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Full Length Research Paper

Growth, yield and content of trace elements in coffee fruits grown in soils under successive application of sewage sludge

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Brazil seeks to optimize the use of fertilizers by taking advantage of agricultural residues. The sewage sludge is used as organic matter in various crops such as coffee. However, an experiment in lysimeter was performed seeking to assess the growth, yield and content of zinc, copper, nickel, cadmium, lead and chromium in coffee fruits grown on Dystrophic Red Yellow Latosol (dRYL), Dystropherric Red Latosol (dRL) and Dystrophic Red Yellow Argisoil (dRYA); under successive application of sewage sludge. The sewage sludge increased the content of Cd, Cr, Cu, Pb and Zn found in soils under study. The sewage sludge decreased coffee growth and productivity and increased the content of Cu, Zn and Cd found in coffee peel. Greater content of Cd in coffee seeds for all three soils, as well as greater content of Zn and Pb in coffee seeds grown on Dystropherric Red Latosol was also found.

Key words: *Coffea arabica,* coffee plants height, quality of coffee fruit, heavy metals.

INTRODUCTION

To recycle organic residues in farming is the basic condition for increasing sustainability in agricultural systems, because it allows possibilities of using nutrients, energy, organic matter, and avoid pollution of essential matrices such as water, used for food production (Chen and Jiang, 2014). The urban population growth is the greatest source of various residues, which are often accumulated in the environment without adequate treatment or use that allows its recycling. The sewage sludge, a semisolid residue, is highlighted among those residues, with considerable percentage of organic matter and essential elements for plants (Berton and Nogueira, 2010). This residue, when adequately hygienised and dry, can be used as organic fertilizer and can be incorporated into the soil as source of nutrients for plants (Bettiol and Camargo, 2006).

Although the agricultural use of sewage sludge is considered the most promising alternative of its final disposal (Campos and Alves, 2008), the presence of heavy metals may limit its use. Thus, the recycling of nutrients contained in sewage sludge may be by means of its use in agricultural areas, since the residue is within

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Table 1. Reference values and chemical composition of sewage sludge obtained from the Jundiaí Treatment Station, State of São Paulo.

Element	Concentration ($mg\ kg^{-1}$)	Maximum value allowed[1] ($mg\ kg^{-1}$)
Cd	6	39
Pb	122	300
Cu	170	1500
Cr	179	1000
Ni	42	420
Zn	367	2800

[1]Conama Resolution n. 375 (BRASIL, 2006).

the minimum standards required by environmental agencies in relation to presence of heavy metals and human pathogens, among others (Bettiol and Camargo, 2001; Brasil, 2006).

The accumulation of heavy metals in soils is the major concern regarding the environmental safety (Soares et al., 2005); because these elements may express their polluting potential directly on soil organisms by means of their availability to plants in phytotoxic levels, besides their transfer possibility into the food chain by means of plants or through contamination of surface and subsurface water (Chang et al., 1984).

Therefore, considering that Brazil is a greater producing and consuming country of coffee in the world, we performed this research aiming to assess the growth, yield and content of zinc, copper, nickel, cadmium, lead and chromium found in coffee fruits grown on Dystrophic Red Yellow Latosol, Dystropherric Red Latosol, and Dystrophic Red Yellow Argisoil; under successive application of sewage sludge.

MATERIALS AND METHODS

The experiment was performed between the year of 2009 and 2013 in a set of 36 drainage lysimeters with 0.9 m deep and 1 m diameter. Lysimeters contain material with undisturbed structure of Dystrophic Red Yellow Latosol (dRYL), Dystropherric Red Latosol (dRL), and Dystrophic Red Yellow Argisoil (dRYA). The soil granulometry in the layer from 0 to 20 cm was estimated using the pipette-based method (Donagema et al., 2011), and the contents of clay, silt and sand were respectively 592, 48 and 360 g kg^{-1} for dRYL; 677, 91 and 232 g kg^{-1} for dRL; and 330, 203 and 467 g kg^{-1} for dRYA.

Bean and corn were cropped at the experimental area (column) before planting coffee seedlings. The bean was planted in late June and harvested in late October, 2009. The corn was planted in late November, 2009 and harvested in late March, 2010. The planting of coffee seedlings was in late December 2009, before harvesting corn, seeking to reduce the light intensity on coffee seedlings.

Soil samples were collected at the layer from 0 to 20 cm, before planting bean, seeking to estimate contents of Cu and Zn by means of the Mehlich-1 extractor. The study found respectively 1.5 and 4.0 mg dm^{-3} for dRYL; 2.7 and 4.8 mg dm^{-3} for dRL; and 0.7 and 4.2 mg dm^{-3} for dRYA. By using the same extraction method, contents of Cd, Pb and Cr were also estimated, which were found to be less than the detection limit, <0.01 mg.dm^{-3} (Silva, 2009).

Liming and corrective phosphate fertilization were carried out based on results of chemical analysis carried out in 15 April, 2009 and 8 May, 2009 in all lysimeters. The amount of lime was estimated by means of bases saturation method to elevate them to 70%. Thus, the adding quantities of chalky (36% of CaO, 14% of MgO and total reactive potential of neutralization equal to 95%) were about 1.6, 2.6 and 5.7 Mg ha^{-1} respectively for dRYL, dRL and dRYA. The amount of phosphorus added to the soil was about 2.0 Mg ha^{-1} P_2O_5, used as single superphosphate. The second liming was at 21 November, 2011, and quantities of lime were 2.7, 3.3 and 0.5 Mg ha^{-1}, respectively for dRYL, dRL and dRYL.

Doses of sewage sludge were estimated according to the content of total nitrogen (N), ammonium (NH_4^+) and nitrate (NO_3^-) present in the sludge, as well as the nitrogen required for coffee trees. The D3 dose corresponded to the requirement in total nitrogen for the crop, and the doses D0, D1 and D2 were 0, ¼ and ½ of D3, respectively. The sewage sludge was applied for four times. The first application was about seven months before plantation of coffee seedlings (*Coffea arabica* L. cv. Mundo Novo 379/19) at doses about 0, 11, 22 and 44 Mg ha^{-1}. The second application was four months after plantation at doses about 0, 8, 16 and 32 Mg ha^{-1}; and the third and fourth applications were done respectively at the first and second year after plantation at doses about 0, 15, 31 and 61 Mg ha^{-1}. Then, applications of sewage sludge were in 15 May, 2009, 16 April, 2010, 14 April, 2011, and 28 April, 2012, respectively.

The sewage sludge was distributed on the whole soil surface of each column, and incorporated at the first 10 cm deep, avoiding the soil revolving for the deepest layers. Considering the content of N found in the sludge and 28% of mineralization rate - in kg ha^{-1} - in the first year after application (Chiaradia et al., 2009), the amount of N potentially mineralized in a year is equivalent to 6.26 × dose of sewage sludge in Mg ha^{-1}. The amount of K_2O provided by the sewage sludge in kg ha^{-1} is equal to 4.23 × dose of sewage sludge in Mg ha^{-1}.

The sewage sludge was obtained from the Jundiaí Treatment Station, State of São Paulo, which predominantly receives domestic sludges. This material passed through the composting process for about 90 days. The content of metals in the sewage sludge was estimated by means of the 3051A method (United States Environmental Protection Agency - USEPA, 1998), and results are shown in the Table 1.

The ammonium sulphate was added to each lysimeter during the first application of sewage sludge and about four months after, at doses about 188 and 97 kg ha^{-1}. The potassium chloride was also added to each lysimeter during the first application, according to doses of sewage sludge as follows: 177, 132, 87 and 0 kg ha^{-1} for doses 0, 11, 22 and 44 Mg ha^{-1}. Five months after the first application of sewage sludge, the ammonium sulphate was added according to doses of sewage sludge as follows: 570, 430, 145 and

0 kg ha^{-1}, respectively for doses 0, 11, 22 and 44 Mg ha^{-1}. The potassium chloride was also added to the lysimeter during the same season, at the dose about 69 kg ha^{-1}. During the third and fourth applications of sewage sludge, the ammonium sulphate was added according to doses of sewage sludge as follows: 1.819, 1.364, 909 and 0 kg ha^{-1}, respectively for doses 0, 15, 31 and 61 Mg ha^{-1}. The potassium chloride was also added to each lysimeter according to doses of sewage sludge as follows: 670, 556, 442 and 214 kg ha^{-1}, respectively for doses of 0, 15, 31 and 61 Mg ha^{-1}.

Samples of soil were collected in 16 December, 2013 on the layer from 0 to 10 cm. These samples were used to estimate contents of Cd, Cu, Pb, Cr and Zn by means of Mehlich 1 extractor (Silva, 2009). The height, stem diameter and number of plagiotropic branches were measured monthly for plants growth assessment from January 2011 to July 2012. Coffee fruits were hand harvested from May to August 2012 and, then, dried in the hothouse at 60°C until obtaining about 11% of humidity. Fruits were mechanically peeled using simple manual peeler. The removal of peels from grains was made using a simple automatic blower. Peels were milled on a Willey type mill, and grains were milled in an analytical coffee-mill model A11, brand IKA, using liquid nitrogen.

A sample about 0.5 g was placed in a Teflon tube and 5 mL of nitric acid (HNO_3) was added. Then, tubes were hermetically sealed to allow digestion according to the method 3051A (USEPA, 1998). This method allows digestion of samples with HNO_3, under high pressure (65 pi) and high temperature (180°C), for 10 min. After digestion, the extract was filtered and the volume filled until 10 mL using distilled water. From these extracts, contents of Cu and Zn were estimated by means of atomic absorbance spectrometry in air-acetylene flame. Contents of Cr, Ni, Cd and Pb in graphite furnace were also estimated. The AAnalyst 800 equipment of Perkin Elmer was used.

The control and results quality guarantee were ensured by using Plankton BCR-414 reference material, certified for vegetable matrix analysis in each samples group analysis, as well as blank samples. Results were satisfactory, with recovery about 114% for Cd, 61% for Cr, 58% for Ni, 70% for Pb, 111% for Cu and 112% for Zn. The metal detection limits were also estimated and, the following values were obtained: Cd = 0.24 µg kg^{-1}, Cr = 2.13 µg kg^{-1}, Ni = 3.37 µg kg^{-1}, Pb = 2.31 µg kg^{-1}, Zn = 0.03 mg kg^{-1} and Cu = 0.09 mg kg^{-1}.

Statistical analysis

The effect of soils and doses of sewage sludge for each soil was analyzed using the first and second degrees polynomial regressions. The experiment was performed in randomized complete block design. Contents of Cd, Cr, Cu, Pb and Zn were subjected to analysis of variance using the F test. Standardized results of trace elements were subjected to principal component analysis, seeking to analyze the effect of soils, doses of sewage sludge and interrelations between variables. Seeking to represent results, the scores and loadings were used to organize soils and doses of sewage sludge in a bidimensional plane. All analyses were carried out using the R Statistical Software (R Development Core Team, 2014), running the arm and ggplot2 packages.

RESULTS AND DISCUSSION

The principal components analysis allowed to explain 80.4% of total variance of data about soil metals on the first two components. The first component contributed about 58.4% of total variance, and the second with 22% (Figure 1). There was dissociation of doses of sewage sludge in the component 1, which allowed to understand the pattern of dissimilarity gradient. This gradient was increased as doses of residue increased. Averages of positions of centroids of doses of sewage sludge, namely, D0, D1, D2 and D3 were, respectively 2.13, 0.49, -1.34 and -1.43 in the component 1. In addition, it was also possible to understand the grouping of dRYL and dRYA, which were dissociated from dRL. Averages of positions of centroids for dRYL, dRYA and dRYL in the component 1 were, respectively 0.39, 0.27 and -0.63; showing that the soil dRL was the most affected by doses of sewage sludge. Regarding the component 2, the dRYL and dRL were dissociated from dRYA, with averages of positions of centroids equals to 0.81, 0.28 and -1.03 for dRYA, dRL and dRYL, respectively.

The structure of principal components analysis, which indicates a correlation between original variables and components 1 and 2, is represented by the direction, orientation and length of arrows shown on the biplot. Then, it is possible to see that contents of Cd, Cr, Cu, Pb and Zn were increased as doses of sewage sludge increased. Besides, contents of Cd, Cr, Cu, Pb and Zn were lesser than the reference values established in the context of preventing contamination (Brasil, 2009) (Table 2). The height, stem diameter and number of coffee plagiotropic branches showed statistical differences between soils and between doses of sewage sludge within each soil from 1 and 499 days (Table 3).The height, diameter of stem and number of coffee plagiotropic branches did not show statistical differences between soils for the treatment without sewage sludge (Figure 2). The coffee height grown on soils dRYA and dRL reduced significantly with applying sewage sludge, however, causing no effect on dRYL. The stem diameter of coffee grown on dRL and dRYL increased with applying sewage sludge. However, there was no effect of doses of sewage sludge for coffee grown on dRYA soil. Besides, there was positive and significant effect for coefficient that correlated doses of sewage sludge and stem diameter for dRL and dRYL. The number of coffee plagiotropic branches grown on dRYA and dRL decreased with applying sewage sludge, but they increased on dRYL.

The coffee productivity was significantly different in studied soils ($F_{2,28}$ = 5.38 and p-value = 0.0107), and for doses of sewage sludge in each soil ($F_{3,28}$ = 18.6 and p-value < 0.0001). In addition, the productivity was lesser on dRYL (0.77 kg plant^{-1}) when compared to dRL and dRYA (0.95 and 1.11 kg plant^{-1}, respectively). Martins (2003), studying the coffee productivity (variety Acaiá IAC-474) with about 10 years, on Eutropherric Red Latosol, found similar results in terms of fruit yield, about 1.0 kg plant^{-1}. Catani *et al.* (1967) found fruit yield about 1.2 kg plant^{-1} for Mundo Novo variety with about 10 years on Red Latosol. The fruit yield reduction found with application of sewage sludge in this study was slight on Drl and evident on dRYA (Figure 3). Martins (2003)

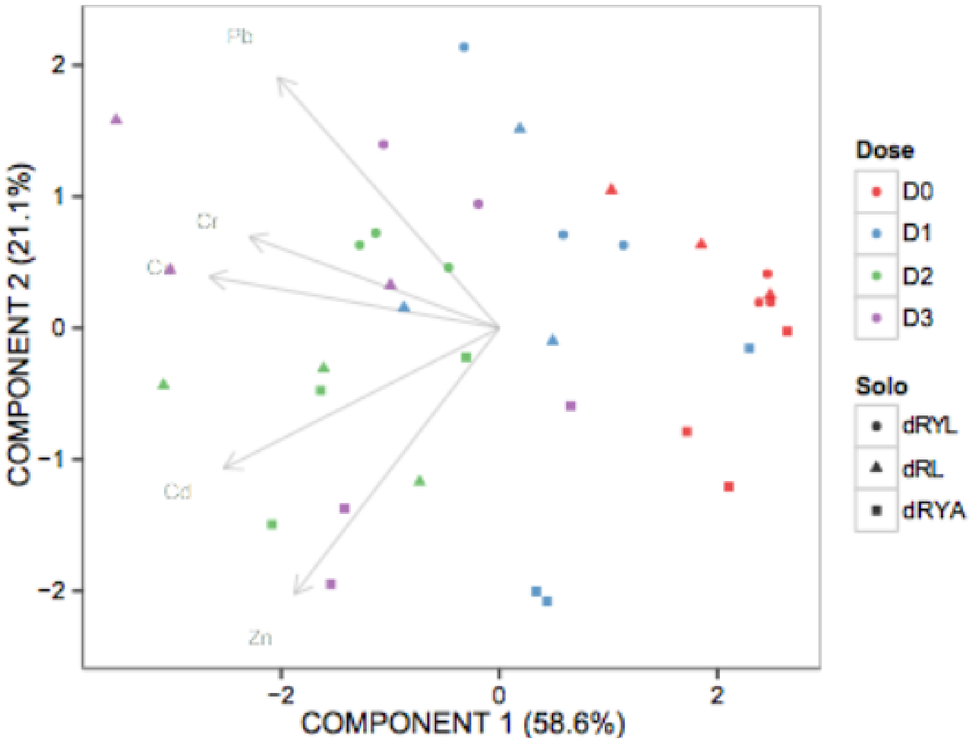

Figure 1. Principal components analysis for Cd, Cr, Cu, Pb and Zn in the soils under successive application of sewage sludge.

