcium (Ca) showed higher values in the litter because it is immobilized in the leaves, and is rarely redistributed to other parts of the plant when it is in senescence. Calcium is a movable element in soil, which causes a loss of Ca by erosion and leaching to deeper layers of the soil. The roots of the trees are pivotable, and so reach greater soil depths, facilitating the reabsorption of the elements that were leached. Grasses have fasciculated roots, therefore occupy mostly the surface of the soil (Vitti, et al., 2006; Silva-Neto et al., 2015).

Micronutrient levels in the litter followed a descending order of Fe, Mn, Zn, Cu. The same result was reported in a research conducted by Caldeira et al. (2007), studying the production of accumulated litter and nutrient contents in a Mixed Rain Forest, at General Carneiro, state of Paraná. Iron (Fe) is considered an immobile element in the plant. Therefore, iron deficiency symptoms are observed faster than is the deficiency of other mobile elements. Because of the limited redistribution in the plant, it is possible to find young leaves with Fe deficiency while old leaves and roots have accumulated Fe (Dechen and Nachtigall, 2006). The high iron content found in the litter may be associated with the high contents found in some species or the contamination of litter samples with soil (Caldeira et al., 2007).

Oxides and sulfides (Mn) are the forms of manganese found most frequently in the soil, and are commonly found associated with Fe. The Mn concentration varies between plant parts and growth period. Older leaves accumulate higher concentrations of manganese and a small portion is translocated to young leaves (Dechen and Nachtigall, 2006). Thus, the accumulated litter showed higher amounts of manganese due to the accumulation in the leaves.

Nitrogen (N) is one of the elements found in larger quantities in the leaves due to their participation in the metabolic reactions of the plant, especially photosynthesis (Malavolta, 1985). Phosphorus (P) is one of the primary macronutrients, along with N and K. Phosphorus is crucial for the process of photosynthesis. Still, it is not required in large amounts in plant, leading to the low values recorded. In addition, the need of the element decreases when the plant is already established, that is, adult (Raij, 1991)

Pastures grown under the baru and pequi trees showed better nutritional aspects with respect to NPK. Oliveira et al. (2005) obtained similar results and state that the increased levels of these minerals must be directly connected to the greater accumulation of organic matter in soil arising from the litter deposition under the canopies.

Trees exploit nutrients found in deep soil layers through the range of their root system. However, forage species only access the most superficial nutrients. Therefore, trees assist in the gradual incorporation of nutrients into the soil/pasture system by depositing biomass onto the soil (Nair, 1999). Carvalho and Xavier (1999) also found higher mineral contents in soils under *B. decumbens* grassland that were intercropped with baru and pequi trees. The trees in silvopastoral systems affect the development of the herbaceous forage increasing soil nutrient and changing the microclimate under the canopies (Carvalho and Xavier, 1999).

Cavalcante et al. (2007) evaluated the spatial variability of MO, P, K and CTC under different managements, recording higher values in Cerrado and no-till areas and a reduction in organic matter contents in the other cultivated areas. This fact can be explained by litter accumulation in the larger forest than in other systems. In addition, P, K and CTC values were higher in the no-till and conventional farming systems. The P and K levels were higher in surface, decreasing the deeper layers.

The benefits of the integrated system in the Cerrado directly improves productivity and quality of the pastures, and enables the production of alternative products, such as fruits of the Cerrado and even wood from sustainable plantations as the CLFi (Abreu et al., 2016, in press). The Cerrado has a richness of native trees with potential of being used in commercial production and in the integrated systems. However, the lack of studies on the behavior of these tree species, forages and crops in a mixed system is a limiting factor.

Conclusion

Systems integrated with Baru trees had higher productivity, that is, higher biomass, for both Brachiaria pasture and litter production in this study. The production of biomass and litter was higher in line among the trees.