Table 2. Content of Cd, Cr, Cu, Pb and Zn in the layer from 0-10 centimeters for soils under different doses of sewage sludge.

Soil	Dose[1] ($mg\ ha^{-1}$)	Cd	Cr	Cu	Pb	Zn
				$mg\ dm^{-3}$		
dRYL[2]	0	0.00	0.14	1.96	0.04	19.70
	49	0.02	0.67	4.22	0.67	72.35
	100	0.21	1.08	7.06	0.58	86.79
	198	0.12	0.79	9.41	1.90	57.19
dRL[3]	0	0.00	0.27	3.32	0.24	31.01
	49	0.08	1.23	5.82	0.33	80.98
	100	0.43	1.34	8.46	0.37	160.67
	198	0.48	1.19	10.81	1.09	174.16
dRYA[4]	0	0.00	0.22	0.74	0.00	93.01
	49	0.06	0.45	2.66	0.00	157.62
	100	0.33	0.82	6.99	0.43	143.05
	198	0.32	0.27	8.77	0.15	133.94

[1]Cumulative dose of sewage sludge; [2]Dystrophic Red Yellow Latosol; [3]Dystropherric Red Latosol; [4]Dystrophic Red Yellow Argisoil.

Table 3. Analysis of variance of the mixed linear model with p-value for fixed effects according to F test and based on the Satterthwaite approximation; and p-value for random effects based on the ration log - likelihood for height, diameter of stem and number of plagiotropic branches.

	Height			Stem diameter			Number of plagiotropic branches		
Fixed effects									
Parameter	**DF**	**Sq**	**p-value**	**DF**	**Sq**	**p-value**	**DF**	**Sq**	**p-value**
Soil	2	0.45	<0.0001***	2	306.38	<0.0007***	2	577.25	<0.0001***
Day	1	1.01	<0.0001***	1	1491.75	<0.0001***	1	859.48	<0.0001***
Block	2	0.11	0.0005***	2	101.03	0.0063**	2	76.42	0.0073**
Soil: Dose	3	0.13	0.0005***	3	173.79	<0.0003***	3	190.48	<0.0001***
Random effects									
Variable	**Chi DF**	**Chi Sq**	**p-value**	**Chi DF**	**Chi Sq**	**p-value**	**Chi DF**	**Chi Sq**	**p-value**
(1 | Day)	1	116.61	<0.0001***	1	62.23	<0.0001***	1	200.94	<0.0001***
R^2 marginal*	-	89.3%	-	-	85.1%	-	-	93.6%	-
R^2 conditional*	-	95.4%	-	-	89.7%	-	-	100%	-

found coffee fruit yield about 0.62 and 0.59 kg plant^{-1} for Acaiá IAC-474 variety with about 9 and 6 years. This variety was grown on Dystrophic Red Latosol and Dystrophic Red Argisoil; with cumulative doses of sewage sludge respectively about 29.8 and 18 Mg ha^{-1}, dry basis.

The amount of Cu found in coffee peel was statistically different in studied soils ($F_{2,28}$ = 16.69 and p-value < 0.0001), as well as for doses of sewage sludge in each soil ($F_{3,28}$ = 34.66 and p-value < 0.0001). The content of Cu coffee peel grown in soils without sewage sludge was as follows: dRYA (7.7 mg kg^{-1}) < dRL (10.9 mg kg^{-1}) < dRYL (12.5 mg kg^{-1}) (Figure 4 and Table 4). Martins (2003) found similar amount of Cu of coffee peel, about 14.6 mg kg^{-1}, for coffee grown on Eutropherric Red Latosol without application of sewage sludge. Malavolta et al. (1963) found the highest content of Cu in coffee peel (18 mg kg^{-1}) for varieties Mundo Novo, Caturra Amarelo and Bourbon Amarelo grown on Red Latosol.

The amount of Cu found in coffee seeds was not statistically different in studied soils ($F_{2,28}$ = 1.42 and p-value = 0.26), as well as for doses of sewage sludge in each soil ($F_{3,28}$ = 1.89 and p-value = 0.15). The content of this element in coffee seeds did not change according to type of soil and doses of sewage sludge (Figure 4). The content of Cu in coffee seeds was about 13.1 mg kg^{-1} (Table 4). Similar results were obtained by Martins (2003), which did also not found differences between doses of sewage sludge (the content of Cu in coffee seed was 14.6 mg kg^{-1}). Malavolta et al. (1963) found similar amount of Cu in coffee seed of three different varieties (15 mg kg^{-1}).

The amount of Zn found in coffee peel did not show statistical differences in studied soils ($F_{2,28}$ = 0.63 and p-value = 0.54). However, it was changed by doses of sewage sludge in each soil ($F_{3,28}$ = 25.37 and p-value <0.0001). The amount of Zn found in coffee peel grown on soils with no sewage sludge was 3.1 mg kg^{-1} (Table 4). Martins (2003) found content of Zn greater in the peel (6.4 mg kg^{-1}) for coffee grown on Eutropherric Red Latosol with no sewage sludge. However, this result differs to that obtained in Malavolta et al. (1963), which reported contents of Zn about 10 times higher (70 mg kg^{-1}) in coffee peel of three varieties.

The amount of Zn found in coffee peel increased with the increase of doses of sewage sludge in the studied soils. This effect was most expressive on dRL in relation to others (Figure 5). Concentrations of Zn obtained on dRL were around 2.6 and 5.6 mg kg^{-1} respectively for lesser and greater doses of sewage sludge (Table 4). Martins (2003) found content of Zn in coffee peel about 5.6 mg kg^{-1} for Acaiá IAC-474 variety with 9 years. This variety was grown on Dystrophic Red Latosol, with cumulative doses of sewage sludge about 29.8 Mg ha^{-1}, dry basis.

The amount of Zn found in coffee seed was statistically different in studied soils ($F_{2,28}$ = 5.75 and p-value = 0.008) and was changed with application of different doses of sewage sludge in each soil ($F_{3,28}$ = 5.29 and p-value = 0.005). The content of Zn found in coffee seed grown on soils with no sewage sludge was 5.1, 6.7 and 6.8 mg.kg^{-1} respectively for dRL, dRYL and dRYA (Table 4). Martins (2003) found similar content of Zn in coffee seeds, about 6.9 mg kg^{-1}, for coffee grown on Eutropherric Red Latosol without sewage sludge. However, these results differ from those reported by Malavolta et al. (1963), which found the highest values of Zn (12 mg kg^{-1}) in coffee seed for three varieties.

The content of Zn in coffee seed was increased with increasing of doses of sewage sludge on dRL.

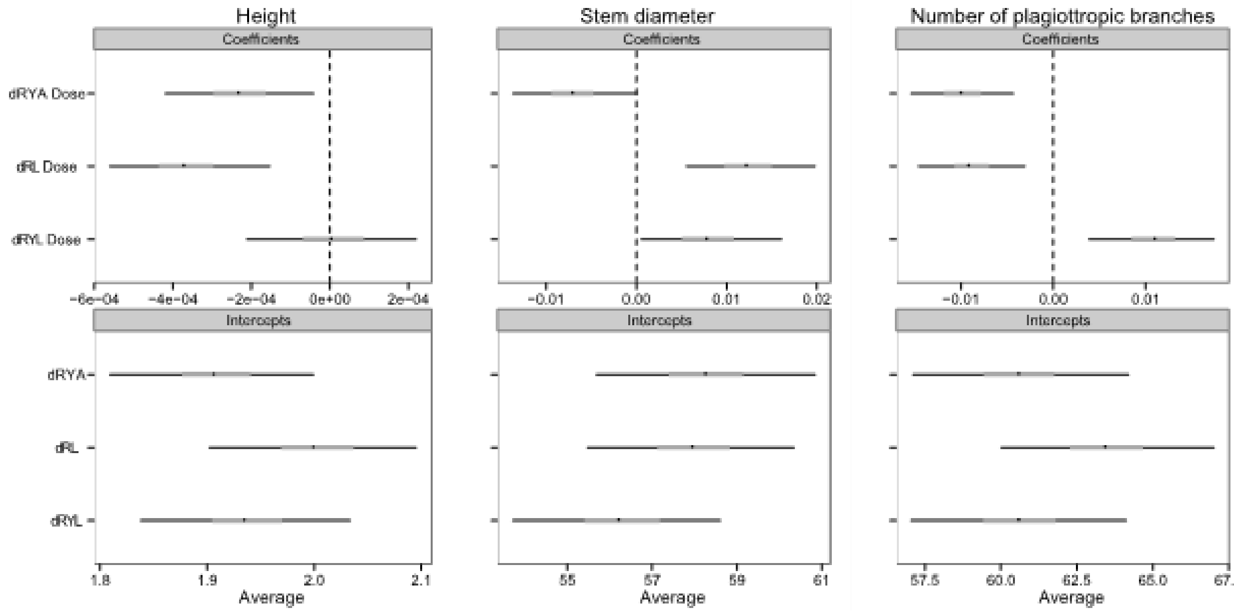

Figure 2. Estimate, standard error, and confidence interval for coefficients of mixed linear model for height (m), stem diameter (mm) and number of plagiotropic branches. These parameters are shown on the model describing the effect of soils and doses of sewage sludge in each soil. The intercepts refer to soil with no application of sewage sludge. Coefficients refer to estimates of doses of sewage sludge in soils under study.

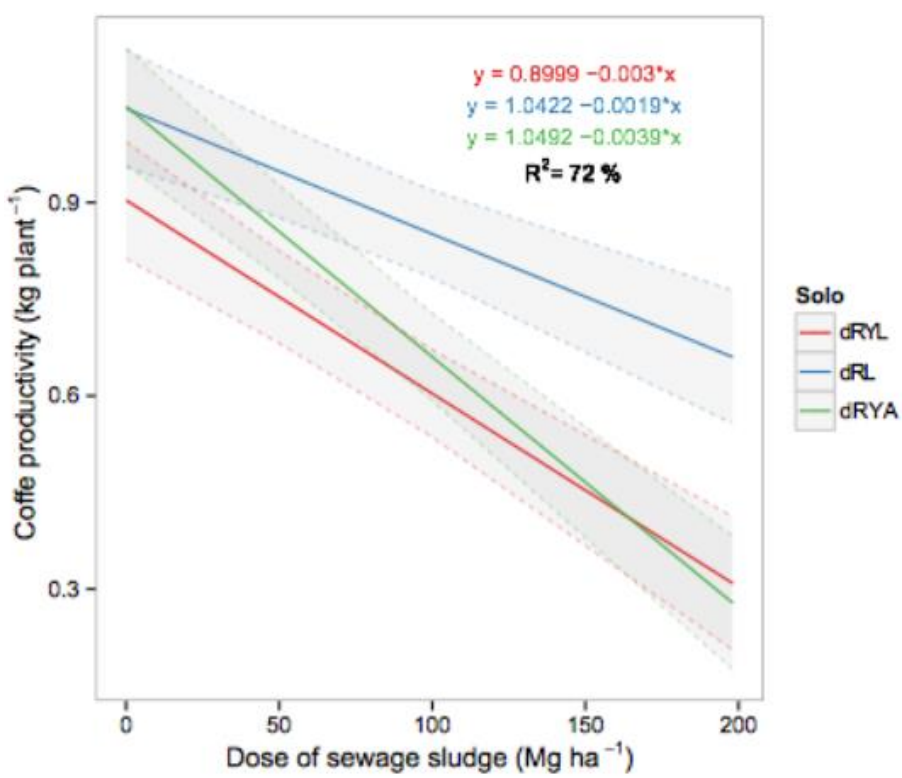

Figure 3. Coffee fruits productivity in the first post-planting year under different doses of sewage sludge. The coffee was grown on Dystrophic Red Yellow Latosol (dRYL), Dystropherric Red Latosol (dRL), and Dystrophic Red Yellow Argisoil (dRYA). The solid line refers to estimated average and the dotted line shows the interval of standard error.

Table 4. Content of Cd, Cu, Ni, Pb and Zn found in coffee peel and seed obtained under different doses of sewage sludge.

Soil	Dose[1] Mg ha^{-1}	Cu	Zn	Cd	Ni	Pb
		------ mg kg^{-3} ------		-------------- µg kg^{-3} --------------		
Coffee peel						
dRYL[2]	0	12.54	3.52	0.00	524.55	228.70
	49	13.39	3.82	44.22	643.29	215.29
	100	13.78	4.09	28.41	590.85	187.75
	198	14.31	4.80	40.64	504.19	181.44
dRL[3]	0	10.94	2.57	0.00	0.00	113.55
	49	11.23	3.46	24.53	65.33	128.62
	100	13.68	3.94	37.77	279.39	199.18
	198	15.20	5.62	49.22	471.54	132.48
dRYA[4]	0	7.74	3.66	0.00	0.00	177.24
	49	9.36	3.79	35.36	227.13	156.18
	100	12.77	4.21	42.54	189.15	266.54
	198	14.40	4.83	45.32	173.35	229.05
Coffee seed						
dRYL	0	13.84	6.66	7.02	657.28	205.21
	49	13.72	7.24	31.58	1018.88	129.76
	100	14.56	7.38	34.50	772.69	145.23
	198	14.79	7.71	34.61	607.86	157.43
dRL	0	13.26	5.13	30.47	367.04	194.26
	49	12.02	6.72	19.48	436.57	111.82
	100	14.51	7.47	24.76	508.58	133.07
	198	13.68	7.80	50.46	620.30	599.80
dRYA	0	12.31	6.75	0.00	112.35	205.09
	49	13.38	8.68	20.67	200.02	139.74
	100	15.08	8.55	36.61	370.14	195.37
	198	14.10	8.15	26.79	256.78	102.10

[1]Cumulative dose of sewage sludge; [2]Dystrophic Red Yellow Latosol; [3]Dystropherric Red Latosol; [4]Dystrophic Red Yellow Argisoil.

Concentrations of Zn in coffee seeds from this soil were around 5.1 and 7.8 mg kg^{-1}, respectively for lesser and greater dose of sewage sludge (Table 4). Martins (2003) reported content of Zn in coffee seeds about 6.6 mg kg^{-1}. This content was found in Acaiá IAC-474 varieties with about 9 years, grown on Dystrophic Red Latosol; with cumulative dose of sewage sludge about 29.8 Mg ha^{-1}, dry basis.

The amount of Cd found in coffee peel and seed did not differ statistically in the studied soils ($F_{2,25}$ = 0.17 and 2.29, and p-value = 0.8446 and 0.1225 respectively for peel and seed). However, it differed significantly for doses of sewage sludge in each soil ($F_{3,25}$ = 9.56 and 6.28, and p-value < 0.0001 respectively for peel and seed). The amount of Cd found in coffee peel and seed increased with application of sewage sludge (Figure 6). Concentrations of Cd obtained in the greater dose of sewage sludge resulted in 49.2 and 50.5 µg kg^{-1} of Cd respectively in the peel and seed (Table 4). Martins (2003) found content of Cd about 0 and 70 µg kg^{-1} in coffee peel, and 70 and 30 µg kg^{-1} in coffee seed. These were obtained from Acaiá IAC-474 varieties with 9 and 6 years, grown on Dystrophic Red Latosol and Dystrophic Red Argisoil, with cumulative doses of sewage sludge, respectively about 29.8 and 18 Mg ha^{-1}.

The amount of Ni found in coffee peel and seed was significantly different in studied soils ($F_{2,28}$ = 44.39 and p-value < 0.0001), as well as for doses of sewage sludge in each soil ($F_{3,28}$ = 10.31 and p-value <0.0001). The amount of Ni found in peel from coffee grown on soils without

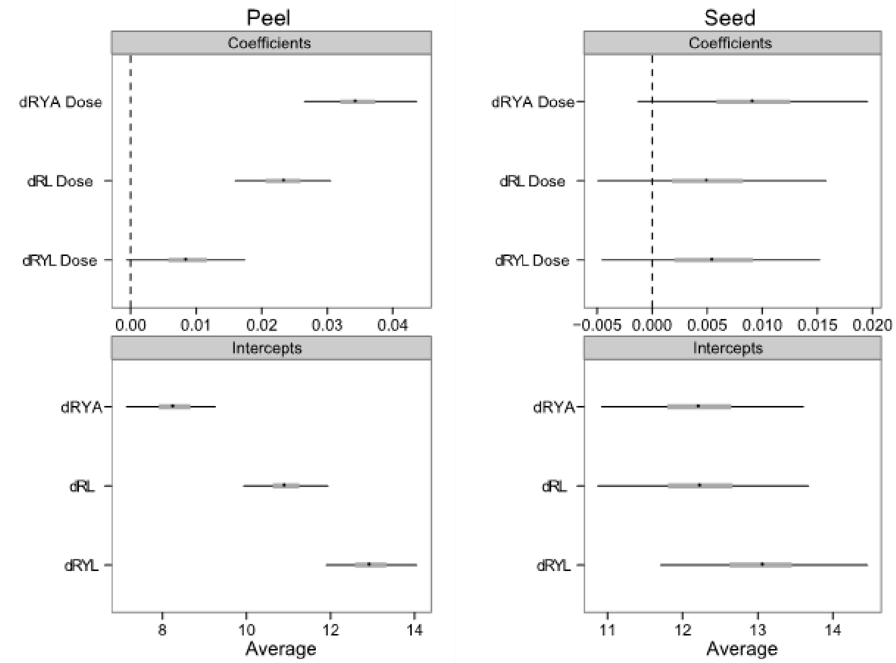

Figure 4. Estimate, standard error, and confidence interval for coefficients of linear model for content of copper (mg kg^{-1}) found in coffee peel and seed. These parameters are shown on the model describing the effect of soils and doses of sewage sludge in each soil. The intercepts refer to soil with no application of sewage sludge. Coefficients refer to estimates of doses of sewage sludge in soils under study.

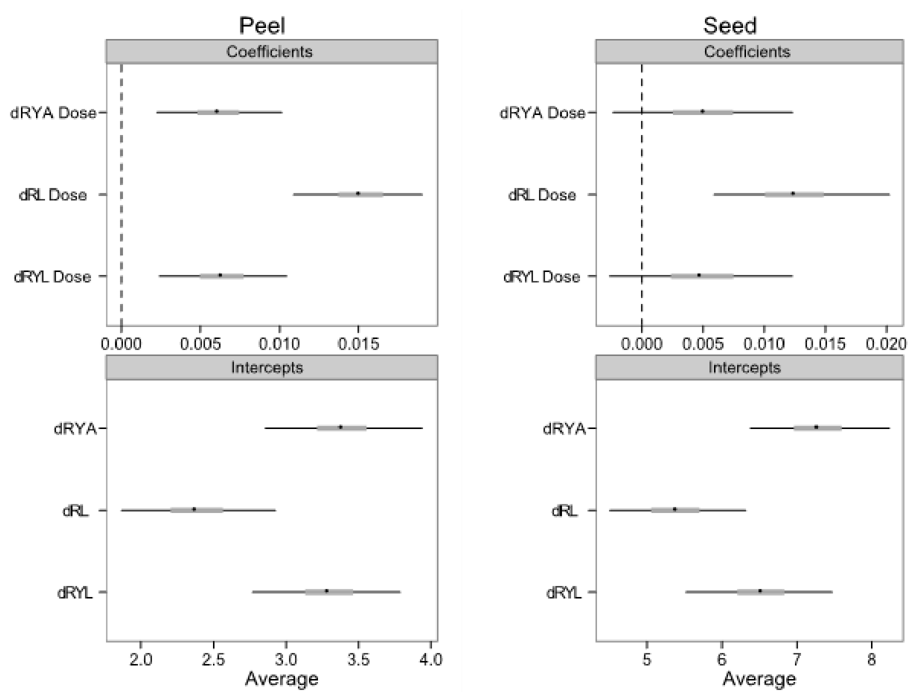

Figure 5. Estimate, standard error, and confidence interval for coefficients of linear model for content of zinc (mg kg^{-1}) found in coffee peel and seed. These parameters are shown on the model describing the effect of soils and doses of sewage sludge in each soil. The intercepts refer to soil with no application of sewage sludge. Coefficients refer to estimates of doses of sewage sludge in soils under study.

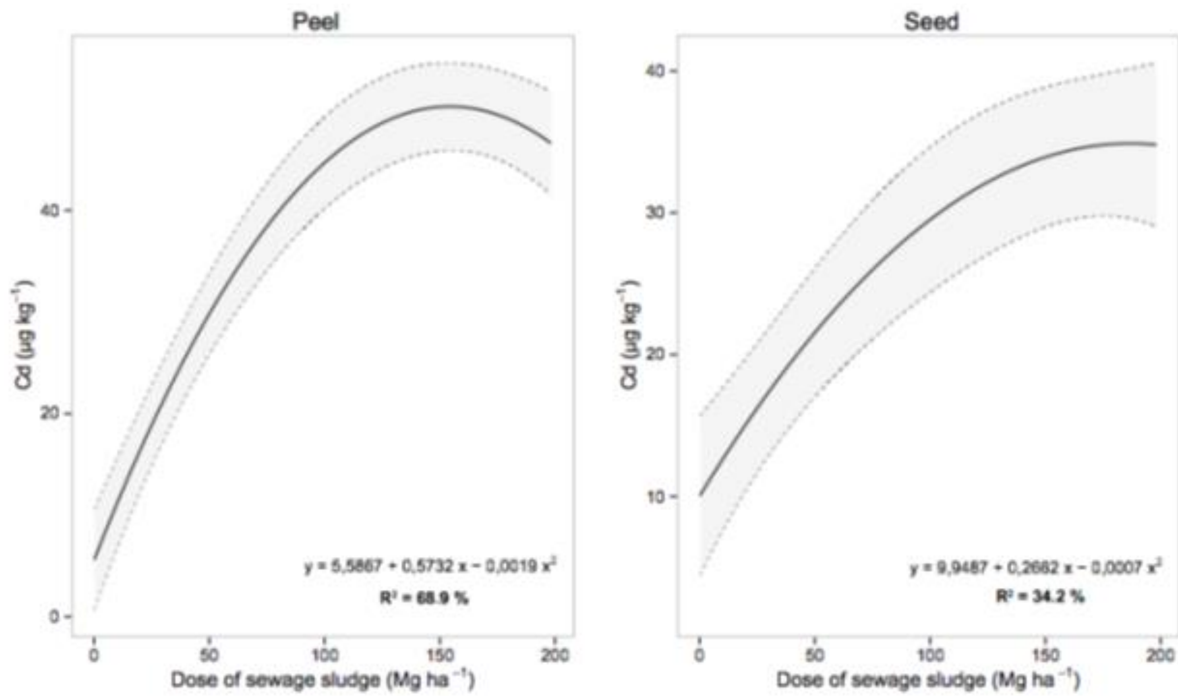

Figure 6. Content of Cadmium in coffee peel and seed in the first year post-planting under different doses of sewage sludge. These amounts were found from coffee grown on Dystrophic Red Yellow Latosol, Dystropherric Red Latosol and Dystrophic Red Yellow Argisoil. The solid line refers to estimated average, and the dotted lines show the interval of standard error.

sewage sludge were 0 for dRL and dRYA, and 0.5 mg kg^{-1} for dRYL (Table 4). Martins (2003) found similar amount of Ni in coffee peel, about 0.2 mg kg^{-1}, obtained from coffee grown on Eutropherric Red Latosol without sewage sludge.

The content of Ni found in coffee peel, obtained from coffee grown on dRL, increased with application of sewage sludge. In addition, it was not affected on dRYL and dRYA (Figure 7). Concentrations of Ni obtained in this soil were around 0 and 0.4 mg kg^{-1}, respectively for the lesser and greater dose of sewage sludge (Table 4). The amount of Ni found in coffee seed was statistically different in soils under study ($F_{2,28}$ = 32.59 and p-value < 0.0001), and did not differ for doses of sewage sludge in each soil ($F_{3,28}$ = 2.37 and p-value = 0.092). The content of Ni found in coffee seed for coffee grown in soils without sewage sludge was greater on dRYL (0.7 mg kg^{-1}) than on dRL and dRYA (0.4 and 0.1 mg kg^{-1}, respectively) (Table 4). Martins (2003) found similar amount of Ni in coffee seed, about 0.5 mg kg^{-1}, from coffee grown on Eutropherric Red Latosol, without sewage sludge.

The amount of Ni found in coffee peel, obtained from coffee grown on dRL soil increased with application of sewage sludge (Figure 7). Concentrations of Ni obtained from this soil were around 0.4 and 0.6 mg kg^{-1}, respectively for lesser and greater dose of sewage sludge (Table 4). Martins (2003), found similar amount of Ni in coffee seed, about 0.1 and 0.3 mg.kg^{-1}, respectively from coffee grown on Dystrophic Red Latosol and Dystrophic Red Argisoil; with cumulative doses of sewage sludge, respectively about 29.8 and 18 Mg ha^{-1}, dry basis.

The amount of Pb found in coffee peel was statistically different in the studied soils ($F_{2,28}$ = 4.31 and p-value = 0.023), and did not differ in the coffee seed ($F_{2,28}$ = 2.00 and p-value = 0.154). The content of Pb found in the coffee peel was not affected by doses of sewage sludge in each soil ($F_{3,28}$ = 1.22 and p-value = 0.32), but it was affected in the coffee seed ($F_{3,28}$ = 5.51 and p-value = 0.004). The content of Pb found in coffee seeds grown on dRL increased only with application of sewage sludge (Figure 8).

Estimates obtained for Cr in the coffee peel and seed are lesser than the detection limit of the equipment (2.13 µg kg^{-1}). Martins (2003) also found lesser content of Cr in coffee fruits, about 3.5 µg kg^{-1} in the peel and 1.2 µg kg^{-1} in the grain. However, factors related with soil, plant, and sewage sludge exert control over the availability of Cr.

Conclusions

The sewage sludge increased the content of Cd, Cr, Cu,

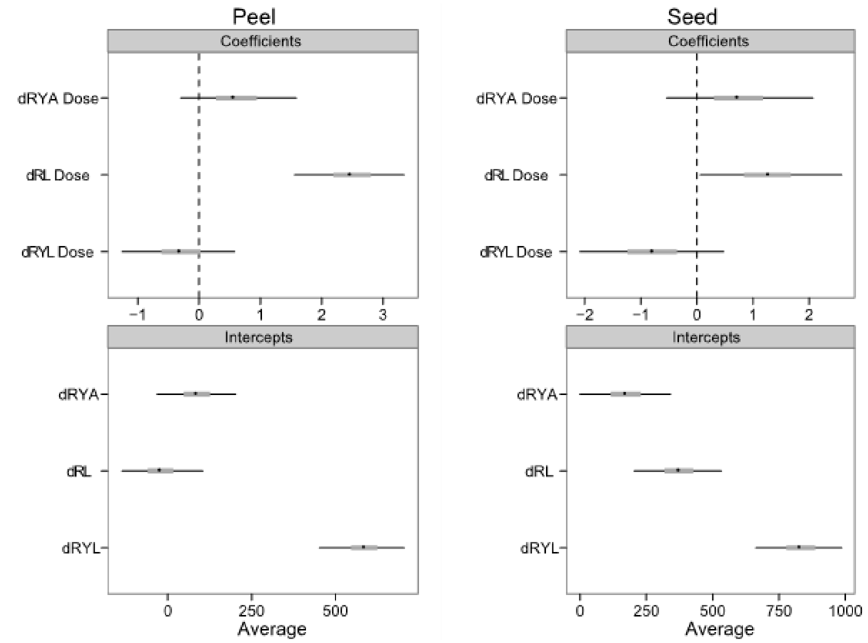

Figure 7. Estimate, standard error, and confidence interval for coefficients of linear model for content of nickel (mg kg^{-1}) found in coffee peel and seed. These parameters are shown on the model describing the effect of soils and doses of sewage sludge in each soil. The intercepts refer to soil with no application of sewage sludge. Coefficients refer to estimates of doses of sewage sludge in soils under study.

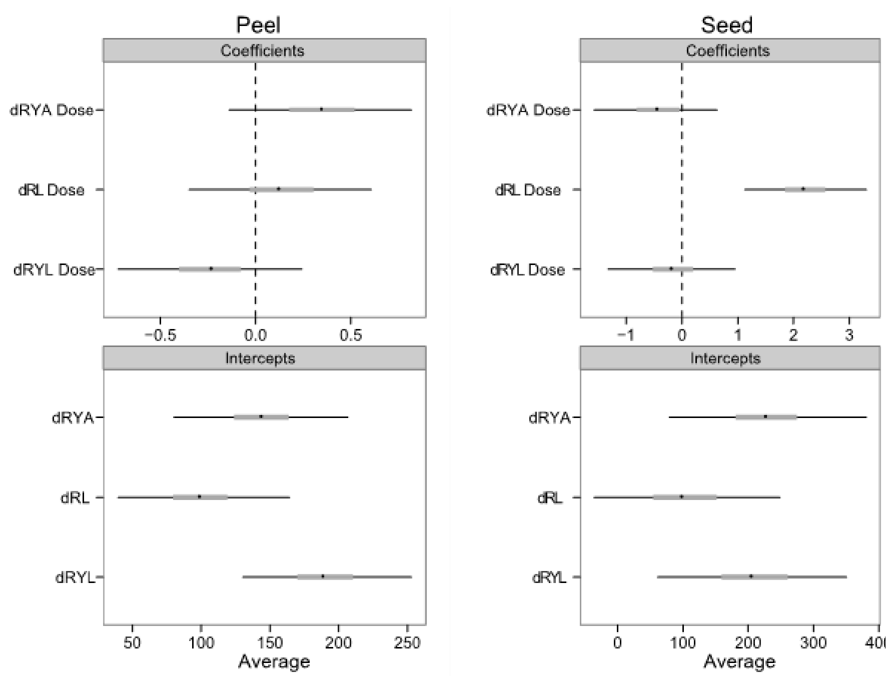

Figure 8. Estimate, standard error, and confidence interval for coefficients of linear model for content of lead (µg kg^{-1}) found in coffee peel and seed. These parameters are shown on the model describing the effect of soils and doses of sewage sludge in each soil. The intercepts refer to soil with no application of sewage sludge. Coefficients refer to estimates of doses of sewage sludge in soils under study.

Pb and Zn found in studied soils, but their estimates were found to be less than those established for contamination prevention.

The sewage sludge decreased growth and productivity of coffee plants in studied soils. The sewage sludge increased the content of Cu, Zn and Cd found in coffee peel, as well as the content of Cd found in coffee seed in the studied soils. The sewage sludge increased the content of Zn and Pb found in coffee seed, for that grown on Dystropherric Red Latosol. Therefore, the content of trace elements found in coffee fruits are within allowed levels. So sewage sludge application to soils under coffee crops is able to increase trace elements in soils and decrease coffee productivity without any risk for human beings.

Conflict of interests

The authors have not declared any conflict of interests.

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Hydro-physical properties and organic carbon of a yellow oxysol under different uses

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Intensive soil use without a management plan based on its structures and limitations is the main cause of soil erosion and degradation. The physical properties changes of a studied agricultural area are evaluated by comparing with an area that did not suffer any modification caused by human activities, which is considered the ideal soil condition. This research evaluated the alterations in the hydro-physical properties and the organic carbon content of a Eutrophic Yellow Oxysol under different use and management systems in the Triângulo Mineiro, an important agriculture region in Brazil. The systems studied were, Cerrado with typical *stricto sensu* vegetation preserved for over 30 years; eucalyptus planted about 30 years ago, without fertilizer or cultivation since the implantation period; corn grown under the no tillage system intercropped with pasture; and *Brachiaria* sp. under no-tillage intercropped with corn, managed with cattle and annually fertilized with turkey litter. The soil bulk density, total porosity, size distribution of the pores, flocculation degree, geometric mean diameter, water infiltration and organic carbon were evaluated. The effects of land use and management system alterations on soil physical properties were similar at both depths, except the geometric mean diameter, which was higher in the surface layer. The eucalyptus and no-tillage systems with corn and pasture recovered the soil organic carbon contents as compared to the preserved Cerrado area, which was more significant in the surface layer. The soil with native Cerrado vegetation presented faster infiltration in relation to other systems followed by, in descending order, the areas planted with eucalyptus, pasture and corn. The infiltration rate and cumulative infiltration curves were similar to values obtained in the field.