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Full Length Research Paper

Genetic diversity in sweet cassava from the Brazilian Middle North Region and selection of genotypes based on morpho-agronomical descriptors

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This work had as objective the morpho-agronomic characterization of germplasm of cassava (*Manhiot esculenta* Crantz) from the Brazilian Region Middle North and the selection of genotypes. The germplasm collection was carried out in autochthonous fields. 10 genotypes of which, 8 were ethno-varieties and 2 modern varieties (BRS Dourada and BRS Gema de Ovo), were collected. A completely randomized blocks design was used and the experiment was carried out in January of 2013 in Chapadinha-MA, Brazil. The morphological and agronomical characterizations were carried out at eight months after planting and at the harvesting time, respectively. The frequency of genotypes in each descriptor class and the entropy level for each descriptor were calculated. There was high phenotypic difference in the germplasm for most of the characters evaluated. Lowest entropy levels corresponded to the descriptors sinuosity of foliar lobe, margins of stipules, pubescence of apical bud, growing habit and color of stem epidermis. Highest entropy and consequently higher variability were noticed in petiole color, color of stem cortex, plant kind and peduncle presence. The genotypes Rampa and Turiaçu have higher productive and marketing value, thus are excellent alternatives for adoption and cultivation in the Middle North Region. The work proved to be a prominent approach in the screening and selection of promising and contrasting cassava genotypes. Cassava genetic resources occurring in the Brazilian Middle North Region represent a valuable resource in breeding programs of this crop.

Key words: Characterization, genotypes selection, *M. esculenta*.

INTRODUCTION

Cassava (*Manhiot esculenta* Crantz) is an important source of carbohydrate for over 700 million people worldwide, mainly in developing countries of tropical regions. This crop presents a wide genetic diversity (Lebot

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et al., 2015; Siqueira et al., 2009). Although cassava has been propagated mainly asexually since its domestication, it has kept its ability to reproduce sexually, therefore enhancing its genetic constitution (Elias et al., 2001). The genetic diversity of cassava germplasm results mainly from its reproductive characteristics, besides a significant anthropological contribution. All of these factors isolated or in association, have enabled this crop to maintain a wide genetic base.

Cassava genetic diversity is closely associated to its reproductive characteristics. This crop presents high cross-pollination (Ly et al., 2013), abrupt dehiscence of fruits (Souza et al., 2006) and high heterozygosis (Chavarriaga-Aguirre, et al., 1998; Duputié et al., 2007). Associated to these aspects, a fragile reproductive barriers of isolation between this crop and its wild congeners (Blaer et al., 2007; Bredeson et al., 2016; Nassar et al., 1995), allow the interspecific hybridization between cassava and wild species of *Manhiot* such as *Manihot glaziovii* (Nichols, 1947), *Manihot neusann* Nassar (Nassar et al., 1995), enhancing the genetic variability in this genus. In nature, there are diverse interspecific hybrids of cassava (Second et al., 1997). These individuals occur especially in the Brazilian biome "*Caatinga*'' place of higher occurrence of the species, consisting of valuable resources in the transference of desirable genes from wild to cultivated species.

Anthropological aspects have significantly altered the genetic makeup of cassava germplasm. This crop has been cultivated for more than 5000 years since its domestication in South America (Allen, 2002). Indigenous peoples have altered the species gene flow in the region since its domestication, by selecting and keeping desirable individuals from determined population. Nowadays, this crop is still the feeding basis for most of the remaining indigenous populations and traditional farmers in Brazil (Silva and Murrieta, 2014; Valle and Lorenzi, 2014). Intrinsic traits regarding this crop such as cultural aspects of peoples involved on its cultivation, as well as peculiarities on its agricultural management have enabled the keeping of high levels of genetic diversity into cassava genetic resources. These resources are mostly landraces and represent a diverse and valuable genetic resource for the species as a source of desirable genes in the genetic improvement of this crop.

Cassava germplasm found in the Brazilian Middle North Region represents genetic resources especially valuable for this crop. This region is known for an overlapping of biomes, which has a pronounced effect on the genetic resources occurring in it. These resources present considerable phenotypic plasticity and homeostasis, which makes them possess great adaptation ability (Moura, 2013). Additionally, cassava present remarkable resilience in terms of adaptation to poor soils conditions, a predominated condition of the Brazilian soils, especially in the Middle North Region. Even though cassava indexes of production in this region is still low, much lower than the Brazilian average (IBGE, 2010). The crop regional lower performance stems from the lack of proper varieties developed especially for the region as well as from the adoption of no-adopted cultivars. This points out for the need of a study program in the screening and development of more productive genotypes and incentive in the adoption of such cultivars in order to strengthen regional cassava production.