Key words: Management systems, no-tillage, soil structure, water infiltration.

INTRODUCTION

Soil degradation is a serious environmental problem throughout the world, which may lead to a decline in soil quality with a continuing reduction of productivity. Inappropriate agricultural practices, among deforestation and other human activities, is an important driving force of soil degradation and the need to reduce the environmental impact of agricultural activities and to control soil degradation is one of the main aims of management systems.

Changes due to different management systems are evaluated by accessing chemical, physical and biological soil properties by comparing managed areas and areas under natural vegetation (Barros and Comerford, 2002). Studies by Tollnerm et al. (1990) and Broersma et al. (1995) showed that land use changes from natural and semi-natural vegetation to cultivated and grazed lands could affect soil bulk density, porosity and water storage, water infiltration, and water flow characteristics and surface runoff.

In Brazil, most of the central part is covered by seasonal savanna, known as "Cerrado", in which Oxisols are the predominant soils in the region (46%). These soils are well drained, but strongly and deeply weathered and have natural poor fertility. Even presenting all these chemical constraints, the potential for arable land use and technological advances in soil and crop management in these vast areas have made the Cerrado the agricultural frontier of Brazil since the early 1970s, emphasizing its importance to the agriculture in the country.

In tropical soils, organic matter is one of the main properties responsible for the soil quality. Adequate levels of organic matter can minimize the impacts of agricultural practices and maintain the soil fertility. Thus, the adoption of conservation cropping systems, such as no-tillage, is fundamental to promote increased stocks of organic matter.

The water infiltration rate is another property that best reflects the soil quality and its structural stability. For Pott and de Maria (2003), the infiltration rate is one of the processes that best demonstrates the soil hydro-physical conditions due to the structural quality conditions such as the pore size distribution which is favorable to the root growth, aeration and soil water infiltration.

The studies on land use impacts have been focused mostly on soil physical and chemical properties, and few researches have investigated the effects on soil hydraulic properties. Soil hydraulic properties consist of soil water retention and hydraulic conductivity functions (Hussein and Warrick, 1995). These properties are influenced by several factors including soil texture, structure, bulk density and organic carbon. It is, therefore important to explore the effects of soil management systems on water infiltration capacity and its relationships with chemical and physical soil properties for a better understanding of the soil quality.

Thus, this study was conducted in the central region of Brazil named Triângulo Mineiro with the objective of evaluating alterations in hydro-physical properties and organic carbon content of an Oxysol under different uses and management systems.

MATERIALS AND METHODS

Characterization of the study area

The work was conducted on the Santa Terezinha farm, Uberaba, MG (19° 12' 11" S, 48° 11' 30" W at an altitude of 830 m). The climate is characterized as rainy tropical with a dry winter (Antunes, 1986). The original vegetation of the study area was the cerrado type (natural vegetation in Brazil).

Four areas were selected, the use of which can be characterized as: (a) Cerrado: vegetation typical of *stricto sensu* cerrado, without history of anthropic interference, preserved for more than 30 years. This area was chosen as a reference for comparison of the alteration of the evaluated characteristics; (b) Eucalyptus: Eucalyptus forested area, established for 30 years, without fertilization or cultivation since the implantation period; (c) Corn: Corn crop in no-tillage (NT), intercropped with pasture, according to the Santa Fé cultivation system - SSF; (Kluthcouski et al., 2000); (d) Pasture: area with *Brachiaria* sp. under NT, intercropped with maize, being managed with cattle and fertilized annually with turkey litter.

The physical and chemical characteristics of the soil, according to methods described in EMBRAPA (1997) are presented in Table 1. All these areas have the same soil type, which was classified as Eutrophic Yellow Oxysol, sandy loam texture, according to the Brazilian Soil Classification System (Santos et al., 2013).

Soil sample and variables analyzed

In June 2012, soil sample collections and the soil water infiltration rate measurements were carried out. During the assessments there was total absence of rain in the experimental area. In each area, four samples were taken in the 0 to 20 and 20 to 40 cm layers, in four points distributed at random. At each point, samples with disturbed structure were collected, placed in plastic bags, sieved at 4 mm and air-dried. These samples were used for particle size determination by the pipette method (Gee et al., 1986) and geometric mean diameter (GMD) by the method of Kemper and Chepil (1965).

Undisturbed soil samples were collected at the same locations mentioned above, using a Kopeck volumetric ring and Uland sampler. After collection, the samples were wrapped in screen fabric and sent to the laboratory for determination of bulk density (BD), macro- (Ma) and micro porosity (Mi) and total porosity (Pt) by the voltage table method, while the bulk density (BD), conducted by the volumetric ring method, was calculated as the ratio between the weight of dry sample in the oven and the ring volume (EMBRAPA, 1997).

For the organic carbon (OC) analyzes, single samples were collected at two depths and four points in each area, and after thoroughly mixing, they were air dried (ADFS) and analyzed as proposed by Yeomans and Bremner (1988).

The determination of the water-dispersible clay (WDC) was performed by the volumetric pipette method according to the

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Table 1. Physical and chemical characteristics of a *Eutrophic Yellow Oxysol* under different use and management systems in the Fazenda Santa Terezinha, Uberaba, MG.

Systems	Sand	Silt	Clay	pH	P (mg dm^{-3})	Mg^{2+}	Ca^{2+}	K^{+}	Al^{3+}	H+Al
	g kg^{-1}					cmol$_c$ dm^{-3}				
					0-20 cm					
Cerrado	784	25.2	190.8	5.1	1.5	0.1	0.1	0.06	0.4	2.7
Eucalyptus	794.8	44.0	161.2	5.2	4.5	0.3	0.3	0.09	0.4	3.3
Pasture	780.4	54.8	164.8	6.1	37.5	0.2	1.5	0.07	0	1.6
Corn	674.2	29.8	296.0	5.5	82.4	0.4	2.0	0.09	0	2.4
					20-40 cm					
Cerrado	759.2	25.7	215.5	5.5	0.7	0.1	0.1	0.05	0.3	2.3
Eucalyptus	794.8	44.0	161.2	5.2	4.5	0.3	0.3	0.09	0.4	3.3
Pasture	785.3	41.0	173.7	6.0	4.2	0.1	0.8	0.07	0	1.6
Corn	698	32.0	270.0	5.5	17.4	0.3	1.1	0.05	0	2.4

pH in water, ratio 1:2.5. Ca^{2+} and Al^{3+}: extractor KCl 1 mol L^{-1}. H+Al: extractor Ca $(OAc)_2$ 0.5 mol L^{-1} pH 7.0. P and K: extractor Mehlich-1.

methodology described by EMBRAPA (2009), and with this result the flocculation degree (FD, %) of the clay fraction was calculated: x cylinders method described by Bernard et al. (2006) was used. After soil surface cleaning in each system used, the cylinders cylinders method described by Bernard et al. (2006) was used. After soil surface cleaning in each system used, the cylinders (external and internal) were fixed on the ground concentrically, to 15 cm deep. The infiltration was measured in the inner ring, with the aid of a graduated ruler, since the purpose of the outer ring was to prevent the infiltration from laterally proceeding into the soil. The water was added simultaneously in the two rings to a height of 9 cm of depth, allowing a maximum oscillation of 4 cm. Timing counting began when the water level reached a height of 7 cm and finished when it reached 5 cm. Constant infiltration was considered as when the value of the reading was repeated at least three times; at this point, the process was suspended, and it was considered that the infiltration rate had been reached.

With the results collected in the field, the cumulative infiltration (Z) and instantaneous infiltration rate (I) curves were obtained. A considerable number of readings were taken in the use system with eucalyptus (N=73) and cerrado (N=21), but in some systems used such as pasture (N=9) and corn (n=8), fewer readings were taken due to the rapid attainment of the constant infiltration rate.

To empirically estimate the water infiltration rate, the model proposed by Kostiakov in 1932 (Equation 1) was used, which describes well the water infiltration in short periods (Bernardo et al., 2006):

$$Z = kt^{a} \quad (1)$$

Where Z is the cumulative infiltration (mm), t is the infiltration time (min), k and a are two constants dependent on soil type. The parameters (k and a) of the Kostiakov equation were obtained by linear regression, as follows: Applying the logarithm of both terms of the equation, a straight line equation log I = log k + a log t is obtained, that is, the log I graphic as a function of log t, which is a straight line, a is the angular coefficient and log k, the linear coefficient. With this graphic device, I = *f*(t) we obtained, a very useful equation because with it I can be calculated for any time t.

The instantaneous infiltration rate (I) of water into soil (Equation 2) was obtained by deriving the cumulative infiltration versus time equation. Where I is the instantaneous infiltration rate (cm min^{-1}), t is the infiltration time (minutes), and k and a are two soil-type dependent constants.

$$I = k\,a\,t^{a-1} \quad (2)$$

Data processing and statistical analysis

The results were submitted for analysis of variance using a completely randomized design in a split plot design, with the treatments (use systems) considered as plots and soil layers as subplots. The means were compared by Tukey test at 0.05 of significance.

To verify the suitability of the estimated I and Z via the Kostiakov model compared with the values obtained in the field with the ring infiltrometer, hierarchical cluster analysis was used, by calculating the Euclidean distance between accesses. Among the algorithms, Ward was used to obtain similar access clusters, since in this method, the distance between the two groups is defined as the sum of squares between the two groups by all the variables. The results of the analysis were presented in graphical form (dendrogram) which aided in the identification of access groups (Hair et al., 2005).

RESULTS AND DISCUSSION

Analyzing the soil physical properties, the macroporosity (Ma), bulk density (Ds) and the flocculation degree (FD) differed according to systems use and management, without variation between depths, as shown in Table 2.

It can be seen that the two NT systems used, corn and pasture, presented lower Ma values (0.08 and 0.11 cm^{3} cm^{-3}) as compared to cerrado vegetation and eucalyptus (0.19 cm^{3} cm^{-3}) which may be due to the absence of tillage and the intense machine traffic (planting, treatments, harvesting) or animals in the pasture (trampling) that provide reduction of this parameter. The Mi and Pt did not differ between the systems evaluated, confirming the results of Melloni et al. (2008), who also found no differences in Mi between eucalyptus, pasture and cerrado systems.

Table 2. Macroporosity (Ma), microporosity (Mi), total porosity (Pt), bulk density (D_S),, flocculation degree (FD) and organic carbon (OC) in different land use and soil management systems and at two depths, evaluated on the Santa Terezinha farm, Uberaba, MG.

Systems	Ma	Mi	Pt	Ds	FD	OC
	$cm^3\ cm^{-3}$			$g\ cm^{-3}$	$g\ kg^{-1}$	
Cerrado	0.19^a	0.28^a	0.47^a	1.56^{ab}	319^{ab}	9.12^b
Eucalyptus	0.19^a	0.28^a	0.47^a	1.46^b	489^a	10.38^{ab}
Pasture	0.11^b	0.30^a	0.41^a	1.60^a	266^b	9.88^{ab}
Corn	0.10^b	0.31^a	0.41^a	1.55^{ab}	439^{ab}	13.12^a
Depths						
0-20 cm	0.13^a	0.29^a	0.42^a	1.54^a	391^a	13.19^a
20-40 cm	0.14^a	0.28^a	0.42^a	1.55^a	366^a	8.06^b

Averages followed by the same letter in column, between systems and depths, were not statistically different by the Tukey test ($p < 0.05$).

Regarding soil density, the values found in all the land uses (between 1.46 and 1.60 g cm^{-3}) are below the range classified by Reichert et al. (2003) for sandy loam soils, ranging from 1.70 to 1.80 g $cm^{-3.}$ Thus, the Ds values found in the present study in the four use systems are not considered harmful to plant development in sandy loam textured soils and therefore do not limit root growth of the species. In Arshad et al. (1996) study, the minimum density value for sandy soils above which there would be restricted to root development ranges from 1.70 to 1.75 g cm^{-3}.

The clay flocculation degree (FD) remained between 266 and 489 g kg^{-1} among soil use and management systems, without variation between depths. Eucalyptus had the highest clay FD in relation to other systems, while pasture had the lowest FD. These results may be related to chemical factors, such as the H+Al contents, which are the main flocculation agents in acid soils (Morelli and Ferreira, 1987). In this work, the H+Al contents were higher in the use system with eucalyptus, followed by corn, cerrado and pasture, in the two layers evaluated (Table 1), which suggests that most of the clay flocculation in the eucalyptus system is due to the aggregating effect of the H^+ and Al^{3+} cations. The exchangeable soil cations such as Ca^{2+} and Mg^{2+}, significantly influenced the aggregation process, but in acid soils, the effect of Ca^{2+} on aggregation is not as important because H^+ saturated soils flocculate more than Ca^{2+} saturated soils (Baver, 1952). Another important factor in the aggregation process refers to organic matter, which was significantly higher in the surface layer, as seen from the OC content (Table 2), which justifies the beneficial effect of organic matter on clay flocculation in areas with eucalyptus and corn.

Use systems and soil management significantly affected the OC, which varied from 9.12 to 13.12 g kg^{-1}. The area under maize grown in NT had the highest OC content, differing from the cerrado area, where the lowest levels were observed. The non-soil tillage combined with the contribution of grass residue, which is more lignified, on the surface in no-tillage, can lead to the slower residue decomposition, promoting the increase in soil organic matter stocks, as seen in the area under maize.

In the cerrado area, on the other hand, the diversity of plant species, notably higher than that of other systems evaluated, will result in litter deposits of organic substrates with varied composition. Moreover, there is greater diversity of organic compounds deposited in the rhizosphere, which favors the growth of different groups of microorganisms in the soil and may help stimulate its decomposition activity. Thus, the different soil conditions under cerrado vegetation make the existence of lower OC levels possible, given the mineralization of organic matter by a more intense activity of the microbial biomass. The areas under eucalyptus and pasture have similar behavior in relation to the OC content, with intermediate values for this characteristic.

The highest OC content occurred in the upper soil layer, where the residues were concentrated, presenting a reduction of 39% in the 20 - 40 cm layer, regardless of the system of use. Nevertheless, the OC levels are considered low, which may be related to the sandy loam soil texture (high sand content), which provides lower organic compound binding with mineral colloidal constituents and therefore less physical protection, facilitating their microbial decomposition (Bayer et al., 2000).

The aggregate stability, measured by the average aggregate geometric diameter (GMD) shows that in the surface layer, eucalyptus and pasture systems propitiated values similar to the Cerrado, with the exception of corn, which was less efficient with respect to aggregation (Table 3). In the subsurface layer, the soil use systems do not differ from each other, indicating similarity in soil structure of all the investigated areas. By presenting a higher diversity of plant species in various stages of growth, the occurrence of larger aggregates in the cerrado may have been influenced by the volume of

Table 3. Geometric mean diameter (GMD) in different land use and soil management systems and at two depths, measured on the Santa Terezinha farm, Uberaba, MG.

Systems	GMD (mm)	
	0-20 cm	20-40 cm
Cerrado	1.22^{aA}	0.92^{aB}
Eucalyptus	1.15^{abA}	0.75^{aB}
Pasture	1.10^{abA}	0.65^{aB}
Corn	0.72^{bA}	0.66^{aA}

Means followed by the same letter in the column, lowercase at the same depth and uppercase between depths, do not differ by the Tukey test (p <0.05).

roots, emphasizing the physical effect of the roots on the formation, maintenance and size of the aggregates.

High aggregate stability is related to lower soil density, which was found in this study for the use system with eucalyptus. In addition to the eucalyptus, the amount of organic waste produced may have been responsible for improving aggregate stability, contributing to protection from environmental agents and maintenance of carbon stocks in the system. In the pasture, a factor that may have contributed to the highest GMD values at 0-20 cm is the compaction caused by animal trampling, which makes the microaggregates unite into larger aggregates, through compressive forces, which increases the aggregate GMD.

For corn, the OC content was significantly higher in relation to other uses and did not present a relationship with the aggregate stability. The absence of correlation between the OC availability with GMD and the soil aggregate classes was also found by Almeida et al. (2014). However, in the soil there may also be more aggregate stability promoted by increased organic material accumulation (Beutler et al., 2001; Souza et al., 2008).