The characterization of plant germplasm based on morphological descriptors is a preliminary step in breeding programs (Rêgo et al., 2011; Upadhyaya et al., 2008), and it has a paramount importance in the genetic improvement of cassava (Fukuda et al., 2002). This tool provides accurate information about the germplasm, which increases the efficiency of its use and exchange, as well as in the determination of genetic diversity (Araújo et al., 2002). Additional advantages related to its application, consist of its lower cost compared to molecular characterization and ease of analysis.

The use of multicategoric data such as morphological descriptors allows a practical, economic and rapid assessment of the genetic variability in cassava germplasm. This tool is more suitable for regions with low levels of technology, allowing the economic assessment of polymorphism of plant germplasm. This tool should be associated with agronomical information, targeting the discrimination of accessions, particularly as to the attributes of economic importance. Morpho-agronomic descriptors has been widely used in the characterization of cassava and it is indispensable in the determination of strategies of on-farm conservation of germplasm, management of gene banks, selection of desirable genotypes and protection of cultivars (Elias et al., 2001; Mamba-Mbayi et al., 2014; Nick et al., 2008; Oliveira et al., 2015; Rimoldi et al., 2010). Despite its importance, the use of morpho-agronomic has been limited in the characterization of sweet cassava germplasm; therefore, the aim of this work was to study the morpho-agronomic characterization of sweet cassava germplasm from the Brazilian Middle North and the selection of genotypes for this region.

MATERIALS AND METHODS

Collection of germplasm

The collection of germplasm was conducted mainly toward autochthonous fields that have been kept by local families, which represent the most common model of cultivation of this crop in these states. 10 genotypes (Table 1), of which, eight were ethno-varieties that have been grown by traditional farmers were collected. The two checks (BRS Dourada and BRS Gema de Ovo), developed for the Brazilian Northeast Region by the Brazilian Enterprise of Agriculture and Research (Embrapa), where donated by one of the Embrapa Units (Embrapa Meio Norte) based in Teresina, PI.

The autochthonous varieties were collected in 5 different locations: in the Micro Regions of Medio Mearim, Chapadinha, São Luis, Itapecuru Mirim and Gurupi in Maranhão state and Bico do

Table 1. Germplasm of cassava collected in the Middle North Region of Brazil, Chapadinha-MA, 2013.

Code	Genotypes	Origin
56- MA	BRS Dourada	Embrapa-pi
57-MA	BRS Gema de Ovo	Embrapa-PI
58-MA	Orelha Leão	Middle Mearim-MA
59-MA	Gameleira	Middle Mearim-MA
60-MA	Pão	São Luís- MA
61-MA	Turiaçu	Gurupi- MA
62-MA	R-01	Chapadinha-MA
63-MA	Rampa	Itapecuru Mirim- MA
64-MA	Rosa	Chapadinha-MA
65-MA	Talo Vermelho	Bico do Papagaio-TO

Papagaio, in Tocantins State. The autochthonous varieties considered different by farmers, which characterized a fix sampling with a fix and directed model were collected in the fields (Hershey, 1992; Martins, 1994).

Establishment and layout of the experiment

The experiment was established in the January of 2013 in the beginning of the regional raining season, and was conducted in the community of Vila União, 3° 44´ 34''S 43° 21´ 07´´W, rural zone of the municipality of Chapadinha, MA. Brazil.

The climate of the region is Aw, with two well-defined dry and raining seasons (Köppen and Geiger, 1939). Chapadinha municipality has an altitude of 106.2 m, with respective annual means of 1,797 mm and 27°C for precipitation and temperature, respectively. The soil of the region is a yellow latosol with frank-sandy texture (Embrapa, 2006). The experiment was conducted in rain fed conditions and eventual irrigation was used just to compensate the unusual shortage of rainfall that occurred during the conduction of the experiment.

The experiment was arranged in a completely randomized design, with four replication and ten treatments. The experimental plots were constituted of simple rows, 5 m long, with spacing of 1.20 and 0.6 m between and within rows, respectively. Each row was had only one genotype, and 10 plants. Of the ten plants per row, the 6 central plants, totalizing 24 plants for each genotype were evaluated. Repeated evaluation within blocks and replication was performed in order to search for morphological variation within genotypes.

Selections of planting material, soil preparation, planting and fertilizer application were performed according to the recommendation of the cassava system of production for the region of Cerrado (Sousa and Fialho, 2008).