With regard to the depths, the highest GMD values were found in the surface layer, except for under corn, which showed similar values in the two layers. The organic matter is one of the principal soil particle aggregating agents, and therefore, in tropical soils, it is expected that part of the aggregate size variation, and in consequence, the aggregation rates, may be attributed to variation in the soil organic matter content. Thus, the higher soil aggregation in the 0 - 20 cm layer may be related to higher OC levels, while lower levels in the 20 - 40 cm layer may have contributed to the reduction the GMD of the systems evaluated.

The mean infiltration rate (I) and cumulative infiltration (Z) values for water into soil, obtained in field through the ring infiltrometer for the four system uses are shown in Figure 1. The soil with native cerrado vegetation had little variation in I during the evaluation time and had higher average in relation to other use systems followed by, in descending order, the areas planted with eucalyptus, pasture and corn. For Bono et al. (2012), removal of natural vegetation and the introduction, of either pasture or crops, leads to a consequent reduction of the basic water infiltration rate, confirming the findings of the present study.

At the start of the evaluation, the I in the cerrado was higher (320 cm h^{-1}) and decreased to a nearly constant value, stabilizing with about 245 cm h^{-1}. The area with eucalyptus showed the highest amplitude in relation to other areas, with I varying from 154 to15 cm h^{-1}. Corroborating with the results observed in this study, Araújo et al. (2007) evaluated the soil quality in the native cerrado area and in areas under different uses and found values of 204 and 185 cm h^{-1}, respectively, for native cerrado areas and pine forestation.

The higher I values, and thus of Z, observed in the cerrado are associated with increased Ma (Table 2) and larger aggregate size, which can be verified through their GMD (Table 3). Comparing the areas under NT, the corn showed lower I in relation to pasture, initially presenting 10 cm h^{-1} and stabilizing at 9 cm h^{-1}, while the pasture presented 36 cm h^{-1} at the start of the tests, reaching stabilization at 24 cm h^{-1}. The lowest water infiltration rate in the soil under NT is due to the soil structure degradation process (compression) caused by particle densification and the higher density in the surface layers (Pinheiro et al., 2009). Thus, the lower I values observed in soil cultivated with maize may be due to alterations introduced in the soil during the planting and harvesting processes, which caused increase in the Ds (Table 2), with consequent reduction of Ma and infiltration capacity, indicating greater physical degradation as compared to the soil cultivated with pasture.

The inverse of the I behavior can be observed in the Z of water in soil, which increased in all the land uses and reached the highest values in the area with eucalyptus, followed by savannah, pasture and corn.

I and Z curves estimated by the empirical model proposed by Kostiakov, using data observed in the field determinations, are shown in Figure 2. The values estimated by the equation and those from the field via double ring infiltrometer, were significantly and positively correlated for I (r = 0.94) and Z (R = 0.98). This similarity between the methods was also seen in the cluster analysis with the formation of distinct groups, respectively, for Z (Group 1) and I (Group 2) (Figure 3).

Conclusions

1. Alterations in the physical properties of the use and management system soils are similar at both depths, except the geometric mean diameter, which is greater in the surface layer. The eucalyptus system and no-tillage systems with corn and pasture recover the soil organic carbon contents ad compared to the preserved Cerrado area, which is more significant in the surface layer.
2. The soil with native cerrado vegetation presents a

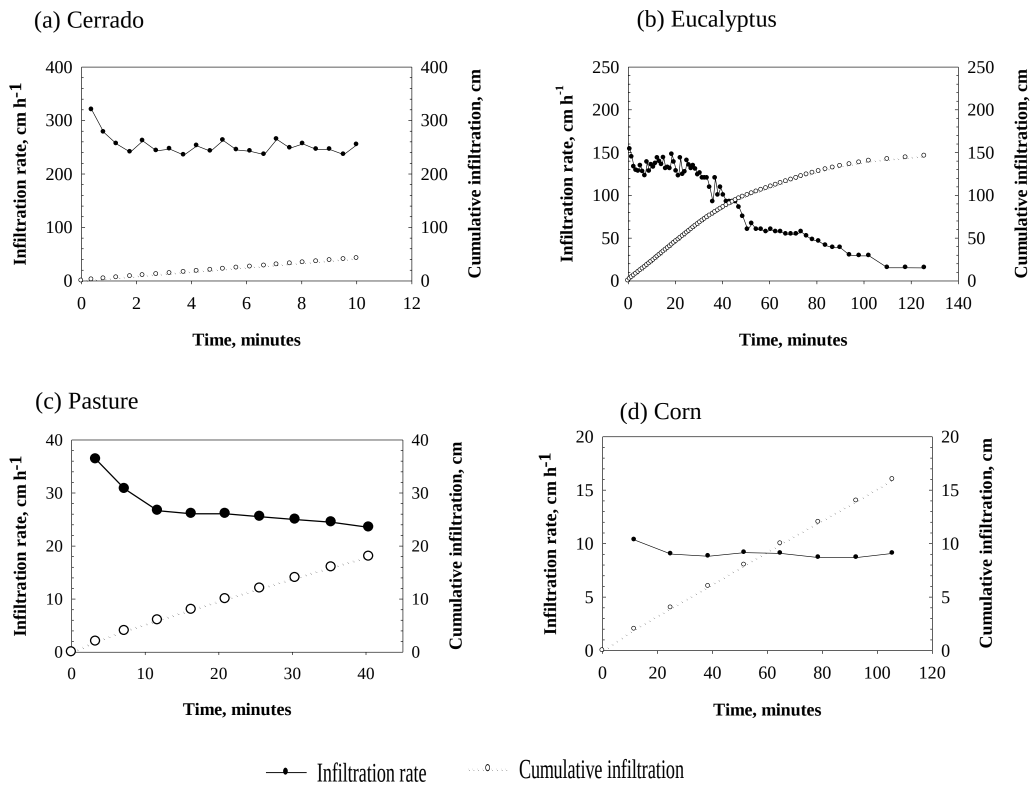

Figure 1. Field values obtained for infiltration rate and cumulative infiltration of water into soil in use systems: Cerrado (a), eucalyptus (b), pasture (c) and corn (d).

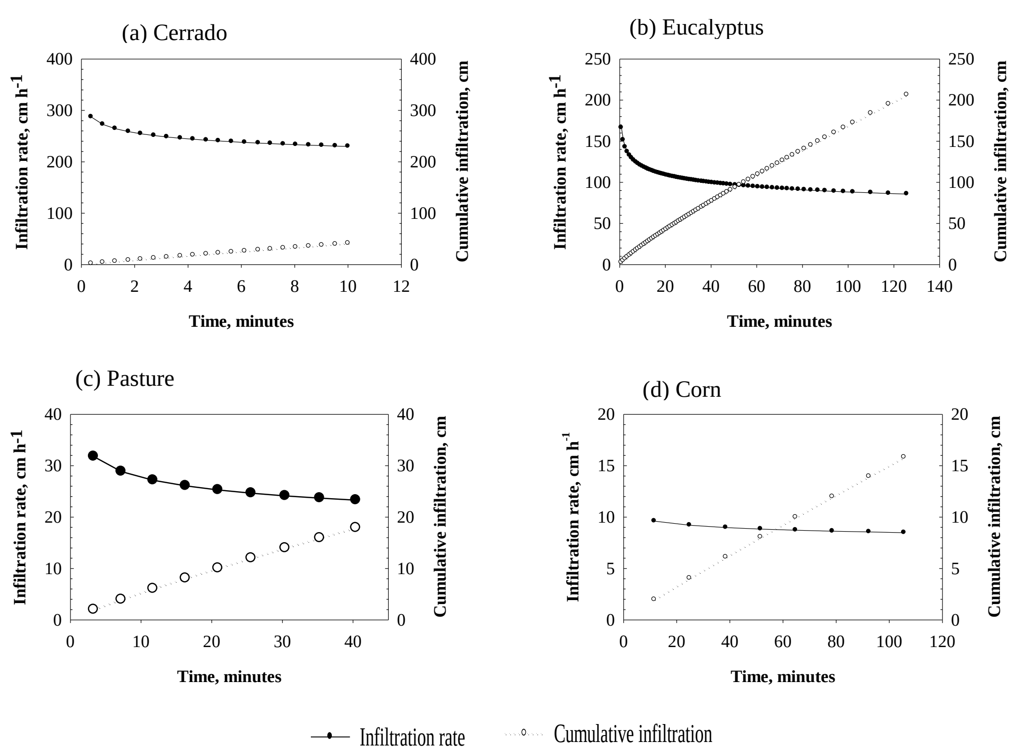

Figure 2. Kostiakov model estimated values of infiltration rate and cumulative infiltration of water into soil in use systems: Cerrado (a), eucalyptus (b), pasture (c) and corn (d).

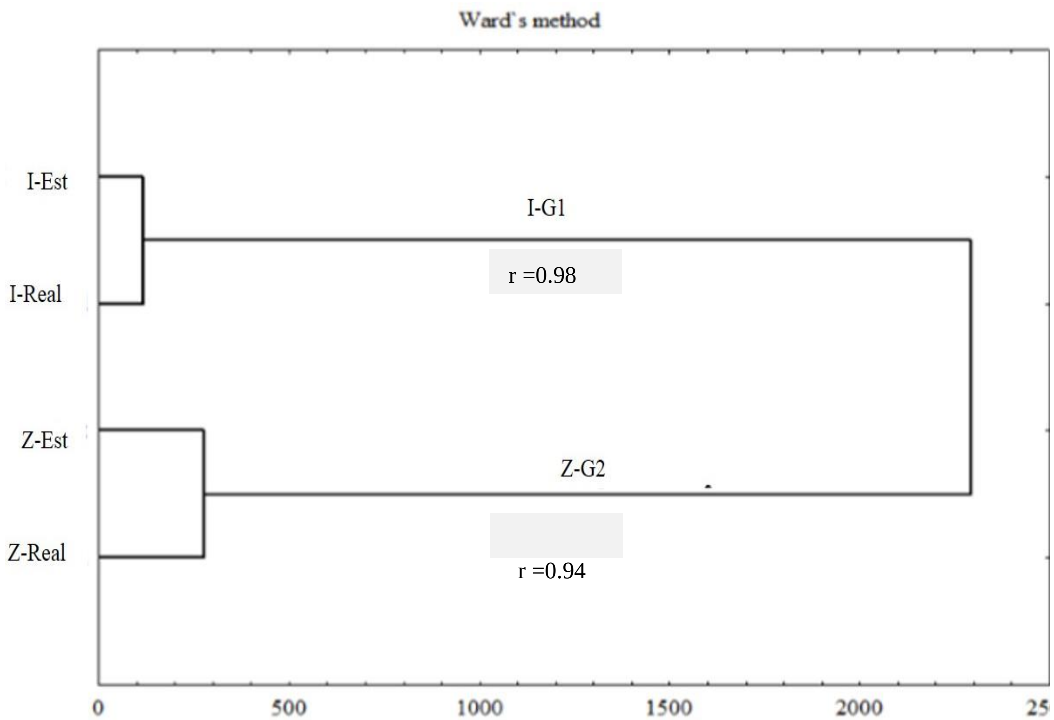

Figure 3. Cluster analysis represented by a dendrogram for the variables: infiltration rate (I) and cumulative infiltration (Z), under real (real) and estimated (Est) conditions. In the graph, r represents the Pearson correlation coefficient ($p < 0.05$).

higher infiltration rate in relation to other systems, followed by, in descending order, the areas planted with eucalyptus, pasture and corn.

3. The infiltration rate and cumulative infiltration curves, estimated by the empirical model proposed by Kostiakov, are similar to values obtained in the field.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Association of weather variables with yield and yield components of cotton (*Gossypium hirsutun* L.) at reproductive phenophase

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Growth and development of cotton is influenced by several environmental factors such as fertility and biology of soil, change in temperature, amount and distribution of rainfall and carbon dioxide concentration which are attributes of climate change. A field experiment was conducted to study the contribution of weather variables for the total variation in yield and yield components during reproductive phenophase of cotton (square initiation to boll opening) during 2013-14 and 2014-15 *kharif* season. The experiment was set out with three sowing time (24, 26 and 28 standard week) as main plot, three deficit irrigation schedules (0.8 IW/CPE, 0.6 IW/CPE and 0.4 IW/CPE) and rainfed as sub plot in split plot design. The result indicated that significant effect of sowing time on yield and yield components of cotton where maximum number of bolls per plant, boll weight, hundred seed weight, ginning percent, and seed cotton yield attained on early sown cotton. Weather variables showed significant correlation with yield and yield components. Results of regression analysis suggested that weather variables such as maximum temperature, mean temperature, relative humidity I and relative humidity II were found to be influential and accounted for over 90% of total variation in seed cotton yield and yield components during the reproductive phenophase.

Key words: Climate, phenophase, standard week, weather variables.

INTRODUCTION

Environment for optimum plant growth and development plays a vital role in realizing crop growth and yields. The time of sowings as varied growth condition for various crops differs depending on climate, varieties and method of cultivation (whether rainfed or irrigated). Knowledge on effects of various elements of environment on crop growth, development and yield is important for agronomists and crop production specialists. Cotton experiences temperature fluctuations ranging from 5 to 45°C during the season, which adversely influence

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growth and development (Reddy, 1994). Study reports also indicated that the high temperatures combined with water stress result in boll shed, small boll size and leaf damage (Hake and Silvertooth, 1990). Reddy et al. (1999) reported a significant decrease in boll growth followed by fruit shed within 3 to 5 days after blossom when there was an increase in temperatures over 32ºC. Sustained changes in temperature during the fiber thickening period will also lead to differences in micronaire (Bange, 2010). Quantitative information regarding plant responses to weather change, soil and management condition essential to design crop adaptation mechanisms and improving productivity.

So far, many researches were undertaken under controlled environment conditions and sowing dates at field conditions. Pettigrew (2002) studied cotton response towards early planting over normal planting date. Dong et al. (2006) compared yield, quality and leaf senescence of cotton of late planting production system and normal planting production system. Moreover, enhancing UV-B radiation effects by Kakani et al. (2003) and temperature influences by Bange (2007) were studied on cotton growth and development. However, these studies did not reveal the relationship among the climatic factors under field situations with yield and yield components. Changes in yields attributes to a single factor such as temperature is not possible due to the confounding effects like rainfall, and solar radiation during crop growing period. Furthermore, controlled environment studies often underestimate yield losses from temperature effects at different phenology of a crop that would occur under field conditions (Paulsen, 1994). Furthermore, studies of crop management practices accompanied with weather conditions plays vital role to provide valuable information for producers. Therefore, this study was designed to identify the critical weather parameters affecting the yield and yield components on cotton.

MATERIALS AND METHODS

A field experiment was carried out for two consecutive *Kharif* seasons (Mansoon) (2013/14 - 2014/15) at Professor Jayashankar Telangana State Agricultural University College Farm, Rajendranagar, Hyderabad, India, located at an elevation of 542.6 m above sea level and lies within 17°19'19.64'' N latitude and 78°24'29.89'' E longitudes. The experiment was designed with three time of sowing at two weeks interval viz., 24 standard week (SW) (D_1), 27 SW (D_2) and 29 SW (D_3) during 2013-14 *kharif* season and 24 SW (D_1), 26 SW (D_2) and 28 SW (D_3) during 2014-15 *kharif* season as main plot and three deficit irrigations schedule as 0.4 IW/CPE (I_1), 0.6 IW/CPE (I_2), 0.8 IW/CPE (I_3) and rainfed as subplot treatments. The treatments were laid out in split plot design in three replications. Irrigation water of 50 mm was applied for the respective treatments when the CPE reaches 125.0, 82.3 and 62.3 mm. Cotton seeds (cultivar Neeraja) were dibbled at 60 × 90 cm spacing and thinned at first two leaf stage to maintain the population to one plant per hill. Recommended fertilizer rate of 120: 60: 60 kg per ha of N, P_2O_5 and K_2O were split applied at 30, 60 and 90 days after sowing to all plots. Harvesting was done manually from a net plot area of 6.48 square meter for four successive pickings and expressed in kilo gram per hectare. Daily weather data on maximum temperature, minimum temperature, mean temperature, relative humidity I, relative humidity II, rainfall, sunshine, wind speed and pan evaporation were collected from weather station, Agricultural Research Institute, Rajendranagar, Hyderabad, India. Data on canopy temperature was recorded on daily bases with infrared thermometer and solar radiation with photo-radiometer at reproductive stage. While the ambient temperature (°C) observed during the period was simultaneously recorded with multi-thermometer.

Daily recorded canopy temperatures and Canopy temperature (T_C) - Ambient temperature (T_A) difference summarized per phonological stages was used to calculate stress degree day and the summarized data during reproductive was used for statistical analysis. Stress degree day (SDD) was calculated using the following formula as suggested by Idso et al. (1977b):

$$SDD = \sum_{n}^{i=1} \left(T_c - T_a\right)_i$$

Total heat requirement for each developmental stage was estimated by accumulating degree days. The following formula was used to compute accumulated degree days from the starting point to predict when the developmental stage was reached.

$$Thermal\ Time\ (Degree\ days) = \sum_{i=1}^{n} (T_{mean} - T_{base})_i$$

Where T_{mean} = mean temperature of daily maximum and minimum, T_{base} = base temperature for cotton (15.5°C), n= number of days between two stages of development.

Mean of weather data of each sowing time that the crop experienced during reproductive stage (73 to 76 mean days after sowing), from square initiation to boll opening stage, were used for correlation regression analysis with yield and yield components of each season mean. After establishing the relationship between weather and yield and yield attributes step down regression analysis (Draper and Smith, 1996) were calculated with SAS software to study the influence of weather parameters on yield and yield attributes. By this analysis, the contribution of respective weather parameters in bringing out the change in yield parameters was known and prediction equation was worked out.

RESULTS AND DISCUSSION

Analysis of variance of yield and yield components

The number of bolls per plant, boll weight, hundred seed weight, ginning percent and seed cotton yield was significantly influenced due to sowing time in both seasons (Table 1). The number of bolls per plant ranged from 16.6 to 27.1, 24.7 to 27.0 and 20.6 to 26.1 during first year (S_1), second year (S_2) and pooled mean, respectively. Higher number of bolls per plant was recorded at D_2 (26 SW) followed by D_1 (24 SW). Boll weight ranged from 2.9 to 3.7, 3.6 to 4.3 and 3.2 to 4.0 g during first year (S_1), second year (S_2) and pooled means, respectively. High boll weight was recorded during early sowing times (D_1 followed by D_2) in both seasons. The HSW of S_1, S_2 and pooled means varied from 8.5 to 9.2, 9.3 to 10.5 and 9.0 to 9.8 g, respectively.

Table 1. Seed cotton yield (kg ha^{-1}) and its components as influenced by sowing time and deficit irrigation during S_1 and S_2 *kharif* seasons.

Treatments	Number of bolls per plant			Boll weight (g)			100 seed weight (g)			Seed cotton yield (kg ha^{-1})			Ginning %		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Sowing time															
D_1	23.6^{a}	27.0^{a}	25.3^{a}	3.7^{a}	4.3^{a}	4.0^{a}	9.2^{a}	10.5^{a}	9.8^{a}	1712^{a}	2228^{a}	1970^{a}	32.5^{a}	32.7^{a}	32.63^{a}
D_2	27.1^{a}	25.1^{b}	26.1^{a}	3.3^{a}	4.0^{ab}	3.7^{a}	8.5^{b}	9.8^{ab}	9.2^{ab}	1711^{a}	1940^{b}	1825^{b}	31.3^{ab}	33.0^{a}	32.19^{a}
D_3	16.6^{b}	24.7^{b}	20.6^{b}	2.9^{b}	3.6^{b}	3.2^{b}	8.7^{ab}	9.3^{b}	9.0^{b}	952^{b}	1798^{b}	1375c	30.2^{b}	34.0^{a}	32.08^{a}
SEm±	0.7	0.7	0.5	0.1	0.1	0.1	0.2	0.2	0.2	74	44	35	0.5	0.4	0.3
CD $_{0.05}$	3.7	1.6	2.1	0.4	0.5	0.3	0.7	1.1	0.7	324	171	136	2.0	1.4	1.1
CV (%)	14.4	5.4	10.9	10.6	10.1	10.2	7.3	9.4	9.5	19.6	7.6	7.0	5.7	3.8	3.0
Irrigation level															
I_1	20.8^{b}	24.5^{ab}	22.6^{b}	3.0^{b}	4.1^{a}	3.5^{a}	8.6^{b}	9.9^{a}	9.3^{ab}	1266^{b}	1845^{b}	1556^{b}	31.2^{ab}	32.7^{a}	31.94^{b}
I_2	23.4^{a}	26.9^{a}	25.2^{a}	3.6^{a}	4.1^{a}	3.9^{a}	9.3^{a}	10.0^{a}	9.6^{a}	1607^{a}	2077^{a}	1842^{a}	31.2^{ab}	33.4^{a}	32.28^{ab}
I_3	25.7^{a}	26.9^{a}	26.3^{a}	3.5^{a}	4.0^{a}	3.8^{a}	8.9^{ab}	9.8^{a}	9.4^{ab}	1551^{a}	2112^{a}	1770^{a}	30.6^{b}	32.7^{a}	31.67^{b}
Rainfed	19.8^{b}	24.1^{b}	21.9^{ab}	2.9^{b}	3.7^{b}	3.3^{a}	8.5^{b}	9.7^{a}	9.1^{b}	1409^{ab}	1919^{b}	1725^{b}	32.5^{a}	34.1^{a}	33.31^{a}
SEm±	0.8	0.8	0.6	0.1	0.1	0.1	0.6	0.2	0.1	85	51	41	0.5	0.7	0.4
CD $_{0.05}$	2.5	2.3	1.8	0.3	0.3	0.2	0.6	0.7	0.4	252	151	122	1.4	2.1	1.3
CV (%)	11.1	9.3	10.5	8.9	6.8	7.5	7.1	5.7	6.2	17.5	7.7	7.1	4.4	6.5	3.9
Grand mean	22.4^{b}	25.6^{a}	24.0	3.3^{b}	4.0^{a}	3.6	8.8^{b}	9.9^{a}	9.3	1458^{b}	1988^{a}	1723	31.4^{b}	33.2^{a}	32.3
Interactions						*P > F*									
Y*D	-	-	0.000	-	-	NS	-	-	NS	-	-	0.001	-	-	0.009
Y*I	-	-	NS	-	-	NS	-	-	NS	-	-	NS	-	-	NS
D*I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.023	NS	NS
D*I*Y	-	-	0.023	-	-	NS	-	-	NS	-	-	NS	-	-	NS

Mean values within columns followed by different letters are significantly different at $P < 0.05$; NS, not significant at 5% level; Y, year; D, time of sowing; I, irrigation level; S_1, 2013-14 *kharif* season; S_2, 2014-15 *kharif* season.

Sowing time one (D_1) followed by D_2 resulted in high mean HSW of 9.8 and 9.2 g, respectively when compared to D_3 (9.0 g). Different sowing times create natural variation in growing conditions for a crop. Ginning percentage ranged from 29.7 to 32.5 and 31.9 to 34.1% during S_1 and S_2, respectively. The result indicated that high ginning percent was recorded for early sowing time during S_1 and late sowing time during S_2. This implied that dry and hot growing conditions showed as positive impact on ginning percent rather than moist and cool conditions. Seed cotton yield ranged from 952 to 1712, 1798 to 2228 and 1375 to 1970 kg ha^{-1} during first year (S_1), second year (S_2) and pooled means. Significantly high mean seed cotton yield was recorded due to D_1 (1970 kg ha^{-1}**)** followed by D_2 (1825 kg ha^{-1}). Cotton grown at sowing time three (D_3) during S_1 was severely affected by heavy rainfall which resulted in mass of square fall, boll shed, lodging, and water logging so that the seed cotton yield of D_3 of S_1 was quite low when compared with seed cotton yield of S_2 and pooled means. The result evidenced strong influence of growing environment on yield and yield components through sowing date variation. All the results supported early sowing times (26 followed by 24 SW) for increasing growth and development of cotton plant. Early sowing time gets favour in day length to attain normal crop growth and development

Table 2. Mean of weather variables prevailed for sowing times during reproductive phenophase of the study seasons.

Year	Sowing time	Tmax	Tmin	Tmean	RHI	RHII	RF	SShr	WS	Epan	GDD	Tc	SDD	InSR	ItSR
	24 SW	29.37	23.27	26.32	85.65	70.12	387.20	234.60	10.33	301.00	746.85	28.00	80.00	146.30	121.80
2013-14	26 SW	28.99	22.56	25.78	88.19	74.21	355.30	223.00	8.04	290.30	698.75	28.00	113.60	146.70	122.30
	28 SW	29.19	21.68	25.44	89.10	72.87	313.00	231.70	6.13	270.70	625.95	28.80	84.70	158.00	133.90
	24 SW	33.19	23.69	28.44	78.98	55.27	118.00	236.50	12.25	269.60	672.85	29.80	60.40	145.10	123.40
2014-15	26 SW	32.17	23.51	27.84	82.78	60.09	154.30	232.80	10.52	201.00	678.75	28.70	55.40	154.20	131.30
	28 SW	31.10	23.17	27.14	85.06	64.50	239.50	223.90	9.27	159.20	628.45	28.80	35.80	168.20	129.10

Tmax, Maximum temperature; SShr, sunshine hours; Tmin, minimum temperature; WS, wind speed; Tmean, mean temperature; Epan, Pan evaporation; RHI, Relative humidity I; GDD, growing degree days; RHII, relative humidity II; Tc, canopy temperature; RF, rainfall; SDD, stress degree days; InSR, incoming solar radiation; ItSR, intercepted solar radiation; SW, standard week.

which escape effect of the cool weather, late rainfall, disease and pest infestation. Similar results were also reported by Dong et al. (2006) who stated significantly low number of bolls in late planting production systems. Reduced boll weight with delayed sowing time was also documented by Ali et al. (2009). Seed weights are components of yield where its contribution strongly influenced by the environment (Johnson et al., 1996). In this connection, significant effect of sowing time on HSW where the higher HSW was produced by early sown maize plants also documented by Dahmardeh (2012). Similarly, Pettigrew (2002) reported that early planted cotton recorded higher lint (about 8%), number of bolls m^{-2} and lint percentage significantly than did cotton in the normal planting.

Correlation of yield and yield components with weather variables

Mean of weather variables prevailed during the reproductive phase of the study seasons and correlation analysis result of weather variables with yield and yield component is presented in Tables 2 and 3, respectively. Weather variables viz., maximum temperature (Tmax), minimum temperature (Tmin), mean temperature (Tmean), relative humidity I and II (RHI and RHII), rainfall (RF) and wind speed (WS) were showed significant effect on boll weight (Bwt), hundred seed weight (HSW) and seed cotton yield (SCY). Boll weight showed positive and significant correlation with Tmax (r = 0.941**), Tmin (r = 0.905*), Tmean (r = 0.985**) and WS (r = 0.936**) and significantly negative with RHI (r = -0.977**), RHII (r = -0.968**) and rainfall (r = -0.827*). Likewise, HSW and SCY showed positive and significant correlation with Tmax (r = 0.954** and 0.751), Tmin (r = 0.805 and 0.945**), Tmean (r = 0.961** and 0.862*) and WS (r = 0.886* and 0.926*). However, the correlation of Tmin with HSW and Tmax with SCY was not significant. Pettigrew (2008) reported that slight elevation in temperature under field conditions was not sufficient to cause a decline in seed weight.

Ginning percent at picking two (GINNII) and four (GINNIV) was negatively and significantly correlated with pan evaporation (r = -0.864* and -0.874*), respectively. Ginning percent at picking four (GINNIV) was also showed negative and significant correlation with stress degree day (r = -0.822*). Low lint percent with insufficient carbohydrate production due to high temperature was demonstrated by Oosterhuis (1999) and Onder et al. (2009); they stated that highest number of opened boll and maximum lint percent resulted from plots under stress condition. Negative effects of relative humidity could be associated with low temperature and disease and pest occurrence which could affect yield and yield components (Blanc et al., 2008).

Regression analysis of yield and yield components with weather variables

Weather variables that showed significant influence on yield and yield components during reproductive phenophase was subjected for step down regression analysis and estimation model for yield and yield components is presented in Table 4.

The mean maximum temperature range between 28.99 to 33.19°C accounted for 89 and 91% of total variation in boll weight and hundred seed weight, respectively. The effect of regressor variable was significant and revealed positive regression coefficient. The result also suggested that other yield and yield components was not

Table 3. Correlation coefficients (r) of weather variables with yield and yield components during flowering.

Weather variable	Yield and yield components						
	NSPP	NBPP	Bwt	HSW	SCY	GINNII	GINNIV
Tmax	-0.096	0.448	0.941**	0.954**	0.751	0.542	0.801
Tmin	-0.340	0.796	0.905*	0.805	0.945**	0.661	0.614
Tmean	-0.170	0.594	0.985**	0.961**	0.862*	0.611	0.788
RHI	0.302	-0.575	-0.977**	-0.982**	-0.854*	-0.435	-0.627
RHII	0.192	-0.453	-0.968**	-0.980**	-0.767	-0.538	-0.768
RF	-0.106	-0.302	-0.827*	-0.862*	-0.601	-0.479	-0.810
SShr	-0.434	-0.143	0.520	0.633	0.180	-0.266	-0.088
WS	-0.419	0.728	0.936**	0.886*	0.926*	0.473	0.508
Epan	-0.174	-0.090	-0.355	-0.282	-0.206	-0.864*	-0.874*
GDD	-0.262	0.462	0.102	0.005	0.342	-0.143	-0.399
Tc	-0.189	0.035	0.635	0.765	0.356	0.115	0.500
SDD	0.320	-0.105	-0.685	-0.671	-0.392	-0.768	-0.822*
ISRad	0.094	-0.379	-0.141	-0.154	-0.330	0.504	0.469
ASRad	0.404	-0.711	-0.154	-0.079	-0.542	0.102	0.290

*, significantly different at P < 0.05; Tmax, maximum temperature; **, significantly different at P < 0.05 and strong correlation; SShr, sunshine hour; Tmin, minimum temperature; WS, wind speed; Tmean, mean temperature; Epan, Pan evaporation; RHI, relative humidity I; GDD, growing degree day; RHII, relative humidity II; Tc, canopy temperature; RF, rainfall; SDD, stress degree day; InSR, incoming solar radiation; ItSR, intercepted solar radiation; NSPP, number of squares per plant; NBPP, number of bolls per plant; Bwt, boll weight; HSW, hundred seed weight; SCY, seed cotton yield; GINNII, ginning percent at picking two; GINNIV, ginning percent at picking four; SW, standard week.

significantly affected due to change in mean maximum temperature. Similarly, significant effect of maximum air temperature on flower and boll production was documented by Sawan (2014) during the study of climatic variables at pre and post anthesis on boll setting. Similar results were reported by Ratnam et al. (2014) who stated positive and significant correlation of boll weight, bolls per plant, dry matter accumulation and seed cotton yield with total rainfall, minimum and maximum temperature and relative humidity.

A change in mean minimum temperature over a range of 21.7 to 23.7°C accounted for 82 and 89 percent of total variation in boll weight and seed cotton yield, respectively, over different sowing times and seasons. The effect of regressor variable was significant and revealed positive regression coefficient. Similar results were reported by Ratnam et al. (2014).

A change in mean temperature over a range of 25.4 to 28.4°C accounted for 97, 92 and 74% of total variation in boll weight, hundred seed weight and seed cotton yield respectively, over different sowing times and seasons. The effect of regressor variable was significant and revealed positive regression coefficient. Similar results on boll weight was reported by Ratnam et al. (2014). Positive effects of increasing average temperatures, especially at the start and end of the cotton season, on growth, development and ultimately yield were reported by Bange (2007). Benefits of temperature under optimum range were also documented by Sankaranarayanan et al. (2010) and Reddy et al. (1991).

A change in mean maximum relative humidity over a range of 79.0 to 89.1% accounted for 95, 96 and 73% of total variation in boll weight, hundred seed weight and seed cotton yield, respectively over different sowing times and seasons. The effect of regressor variable was significant and revealed negative regression coefficient suggesting the reduction in yield and yield components during increase in maximum relative humidity. Similar research reports were documented by Oosterhuis (1999), Oosterhuis (1997) and Ratnam et al. (2014).