Characterization of germplasm

Characterization of germplasm was carried out at eight months after planting, at the recommended age for morphological characterization of cassava germplasm. The descriptors and the methodology used in the characterization were proposed by Fukuda et al. (2010). The genotypes were characterized by 24 morphological descriptors. For practical issue, the descriptors were dived into the following five categories: i) plant descriptor, ii) leaf descriptor, iii) stem descriptor and iv) tuberous root descriptor (Table 2).

In order to determine the germplasm genetic diversity, the percentage frequency of genotypes in each class of the descriptors and the entropy level for each descriptor was calculated. As shown below, the coefficient of entropy was calculated according to the Renyi coefficient of entropy (Renyi, 1960).

$$H = -\sum_{i=1}^{s} pi\, ln\, pi$$

Where: H= Entropy Coefficient; *Pi*= Frequency of genotypes in each class of descriptor.

The entropy measures the frequency of (n) access P = (p_1+ p_2+…p_n), where p_1 = fi/n e (p_1+ p_2+…p_n = 1) since n = (f_1+ f_2 +…f_n), where f_1+ f_2 and f_n are the counting of each descriptor class. The entropy for each descriptor changes according to the number of classes for each descriptor and the equilibrium of accessions frequency in the different phenotypic classes. Thus, the greater the numbers of phenotypical classes and the more balanced the proportion between the access frequencies in the different phenotypical classes, the higher the entropy for a given descriptor will be.

The agronomic characterization was performed at harvest, in the twelfth month after planting. For this, the genotypes were evaluated to mean productivity of tuberous roots; harvest index; number of roots per plant; Root length and diameter; plant height; height of first branching point; branching levels and length of phyllotaxis. The statistical analysis was performed with the help of the statistical software ASISTAT (Silva et al., 2009).

RESULTS AND DISCUSSION

The genotypes Rampa and Turiaçu presented the highest average yield of tuberous roots, which was significantly differentiated them from the others (Table 3). The average of the two genotypes is near the regional average (7400 kg ha^{-1}) and superior to the local cultivar (Rosa) (Table 3). The results demonstrate a good performance of these genotypes in the local conditions. The yield variation 1.56 and 6.08 $t.ha^{-1}$ was expected. This characteristic is controlled by a set of genes, besides being influenced by environmental factors, which justifies its high amplitude. It confirms the degree of difference between cassava germplasm found in the Brazilian Middle North Region and points out the importance of regional selection of genotypes. Although it is desirable to have high productivity in the cultivation of cassava varieties, the practice of early harvesting, inherent to the growing of sweet cassava, prevents the full expression of the productive potential of the genotypes.

A considerable variation (from 0.18 to 0.50%) for the harvest index (HI) among genotypes was observed. The highest average for this trait corresponded to the genotype Rampa, which shows its ability on a greater translocation of photo assimilation to tuberous roots. The HI varies depending on the ratio between the aerial part weight and root production, and purpose of cultivation. Low values for HI in cassava stem from an increased production of the aerial part of the plants at the expense of root production and are acceptable when the crop is

Table 2. Descriptors categories and their classes used in the morphological characterization of cassava germplasm, Chapadinha, MA, 2013.