A change in mean minimum relative humidity over a range of 55.3 to 74.2% accounted for 94 and 96% of total variation in boll weight and hundred seed weight, respectively over different sowing times and seasons. The effect of regressor variable was significant and revealed negative regression coefficient. The present result did not agree with the reports of Ratnam et al. (2014). However, increase in minimum relative humidity could favour the incidence of pest and disease (Sharma and Razdan, 2013) which could adversely affect yield and yield components, so that inverse association could be observed.

A change in total rainfall over a range of 118.0 to 387.2 mm accounted for 68 and 74% of total variation in boll weight and hundred seed weight, respectively over different sowing times and seasons. The effect of regressor variable was significant and revealed negative regression coefficient. A large the amount of rainfall could result in over saturation and water logging which had adverse effect on cotton growth and development. Similarly, in relation to the amount of rainfall, Ogbuene (2010) reported that rice yield was negatively and

Table 4. Estimation of yield and yield components by linear regression functions of weather variables at square initiation and first flower phenophases.

Regressor vs. response variable	Model of estimated parameters	R^2	Probability	Regressor vs. response variable	Model of estimated parameters	R^2	Probability
Tmax vs Bwt	Bwt = -3.4 + 0.23Tmax	0.89	0.0051	RHII vs Bwt	Bwt = 7.5 - 0.06RHII	0.94	0.0015
Tmax vs HSW	HSW = -2.9 + 0.4Tmax	0.91	0.0032	RHII vs HSW	HSW = 15.7 - 0.1RHII	0.96	0.0006
Tmin vs Bwt	Bwt = -8.5 + 0.5Tmin	0.82	0.0130	RF vs Bwt	Bwt = 4.6 - 0.003RF	0.68	0.0421
Tmin vs SCY	SCY = -10790 + 544Tmin	0.89	0.0044	RF vs HSW	HSW = 10.8 - 0.006RF	0.74	0.0272
Tmean vs Bwt	Bwt = -6.0 + 0.36Tmean	0.97	0.0004	WS vs Bwt	Bwt = 2.0 + 0.2WS	0.88	0.0060
Tmean vs HSW	HSW = -6.8 + 0.6Tmean	0.92	0.0023	WS vs HSW	HSW = 6.5 + 0.3WS	0.78	0.0188
Tmean vs SCY	SCY = -6650.1 + 312.4Tmean	0.74	0.0273	WS vs SCY	SCY = 7.1 + 182.2WS	0.86	0.0079
RHI vs Bwt	Bwt = 13.5 - 0.11RHI	0.95	0.0008	Epan vs GINNII	GINNII = 37.3 + 0.02Epan	0.75	0.0267
RHI vs HSW	HSW = 25.9 - 0.2RHI	0.96	0.0005	Epan vs GINNIV	GINNIV= 37.9 - 0.02Epan	0.76	0.0227
RHI vs SCY	SCY = 10053 - 98.02RHI	0.73	0.0305	SDD vs GINNIV	GINNIV = 35.4 - 0.04SDD	0.68	0.0449

Tmax, maximum temperature; SShr, sunshine hour; Tmin, minimum temperature; WS, wind speed; Tmean, mean temperature; Epan, Pan evaporation; RHI, relative humidity I; GDD, growing degree day; RHII, relative humidity II; Tc, canopy temperature; RF, rainfall; SDD, stress degree day; InSR, incoming solar radiation; ItSR, intercepted solar radiation; NSPP, number of squares per plant; NBPP, number of bolls per plant; Bwt, boll weight; HSW, hundred seed weight; SCY, seed cotton yield; GINNII, ginning percent at picking two; GINNIV, ginning percent at picking four.

significantly correlated with rainy days. A change in mean wind speed over a range of 6.1 to 12.3 km h^{-1} accounted for 88, 78 and 86% of total variation in boll weight, hundred seed weight and seed cotton yield, respectively over different sowing times and seasons. The effect of regressor variable was significant and revealed positive regression coefficient which could be expected in relation to turbulence effect of wind on atmospheric CO_2, moisture and leaf temperature favouring rate of photosynthesis in cotton. However, Barker et al. (1985) reported plants grown under partial shelter (reduced wind speed) condition resulted in increased plant height, earlier squaring, earlier boll set and more bolls and biomass.

A change in total pan evaporation over a range of 159.3 to 301.0 mm accounted for 75 and 76% of total variation in ginning percent at picking two and four, respectively over different sowing times and seasons. The effect of regressor variable was significant and recorded positive regression coefficient with ginning percent at picking two and negative with ginning percent at picking four. Similarly, a change in stress degree day over a range of 35.5 to 113.6°C accounted for 68% of total variation in ginning percent at picking four over different sowing times and seasons. The effect of regressor variable was significant and revealed negative regression coefficient. Crop growth and development could be benefited from increasing pan evaporation provided that the soil moisture content maintained during critical crop growth periods, however, excess loss of moisture could result in desiccation which adversely affected ginning percent. Similar findings in relation to deficit irrigation were documented by Onder et al. (2009) who reported high ginning percent under stress conditions.

Conclusion

Yield and yield components of cotton was significantly influenced due to sowing time and deficit irrigation. The result suggested that the different sowing times and deficit irrigation created natural variation in growing conditions for cotton crop were attributed with varied climatic elements affected yield and its components. Weather variables such as maximum temperature, mean temperature, relative humidity I and relative humidity II were found to be influential and accounted for over 90% of total variation in seed cotton yield and yield components during reproductive phenophase.

Conflict of Interests

The authors have not declared any conflict of interests.

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Nutrient intake, nitrogen balance and growth performance in buffalo calves fed citrus pulp as a concentrate source

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The study was planned to investigate the effects of dried citrus pulp on nutrient intake, digestibility, nitrogen balance, blood metabolites, growth performance and economics in *Nilli Ravi* buffalo calves. Twenty buffalo male calves of 18 to 24 months of age having 200 to 250 kg body weight were used in a randomized complete block design. Four iso-caloric and iso-nitrogenous diets containing 5, 10, 15 and 20% dried citrus pulp were formulated. The experiment lasted for adaptation period while last five days of each month served as collection period. Feed was offered *ad libitum* twice a day. Animals were weighed fortnightly before morning feeding to assess their growth performance. The results showed non-significant effects of various levels of dried citrus pulp on nutrient intake and digestibility. Nitrogen metabolism was also remained unaltered among the treatments. There were non-significant differences in weight gain and blood metabolites in calves fed various levels of dried citrus pulp. However, a linear reduction in price per kg diet was observed as the level of dried citrus pulp was increased from 5 to 20% in the diet. The study showed that dried citrus pulp can be used successfully up to 20% in the diet of calves without any ill effect on feed intake, digestibility and growth performance.

Key words: Buffalo calves, citrus pulp, economics, growth performance.

INTRODUCTION

Citrus pulp is one of the major agro-industrial by-products. It is solid residue lefts after squeezing the juice of fresh citrus fruits. It comprises 60 to 65% peels, 0 to 10% seeds and 30 to 35% pulp segments. Fresh citrus pulp has 19.7% dry matter (DM) (Agshaghali and Maheri, 2008). It has high moisture and sugar contents (Rihani, 1991). Properly preserved citrus pulp can be used in the diet of ruminants throughout the year without any chemical change (Caparra et al., 2007). There are two methods for its preservation, that is, ensiling and drying. Drying reduces the moisture content of citrus pulp from 80 to 11% (Grant, 2007). Dried citrus pulp contains 85.5,

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Table 1. Ingredients and chemical composition of experimental diets for calves.

Ingredients	Diets[1]			
	LDCP	MDCP	HDCP	VHDCP
Dried citrus pulp	5	10	15	20
Maize	6	2	1	1
Wheat bran	4	3	1	1
Rice Polishing	3	2	0	0
Sunflower meal (CP 32%)	6	7	7	4
Canola meal (CP 30%)	6	6	7	10
Cotton seed cake	7	7	7	3
Molasses	10	10	9	8
Urea	1	1	1	1
Mineral Mix	2	2	2	2
Corn silage	30	30	30	30
Wheat straw	20	20	20	20
Total	100	100	100	100
Chemical composition (%)				
Dry matter	72.96	73.12	73.39	73.59
Crude protein	14.09	14.03	14.03	14.00
Metabolizeable energy (Mcal/kg)	2.46	2.45	2.45	2.50
Neutral detergent fibre	40.33	40.80	40.68	39.55
Acid detergent fibre	24.30	25.03	25.43	25.64

[1]LDCP, MDCP, HDCP and VHDCP indicates 5, 10, 15 and 20% inclusion level of dried citrus pulp in diets, respectively.

92.8, 6.9, 24.2 and 22.2% of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF), respectively (NRC, 2001).

Dried citrus pulp has greater importance in tropical areas where low to medium quality forages are the common feedstuffs (Villarreal et al., 2006). It is rich in energy content approximately 1.66 Mcal of net energy/kg of DM (Fegeros et al., 1995) and contains 120 to 400 g sugar and less than 10 g starch per kg of DM (Hall, 2000). It also has 250 g pectin contents per kg of DM which are approximately 98% digestible (Arthington et al., 2002). Ruminal bacteria can easily degrade the pectin content of dried citrus pulp (Sunvold et al., 1995). It can be used as an alternate energy source in ruminants' diet (Caparra et al., 2007). Dried citrus pulp can be used as a cereal substitute in concentrate for ruminant feed (Bampidis et al., 2006; Villarreal et al., 2006). Its incorporation in the diet enhances fiber digestion and microbial protein synthesis (Gado et al., 2009). As citrus pulp is high in fiber contents, it produces large amount of saliva that act as a buffer (Faria et al., 2008) that increases fiber digestion. Addition of dried citrus pulp in the diet of ruminants reduces urinary N excretion due to increased microbial growth (NRC, 2000). Caparra et al. (2005) stated that its supplementation in lambs' diet did not affect average daily gain and live body weight. The dried citrus pulp also improves feed intake and economic efficiency (Caparra et al., 2007). It results in better growth performance and feed intake in growing kids (Bueno et al., 2002).

Limited data are available regarding the use of dried citrus pulp as an energy source in *Nilli Ravi* buffalo calves. So, the present study has been planned to evaluate the effects of dried citrus pulp as an energy source on nutrient intake, digestibility, nitrogen metabolism, blood parameters, growth performance and economics in *Nilli Ravi* buffalo calves.

MATERIALS AND METHODS

Citrus pulp was collected from juice extraction unit "Citro Pak. Ltd., Sargodha". After receiving the pulp, it was scattered on a polythene sheet for sun drying at Agronomy Farm, University of Agriculture, Faisalabad. Polythene sheets were spread to prevent the soil contamination. After drying, samples were taken and analyzed for DM, OM and nitrogen (AOAC, 1990), NDF and ADF (Van Soest et al., 1991). Four isocaloric and isonitrogenous diets containing 5, 10, 15, and 20% dried citrus pulp were formulated and represented as low dried citrus pulp (LDCP), medium dried citrus pulp (MDCP), high dried citrus pulp (HDCP) and very high dried citrus pulp (VHDCP), respectively (Table 1).

Twenty *Nilli Ravi* buffalo male calves of 18 to 24 months of age having 200 to 250 kg weight were used to conduct the trial at

Table 2. Chemical composition of dried citrus pulp.

Nutrients (%)	Percentage (DM basis)
Dry matter	90.21
Organic matter	94.40
Crude protein	6.56
Metabolizeable energy (Mcal/kg)	3.06
Neutral detergent fiber NDF	21.19
Acid detergent fiber ADF	14.61
Ash	5.37

Buffalo Research Institute, Pattoki, according to approved protocol for research projects by the University Ethics Committee. These calves were weighed at day zero (initial body weight was 204.8 to 206.4 kg) before morning feeding. Calves were randomly divided into four blocks, 5 animals in each block. The experiment lasted for 80 days where first 21 days were taken as adaptation period while last five days of each month served as collection period. Calves were fed *ad libitum* twice a day. Feed intake was recorded daily. Fresh water availability was ensured round the clock. Animals were weighed fortnightly before the morning feeding.

During the collection period, digestibility of DM, CP, NDF and ADF were determined using total collection method. Fecal samples were collected daily, weighed, mixed thoroughly and 20% were sampled and dried at 55°C. At the end of each collection period, dried fecal samples were composited and 10% of these were taken for analysis. Samples of feed and feces were taken and ground to pass through a 2 mm screen and analyzed for DM and CP (AOAC, 1990), while NDF and ADF were analyzed using method described by Van Soest et al. (1991).

Two calves were selected randomly from each group for blood collection. Blood samples were collected six hours after the last feeding. Ten milliliters of blood was extracted from jugular vein and transferred to vacutainer. Serum was extracted by centrifuging it at 3500 rpm. Blood urea nitrogen was determined according to the method prescribed by Bull et al. (1991). Blood glucose was determined by using crescent diagnostic glucose enzymatic colorimetric god-pap method (Trinder, 1969). Nitrogen balance was calculated by the equation described by NRC (2001). Cost incurred on each diet was also calculated to determine the economics of feeding.

The data collected for nutrient intake, digestibility, nitrogen metabolism, blood metabolites, growth performance and economics were statistically analyzed using General Linear Model procedure of SPSS (SPSS 10.0.1., 1999) and means were compared by Duncan's Multiple Range Test (Steel et al., 1997).

RESULTS

Chemical composition

Chemical analysis of dried citrus pulp revealed that it contained 90.21% DM, 94.40% OM, 6.56% CP, 3056 kcal/kg ME, 21% NDF, 14% ADF, and 5.37% ash (Table 2).

Nutrient intake

The DM intake was not significantly affected. It was 8.00, 8.14, 8.29 and 8.30 kg/day in calves fed LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 3). The CP intake also remained unaltered. It was 1.09, 1.17, 1.19 and 1.19 kg/day in calves fed LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 4). Similarly, a non-significant difference (P>0.05) was observed on NDF and ADF intakes in buffalo calves fed various levels of dried citrus pulp.

Nutrient digestibility

There were non-significant effects of different levels of dried citrus pulp on DM digestibility in buffalo male calves. It was 66.70, 65.38, 65.30 and 65.17% in calves fed LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 3). Similarly, the CP digestibility was also non-significant (P>0.05) among different treatments. It was 69.81, 69.63, 69.14 and 69.01% in calves offered LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 3). NDF and ADF digestibility also remained unaltered across the treatments (Table 3).

Nitrogen metabolism

Various levels of dried citrus pulp in the diet had non-significant effect on nitrogen intake. It was 174.46, 187.15, 190.34 and 190.44 g/day when the calves were fed LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 4). Fecal and urinary nitrogen excretion remained unchanged (P>0.05) across treatments. Nitrogen retention also remained unaffected by dietary treatments (Table 4).

Blood metabolites

Dried citrus pulp had non-significant effects on blood urea in *Nilli Ravi* calves. Blood urea nitrogen also remained unaffected. It was 26.33, 25.97, 25.03 and 25.01 mg/dl in calves fed LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 5). Blood glucose also remained unaltered. It was 53.79, 53.93, 54.41 and 54.88 mg/dl in calves fed LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 5).

Table 3. Effects of varying levels of dried citrus pulp on nutrient intake and digestibility in buffalo calves.

Parameter	Diets[1]				SEM[2]
	LDCP	MDCP	HDCP	VHDCP	
Dry matter					
Intake(kg)	8.0	8.14	8.29	8.30	0.168
Digestibility (%)	66.70	65.38	65.30	65.17	0.602
Crude protein					
Intake(kg)	1.09	1.17	1.19	1.19	0.026
Digestibility (%)	69.81	69.63	69.14	69.01	0.550
Neutral detergent fibre					
Intake(kg)	2.64	2.78	2.84	2.84	0.058
Digestibility (%)	54.60	54.13	53.03	52.52	0.774
Acid detergent fibre					
Intake(kg)	1.44	1.47	1.49	1.49	0.029
Digestibility (%)	47.58	46.16	45.95	45.13	0.910

[1]LDCP, MDCP, HDCP and VHDCP indicates 5, 10, 15 and 20% inclusion level of dried citrus pulp in diet. [2]Standard error mean (P>0.05).

Table 4. Effects of varying levels of dried citrus pulp on nitrogen balance in buffalo calves.