S/N	Plant descriptors	Given categories
1	Branching habit	1-Erect; 2-Dichotomous; 3-Trichotomous and 4-Tetrachotomous.
2	Type of plant	1-Open; 2-Umbrella type and 3-Compact
	Leaf descriptors	
3	Apical leaf color	1- Light green; 2- Dark green; 3- Purplish-green and 4- Purple.
4	Pubescence of apical bud	1-Present and 2- Absente.
5	Petiole color	1-Yellowish-green; 2- Green; 3-Redish-green; 4- greenish-red; 5-red and 6-Purple
6	Developed leaf color	1-Light-green; 2-Dark-green; 3-Purplesh-green and 4-purple
7	Terminal branches color	1-Light-green; 2-Dark-green; 3-Purplesh-green and 4-purple
8	Leave´s rib color	1- Green; 2-Redish-green; and 3- Greenish-red
9	petiole position	1-Tilted up; 2-Horizontal; 3-Angled down and 4-Irregular
10	Prominence of leaf scars	1-Without prominence and 2-Proeminent.
	Stem Descriptors	
11	Color of stem cortex	1-Light yellow; 2-Light green; 3-Green and 4-Dark green.
12	Length of phyllotaxis	1-Short; 2-Middle and 3-Large.
13	External Color of steam	1-Orange; 2-Yllowish-green; 3-Golden; 4-Light brown; 5-Gray; 6- Silvery; 7- Gray; 8-Silvery; 9- Dark brown.
14	Color of stem epidermis	1- Cream; 2- Light brown; 3- Dark brown; 4- Yellow.
15	Growth habit of the stem	1-Straight and 2-Forked.
	Root descriptors	
16	Presence of peduncle in roots	1-Present and 2-Absent.
17	External color of roots	1-White; 2-Yellow; 3-Light brown; 4-Brown and 5-Dark brown.
18	Color of root Cortex	1-White; 2-Yellow and 3-Pinkish.
19	Texture of root epidermis	1-Smooth and 2-Rough.
20	Constriction of roots	1-Absent; 2-Little or none and 3-Average.
21	Root shape	1-Conical; 2-Cylinder and 3-Spindle.
22	Highlight pellicle from roots	1-Easy release and 2- Difficult release.
23	Highlight of roots cortex	1-Easy release and 2-Difficult release.
24	Position of roots	1-Horizontal and 2- Vertical tendency.

Table 3. Means of the production components of sweet cassava germplasm collected in the Brazilian Middle North Region, Chapadinha-MA, 2013.

Genotypes	P (tons ha^{-1})	HI (%)	NTRP (unit)	LR (cm)	RD (cm)	PH (m)	HFB (m)	LB (%)	FL (cm)
Rosa	2.962^{b}	0.271^{c}	7.291^{c}	26.2^{b}	42.532^{a}	199.0^{c}	84.0^{b}	71.5^{a}	19.507^{b}
Dourada	2.064^{b}	0.175^{c}	7.333^{c}	22.946^{b}	40.637^{a}	246.5^{b}	76.25^{b}	10.915^{c}	19.737^{b}
T alo Vermelho	2.643^{b}	0.253^{c}	8.541^{c}	25.278^{b}	35.521^{a}	169.75^{c}	94.0^{b}	79.247^{a}	13.36^{d}
Orelha de Leão	3.207^{b}	0.344^{b}	10.5^{b}	29.0^{a}	24.583^{a}	217.25^{c}	79.0^{b}	65.412^{a}	19.54^{b}
Rampa	6.086^{a}	0.539^{a}	11.0^{b}	27.166^{b}	40.666^{a}	217.0^{c}	101.25^{b}	82.565^{a}	13.845^{d}
Gameleira	1.912^{b}	0.347^{b}	7.375^{c}	29.833^{a}	35.104^{a}	209.75^{c}	142.5^{a}	68.727^{a}	21.867^{a}
R.01	3.686^{b}	0.371^{b}	6.937^{c}	28.983^{a}	48.806^{a}	211.5^{c}	58.75^{b}	38.375^{b}	15.452^{c}
Gema de Ovo	2.983^{b}	0.253^{c}	7.875^{c}	24.4^{b}	35.046^{a}	223.75^{c}	60.75^{b}	42.865^{b}	19.22^{b}
Turiaçu	5.85^{a}	0.42^{b}	14.208^{a}	32.108^{a}	44.641^{a}	289.0^{a}	145.25^{a}	70.442^{a}	21.212^{a}
Pão	1.561^{b}	0.185^{c}	5.925^{c}	23.403^{b}	39.287^{a}	203.25^{c}	65.5^{b}	76.62^{a}	20.927^{a}

Averages followed by the same letter in the column do not differ significantly at 5% level of significance. P-productivity, ton.ha^{-1}; HI-harvest index,%; NTRP- number of tuberous roots per plant; RL-root length, cm; RD- root diameter, cm; HP- plant height, cm; HFB- height of the first branching, cm; LB-level of branching,%; FL-length phyllotaxis, cm.

intended for animal feed. On the other hand, they are undesirable in the production of sweet cassava. Values above 60% for the HI are considered suitable in the production of this crop.

The highest average for the number of tuberous roots per plant (NTRP) corresponded to genotype Turiaçu. This characteristic is influenced by environmental conditions and by the pattern of carbohydrates allocation to the roots during the early stages of plant development. In this study, the genotypes with greater number of roots per plant (Turiaçu and Ramp) corresponded to higher yields (Table 3). Reduction on the number of tuberous roots per plant can occur with the increase in planting density.

The highest averages for root length (RL) corresponded to the genotypes Turiaçu, Gameleira, Orelha de Leão and R.01 (Table 3). This characteristic is an important component of production, which is used as a criterion by farmers in the adoption of sweet cassava varieties.

There was a high variation (from 169 to 289 cm) for plant height among the genotypes, and Turiaçu presented the highest average for this trait (Table 3). Studies on cassava show a positive and significant correlation between the above ground production and plant height. Although there is a positive interaction between plant height and production of tuberous roots, there is no definition of what are ideal heights of plants in cassava cultivation. Concerning the average height of the first branching (HFB), it was observed that the genotypes Turiaçu and Gameleira showed the highest average. As for the branch level, the lowest average corresponded to the genotype BRS Dourada.

Characteristics such as plant height, height of the first branching and branch levels, influence the conduction of cultural practices are crucial in the dynamics of photo assimilated allocation in cassava. Higher plants with greater height of the first branching facilitates cultivation and harvesting operation. Genotypes with lower plant height are more suitable for regions with the incidence of strong winds, where problems with plant toppling have to be overcome. The branching levels, on the other hand, determine the photo-assimilated dynamic of allocation in the plant, besides influencing the performance of cultural practices. As the branching level increases so does the competition for nutrient and water. Thus, the cultivation of more compact plants optimizes cassava cultivation, by allowing greater density in planting without affecting the allocation of photo-assimilate.

For phyllotaxis length (FL), the lowest average corresponded to genotype Talo Vermelho and Ramp, which significantly differed from other genotypes (Table 3). The length of phyllotaxis relates to the yield of propagating material. Plants with lower values for this trait have a higher number of buds per length of stem, being desirable in the optimization of use of the propagating material.

There was a high phenotypic difference between the germplasm for most of the characteristics relating to the descriptors of leaf (Table 4).

The difference in leaf descriptors of the genotypes was expected, considering this crop wide genetic diversity, the different levels of breeding of the genotypes, besides their cultivation in conditions different to those of their place of origin. Considering this, Albuquerque et al. (2009), points to the high environmental interaction of cassava, and this is capable of promoting pronounced phenotypic changes in this crop.

The descriptors sinuosity of leaf lobe, margin of stipules and pubescence of the apical bud presented the lowest entropy levels (Table 4). Smaller entropy for these descriptors occurs due to their small number of classes and the imbalance in the frequency of genotypes in each class of these descriptors. Additionally, the process of conscious or unconscious selection carried out by farmers on certain characteristics explains the predominance of these classes for a particular descriptor.

Characteristics such as the sinuosity of the leaf lobe have a close relationship with the photosynthetic process. According to Fukuda (2006), the smooth sinuosity of the leaf lobe increases the efficiency of this process by increasing the leaf area. Characteristic such as the absence of pubescence in the apical bud reflects genotypes higher level of breeding. Low entropy level for leaf sinuosity has been reported in previous studies in the characterization of cassava germplasm (Albuquerque et al., 2009; Vieira, 2009).

Medium values of entropy correspond to the descriptors position of petiole, and the colors of developed leaves, terminal branches and apical leaves (Table 4). For descriptors such as the color of developed leaf and terminal branches, there was a higher frequency of green (50%) and dark green (60%), respectively. There is a close relationship between the color of the terminal branches and the color of developed leaves. Since these last composes terminal branches, there are only slight differences between both as to color tone. Predominance of green color in developed leaves has been reported in the characterization of cassava germplasm (Albuquerque et al., 2009). In this work, it was observed that the four color categories of apical leaves proposed by Fukuda et al. (2010) and a higher frequency of light green color (Table 4).

Highest entropy values corresponded to petiole color and shape of leaf lobe (Table 4). A larger number of classes for these descriptors and the balance in the frequency of genotypes in these classes justify the high entropy values of these characteristics. Although they have no agronomic importance, the petiole color and shape of leaf lobe are fundamental to farmers in the differentiation of genotypes. These characteristics are additionally important in taxonomic characterization of cassava. High entropy for the shape of leaf lobe, confirms the high genetic diversity for this trait in cassava germplasm. High entropy for the shape of leaf lobe has

Table 4. Descriptors of leaf, their classes, frequency of genotypes in each class and the entropy level for each descriptor in the characterization of cassava germplasm from Brazil Middle North Region, Chapadinha, MA, 2013.

Leaf descriptors		Frequency (%)	Entropy level
Categories	**Classes**		
Petiole color	Reddish green	10	1.503
	Yellowish green	30	
	Red	30	
	Greenish red	20	
	Purple	10	
Petiole position	Horizontal	50	0.692
	Tilted up	50	
Shape of leaflet	Lanceolate shape	40	1.192
	Elliptical	10	
	oblong Lanceolate	10	
	Elliptical Lanceolate	40	
Sinuosity of foliar lobe	Smooth	100	0
Color of developed leaf	Green	50	0.942
	Dark green	40	
	Light green	10	
Color of terminal branches	Green	20	0.948
	Dark green	60	
	Light green	20	
Stipules margins	Entire	100	0
Color of apical leaves	Light green	60	0.948
	Purple	20	
	Purplish green	20	
Pubescence of apical bud	Absent	100	0

been reported in the characterization of cassava germplasm (Vieira, 2009).

There was phenotypic difference for most of stem descriptors (Table 5). Lower entropy levels corresponded to growth habit, phenotypic difference was not observed between the germplasm for this trait. The growth habit in cassava implies in the ease or difficulty of their cultivation. It is a characteristic of economic importance in the culture, which explains the selection on genotypes with ideal growth habit. A straight growth facilitates cultivation, resulting in more uniform areas of cultivation. In addition, it facilitates the harvesting and the marketing of propagative material.

Descriptors such as presence of latex, external color and color of the stem epidermis presented mean entropy values (Table 5). These characteristics have no economic importance, which explains a lower imbalance in the frequency of genotypes on the different classes of these descriptors. A higher frequency of the classes of light brown external color for the descriptors stem and color of stem epidermis as well as predominance of the colors yellow and light green for the color of stem cortex was observed. The color of stem cortex is a highly variable characteristic in cassava germplasm; however it is not agronomically important, which probably contributed to the no selection of specific colors over time. This characteristic is essential in the differentiation cultivars.

Higher entropies were observed for the descriptors color of stem cortex, branching habit and type of plant

Table 5. Descriptors of plant stem, their classes, frequency of genotypes in the classes and the entropy level for each descriptor in the characterization of cassava germplasm from Brazil Middle North Region, Chapadinha, MA, 2013.

Stem descriptors		Frequency (%)	Entropy level
Categories	**Classes**		
Growing habit	Upright	100	0
Branching habit	Dichotomic	20	1.028
	Erect	50	
	Trichotomic	30	
External color of stem	Light brown	50	0.942
	Gray	40	
	Silvery	10	
Color of stem epidermis	Light brown	70	0.800
	Yellow	10	
	Cream	20	
Color of stem cortex	Yellow	40	1.053
	Light green	40	
	Dark green	20	
Latex presence	Medium	50	0.692
	Few	50	
Plant kind	Compact	40	1.053
	Cylindrical	40	
	Open	20	

(Table 5). There was a predominance of erect branching habit (50%), compact class (40%) and cylindrical (40%) for the descriptors branching habit and type of plant, respectively. Characteristics like growth habit and type of plant determine plant structure, being determinants components of leaf area. Agriculturally, these characteristics affect the cultivation of cassava, increasing or decreasing the difficulty of harvesting. Characteristics such as erect branching habit of stem and compact plants were not prevalent in most studies of morphological characterization of cassava (Gusmão and Neto, 2008; Nick et al., 2008).

There was variability for all the descriptors of the tuberous roots (Table 6). The lower levels of entropy corresponded to the descriptors epidermis texture, pulp color and constriction of roots. It a predominance of the classes white and few o absent for the descriptors pulp color and root constriction were observed, respectively. The color of pulp in roots has been one of the most important features in the selection of genotypes of sweet cassava varieties, which explains the lower number of classes and the occurrence of specific classes for this descriptor.

Consideration as to the color of pulp is essential for the adoption of cassava varieties, wherein the preference for varieties with specific color of pulp varies according to the purpose of cultivation and region of production. In Brazil Midwest Region there is a preference for roots of yellow pulp (Vieira, 2009), while there is a greater preference for white pulp varieties in the North and Northeast regions of the country. Varieties of cassava presenting roots with yellow pulp are good sources of β-carotene. These varieties have been strategically incorporated into the diet in areas where nutritional deficiency rates are of concern (Welsh and Graham, 2002), and its adoption can contribute to food security of populations in these regions. A reduced amount of roots constrictions facilitates the processing of roots, especially when this is done manually. On the other hand, the epidermis texture has an aesthetic importance in the marketing of fresh roots.

Descriptors with mean entropy values corresponded to root position, external color and peeling. There was a predominance of the classes' vertical trend and light brown color for the descriptors position of roots and roots color, respectively (Table 6). Regarding the peeling of

Table 6. Descriptors of tuberous roots, their classes, frequency of genotypes in each class and the entropy level for each descriptor in the characterization of cassava germplasm from Brazil Middle North Region, Chapadinha, MA, 2013.

Tuberous root descriptors		Frequency (%)	Entropy level
Categories	**Classes**		
Format	Cylindrical	40	0.942
	Cylindrical-conical	50	
	Irregular	10	
Root position	Horizontal tendency	40	0.672
	Vertical tendency	60	
Epidermis texture	Rough	90	0.324
	Smooth	10	
External color	Dark brown	10	0.8
	Light brown	70	
	Whit or cream	20	
Cortex color	Pinkish	20	1.028
	White or cream	50	
	Purple	30	
Pulp color	White	80	0.499
	Cream	20	
Root constriction	Few or absent	80	0.499
	Medium	20	
Peduncle presence	Sessile	40	1.053
	Mixed	40	
	Pedunculated	20	
Pealing	Easy	50	0.692
	Difficult	50	

roots, there was a balance in the distribution of genotypes to the classes' easy and difficult peeling. This descriptor is greatly influenced by environmental conditions and has a great agronomic importance. Thus, it can be assumed that the prevalence of these classes for this descriptor in cultivated germplasm of cassava results from intensive selection over this crop. Descriptors such as the position, constriction and peeling of roots determine the efficiency of harvesting and processing of roots. Genotypes whose roots arrange predominantly horizontally in the soil make harvest easier because they require less effort in the uprooting of roots. Plants with horizontal roots tend to result in lower losses related to the remaining roots on the soil, compared to genotypes with roots with vertical arrangement. Smaller amounts of constriction in the roots and an easier peeling increase the processing efficiency of roots and the quality of the final product. These characteristics are even more important in traditional cultivation of cassava, where much of the processing is carried out manually.

Higher entropy corresponded to the descriptors presence of peduncle, cortex color and shape. Of these, only the shape of roots has economic importance. All these descriptors showed higher numbers of classes as well as a more balanced frequency of genotypes on these classes, suggesting greater difficulty in the selection of genotypes with specific classes for these descriptors. In addition to the high genetic variability, these descriptors are greatly affected by soil and weather conditions. There was a predominance of the classes conical-cylindrical, light brown and white or cream for the descriptors format of roots, external color and color of the roots cortex, respectively. There was also a predominance of the sessile and mixed classes for root peduncle. The shape of roots is fundamentally important in the marketing of sweet cassava, being preferred the cylindrical and conical-cylindrical shapes. The presence of peduncle facilitates the harvesting process (Albuquerque et al., 2009). The peduncle works as a natural barrier of protection to the roots, protecting them from oxidative processes and against pathogens. Thus, its presence reduces the ongoing problem of roots oxidation and exposure to pathogens, decreasing the rotting of roots and enhancing their shelf in post-harvest.

Conclusions

The genotypes Rampa and Turiaçu were the most promising as the production and consumption aspects are excellent alternatives for cultivation in the Brazilian Middle North Region. The Genotypes Rosa, R.01 and Rampa had the lowest plant heights, which allow easer cultivation and harvest. The genotype Rampa had the highest harvest index showing its high productive potential. The study showed a high genetic diversity of cassava germplasm in the Brazilian Middle North Region. The work proved to be an excellent approach in the screening and selection of promising and contrasting genotypes of cassava. Genetic resources of cassava that occur in the Brazilian Middle North Region represent a valuable asset for the breeding programs of this crop.

Conflict of Interests

The authors have not declared any conflict of interests.

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