Item (g/day)	Diets[1]				SEM[2]
	LDCP	MDCP	HDCP	VHDCP	
Nitrogen intake	174.46	187.15	190.34	190.44	1.348
Nitrogen in feces	52.83	56.61	58.68	58.99	0.615
Nitrogen in urine	103.99	109.21	107.63	103.81	0.468
Nitrogen retention (%)	10.11	11.40	12.62	14.51	0.299

[1]LDCP, MDCP, HDCP and VHDCP indicates 5, 10, 15 and 20% inclusion level of dried citrus pulp in diet. [2]Standard error mean (P>0.05).

Table 5. Effects of varying levels of dried citrus pulp on blood metabolites in buffalo calves.

Blood metabolites (mg/DL)	Diets[1]				SEM[2]
	LDCP	MDCP	HDCP	VHDCP	
Blood urea	46.83	46.58	46.14	45.89	0.212
Blood urea nitrogen	26.33	25.97	25.03	25.01	0.669
Blood glucose	53.79	53.93	54.41	54.88	0.495

[1]LDCP, MDCP, HDCP and VHDCP indicates 5, 10, 15 and 20% inclusion level of dried citrus pulp in diet. [2]Standard error mean (P>0.05).

Growth performance and gain to feed ratio

Body weight gain also remained unaffected (P>0.05) across treatments. Average body weight gain was 38.80, 39.60, 40.00 and 40.40 kg when calves were fed LDCP, MDCP, HDCP and VHDCP diets, respectively (Table 6). A non-significant (P>0.05) effect was observed on gain to feed ratio among the treatments (Table 6).

Economics

Price per kg of diet was reduced by increasing the level of dried citrus pulp. It was 24.19, 23.40, 22.98 and 22.10

Table 6. Effects of varying levels of dried citrus pulp on weight gain and economics inbuffalo calves.

Weight (kg)	Diets[1]				SEM[2]
	LDCP	MDCP	HDCP	VHDCP	
Initial	206.2	206.4	204.8	206.4	3.384
Final	245.0	246.0	244.8	246.8	5.029
Weight gain	38.8	39.6	40.0	40.4	2.331
Avg. Daily gain(g)	517.3	528.0	533.3	538.7	38.845
Feed price (Rs./kg)	24.19	23.40	22.98	22.10	-
Gain to feed ratio	0.12	0.12	0.11	0.11	0.007
Price/kg weight (Rs.)	208.63	201.51	199.97	196.73	10.716

[1]LDCP, MDCP, HDCP and VHDCP indicates 5, 10, 15 and 20% inclusion level of dried citrus pulp in diet. [2]Standard error mean (P>0.05).

Rs. for LDCP, MDCP, HDCP and VHDCP diets, respectively. Price was maximum for LDCP and minimum for VHDCP diet (Table 1).

DISCUSSION

Chemical composition

Chemical composition of dried citrus pulp in this study is in consistent with Abdullah and Sharif (2014), who observed 90.63, 94.57, 6.32, 20.68 and 14.32% DM, OM, CP, NDF and ADF, respectively. Ibrahim et al. (2011) also observed 94.98, 6.40 and 5.02% OM, CP and ash contents, respectively. Similar results were observed by Watanabe et al. (2010) who reported that dried citrus pulp contained 89.10, 6.35, 18.85 and 14.32% f or DM, CP, NDF and ADF, respectively. Contrary to this study, Kour et al. (2014) observed that dried citrus pulp contained 92.05% DM, 7.6% CP, 26.35% NDF and 19.5% ADF. Hernandez et al. (2012) also noticed higher CP (7.6%) and lower ash (3.9%) contents. Variations in chemical composition of citrus pulp might be due to the difference in soil properties used for growing citrus (Lambert et al., 2008) or juice extraction method, which affects chemical composition of citrus pulp (Arthington et al., 2002).

Nutrient intake

Our findings are in accordance with Gobindram et al. (2015) who reported that feed intake was remained unaltered in lambs fed 35% dried citrus pulp in concentrates. Santos et al. (2014) also stated non-significant effect on feed intake using various levels of dried citrus pulp in diet. In accordance to our findings, Gilaverte et al. (2011) observed that nutrient intake of Santa ines sheep was not significantly affected by replacing corn by dried citrus pulp. However, Gawad et al. (2013) reported significant differences in nutrient intake with an increase in dried citrus pulp level. Crosswhite et al. (2013) observed higher DM intake in animals by replacing corn with dried citrus pulp. The reason for increased intake might be likeness of animals for dried citrus pulp due to its specific smell and taste. Another reason might be better palatability of the citrus pulp (Franzolin et al., 2010).

Nutrient digestibility

Lack of effect in our study was in accordance with Gawad et al. (2013) who reported that DM, OM and CP digestibility remained unaffected by various levels of dried citrus pulp. Ahooei et al. (2011) stated that supplementation of dried citrus pulp in the diet had non-significant effect on nutrient digestibility. Gilaverte et al. (2011) also noticed that nutrients digestibility remained unaffected in sheep when corn was replaced by dried citrus pulp. Contrary to our findings, Nam et al. (2009) reported that nutrient digestibility was higher in dried citrus pulp based diet than control. Macedo et al. (2007) found higher digestibility when levels of dried citrus pulp were increased in ruminant rations. Miron et al. (2002) pointed out increase in digestibility when dried citrus pulp was increased (9.6 to 20.7% of dietary DM) in dairy cows diet. This might be due to total soluble solids and neutral detergent soluble carbohydrates in dried citrus pulp that would be rapidly digested in the rumen (Nam et al., 2009).

Nitrogen metabolism

Results of our studies are in agreement with Peixoto et al. (2015) who found non-significant differences of various levels of dried citrus pulp on ammonia nitrogen. This ammonia nitrogen is necessary to promote growth of fiber degrading bacteria which use ammonia nitrogen as nitrogen source, resulting in improved fiber digestion. Williams et al. (1987) also observed lack of effect of dried citrus pulp on nitrogen retention in calves. Chen et al. (1981) stated that dried citrus pulp did not affect nitrogen retention in lambs. This might be due to its non-significant effect on intake and digestibility.

Blood metabolites

Various inclusion levels of dried citrus pulp had non-significant effect on blood metabolites in buffalo calves. Our results are in agreement with Ahooei et al. (2011) who reported non-significant effect of dried citrus pulp on blood urea nitrogen in male fattening calves. Similarly, Belibasakis and Tsirgogianni (1996) also found that blood urea nitrogen remained unaltered in dairy cows fed dried citrus pulp based diets. Findings of our study are similar with Oni et al. (2008) who reported that blood glucose remained unaltered by various levels of dried citrus pulp in West African dwarf goats. Broderick et al. (2002) found lack of effect on blood glucose in cows fed dried citrus pulp based diets. Lack of effect of DCP on blood glucose might be attributed to non-significant intake and digestibility of DCP based diets.

Growth performance and gain to feed ratio

Non-significant effect of dried citrus pulp on weight gain in the present study was supported by Gawad et al. (2013). Santos et al. (2014) also stated that weight gain remained unaltered by different levels of dried citrus pulp. Caparra et al. (2007) found non-significant differences of dried citrus pulp on weight gain in lambs. Scerra et al. (2001) also revealed lack of effect on live body weights of lambs fed dried citrus pulp based diets. This might be attributed to similarity in chemical composition of diet fed to the animals.

Contrary to this study, Bueno et al. (2002) revealed a better growth performance in Saanen kids fed diet having 46% dried citrus pulp. Similarly, Miron et al. (2002) found that there was a higher weight gain in cows fed total mixed ration having 21% dried citrus pulp than those offered total mixed ration having 10% dried citrus pulp. Aregheore (2000) stated better daily live weight gain in sheep and goats fed dried citrus pulp based diets. The reason for higher weight gain might be attributed to more feed consumption by the animals with an increased level of dried citrus pulp (Williams et al., 1987).

Economics

Results of our study are in accordance with Gholizadeh and Naserian (2010) who reported that dried citrus pulp reduced the feeding cost. Oni et al. (2008) noticed that feed cost was decreased when barley grains were replaced with dried citrus pulp in the diet of Saanen kids. Caparra et al. (2007) stated that using dried citrus pulp as concentrate energy source is very economical in diets of lambs. Macedo et al. (2007) also found a reduction in feed prices when citrus pulp replaced sorghum silage as concentrate energy source. Similarly, Broderick et al. (2002) stated that dried citrus pulp is very efficient to minimize feed cost in goat rations. The reason might be that citrus pulp is a waste industrial by-product with excellent nutritional profile for ruminants which is cheaper than cereal grains, resulting in preparing an economical ration (Naserian et al., 2009).

Conclusion

The study showed that dried citrus pulp can be used successfully up to 20% in the diet of calves without any ill effect on feed intake, digestibility and growth performance. It also helps in cost effective ration formulation.

Conflict of interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

Spatial variability of weeds in an Oxisol under no-tillage system

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In the global agribusiness, the herbicide use is a major problem for sustainable production, in this sense, it is necessary to better understand the interaction of weed species and floristic composition such as biodiversity indicators. The objective of this study was to analyze the spatial variability of weeds in an Oxisol under no-tillage system. Samples were taken in an area of 0.5 ha, in 50 sampling points with spacing of 5 m x 10 m. Data were analyzed by means of classical statistics, geostatistics, and spatial variability of the constructed maps by the interpolation by kriging technique. All the species of weeds presented in the study area showed spatial variability with the exception of *Ipomoea triloba* (L.) and *Heliotropium indicum* (L.), which showed pure nugget effect. The range values (a) shows that the spacing between samples can be extended to all species of weeds. The study was unable to determine specifics areas of management in the local since the different species of weed infested different plots of the area.

Key words: Precision agriculture, semivariograms, site-specific management.

INTRODUCTION

The weeds have acquired along the evolutionary process the capacity to establish themselves in areas where the natural vegetation has been eliminated, mainly for agricultural cultivation. Among the developed features by the weeds, there are high reproductive capacity, rapfastid dispersal, and genetic adaptations. These associated characteristics are responsible for a significant part in the reduction of agricultural production (Rodrigues et al., 2010). Once uncontrolled, weeds host several pest insects, nematodes, and pathogens in the crops.

Furthermore, the weeds even compete for water, nutrients which reduce the availability in the crop in the area.

The knowledge of how the populations of weeds develop allows adding to the agricultural production system a lot of information that were previously ignored in most of the cases which herbicide application is made considering an average infestation for all growing area. In

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this sense, the use of precision farming tools allows space and temporal monitoring of weeds variability, mapping the infestation areas, specific areas of management determination (Goel et al., 2003), and herbicides localized application, which reduces the applied amount and costs.

According to Mortensen et al. (1998), the weed species presented temporal stability which favors the management of cropping areas. However, in Brazil, little is known about the spatial variability of weeds. The first works were Shiratsuchi et al. (2004, 2005), Schaffrath et al. (2007) and Monquero et al. (2008) whose studied the spatial distribution of weed in order to determine specific zones of management. Other studies emphasized the importance of studying the weeds distribution and specific management sites. Domingos and Laca-Buendia (2010) studied weeds in the preharvest of the sorghum crop. Calado et al. (2013) studied weed control in winter wheat influenced by different farming systems. Bressan et al. (2006) used geostatistics techniques to classify the risk of weed infestation, and made the decision on the best management for each field area.

Shiratsuchi et al. (2005) also reported that most studies that focused on weeds mapping had as a primary concern mapping the emerging flora during the critical cycle of interference, being the only few studies on spatial variability of weeds in the course of the crop cycle. Allied to this, studies focusing on weed parameters analysis of species diversities of the communities try to determine the degree of infestation, being one of the first steps in studying the weeds dynamics and the choice of strategies control (Lacerda et al., 2005).

Thus, this study aimed to determine the spatial variability of weeds in an Oxisol managed under the no-tillage system in Urutaí (Goiás, Brazil).

MATERIALS AND METHODS

The study area has 0.5 ha (50 m x 100 m), and is located at the Goiano Federal Institute - Campus Urutaí (17°27'50'' South and 48°12'10'' West). The soil of the area is Rhodic Hapludox (USDA, 1999), managed under no-tillage since 2001, and the sampling time was cultivated with sunflower (*Helianthus annuus* L.) cultivate M-734. The climate, according to Köppen is Aw, with two well-defined seasons, dry in winter and humid in the summer, with average temperatures higher than 18°C during all months of the year.

The study area was divided into a sampling grid with 50 points with spacing of 5 m x 10 m. At each sampling point was randomly placed a circle of 0.5 m diameter (0.196 m^2) for identifying the number of individuals per point, the number of species per point and the incidence of each type in each sampling point, by manual identification technique (Lutman and Perry, 1999).

The identification of the presented weeds in the area of study was performed using the Identification Manual and Weed Control (Lorenzi, 2000). The following weed species were identified: *Cenchrus echinatus* L.; *Chamaesyce* sp. (L.) Mill; *Heliotropium indicum* L.; *Ipomoea triloba* L.; *Eleusine indica* (L.) Gaertn and *Bidens pilosa* L. They were evaluated for their quantitative values for density, relative density, frequency, relative frequency, abundance, relative abundance and relative importance index values, according to Mueller-Dombois and Ellenberg (1974).

$$Density = \frac{Total\ number\ of\ individuals\ per\ species}{Total\ number\ of\ circles\ obtained\ (total\ area)} \quad (1)$$

$$Relative\ density = \frac{100\ x\ density\ of\ the\ species}{Total\ density\ of\ all\ species} \quad (2)$$

$$Frequency = \frac{Number\ of\ squares\ containing\ the\ species}{Total\ number\ of\ circles\ obtained\ (total\ area)} \quad (3)$$

$$Relative\ frequency = \frac{\text{100 x frequency of the species}}{\text{overall frequency of all kinds}} \quad (4)$$

$$Abundance = \frac{Total\ number\ of\ individuals\ per\ species}{total\ number\ of\ circles\ containing\ the\ species} \quad (5)$$

$$Relative\ abundance = \frac{100\ x\ abundance\ of\ species}{total\ abundance\ of\ all\ species} \quad (6)$$

$$Relative\ Importance\ Value\ Index = rel.freq + rel.abund. + rel.density \quad (7)$$

Where: *rel. freq*= relative frequency, *rel. abund*=relative abundance and *rel. density*=relative density. Biodiversity indexes were obtained by Species Diversity program (DivEs 3.0.7) (Rodrigues, 2015). The Shannon-Wiener Index is suitable for random samples of species of an interested community or sub-community.

$$H' = \sum_{i=0}^{n} Pi\ x\ logb\ Pi \quad (8)$$

Where: *pi* is the proportion of species in relation to the total number of found species in the conducted surveys logb = logarithm to the basis b (2 or 10). Simpson index takes into consideration the number of species (*S*), the total numbers of individuals (*N*), and the total proportion of occurrence of each species.

$$Ds = 1 - \frac{\sum_{i=0}^{n} ni\ x\ (ni-1)}{N(N-1)} \quad (9)$$

Where: *n* is the number of individuals of each species; *N* is the number of subjects. Simpson Diversity D is determined by the Simpson diversity index.

$$Ds = 1 - \left(\frac{\sum_{i=1}^{n} n_{i\ x\ (n_{i-1})}}{N(N-1)}\right) \quad (10)$$

Where: *ni* is the number of each species individuals; *N* is the number of subjects. Menhinick diversity is a simple diversity index, taking into account only the species number (s) and the square root of the individuals total number. The diversity index of Menhinick (Db) is:

$$Db = \frac{s}{\sqrt{N}} \quad (11)$$

Where: s is the number of sampled specie, N is the total number of individuals in all species and Logb = logarithm base b (2 or 10).

Table 1. Descriptive statistics of weeds populations in an Oxisol under the no-tillage system, Instituto Federal Goiano - Campus Urutaí.

Atributs	*C. echinatus*	*Chamaesyce sp.*	*H. indicum*	*I. triloba.*	*E. indica*	*B. pilosa*
N	41	10	13	5	20	25
Min	1	1	1	1	1	1
Max	40	3	7	2	10	4
Mean	8.9	1.5	2.5	1.2	4	2.6
Variance	57.3	0.72	3.9	0.2	11	1.4
Standard deviation	7.57	0.85	2	0.45	3.3	1.2
Coefficient of variation	85.06	56.65	80.59	37.26	81.88	46.6
Skew	2.014	1.358	1.202	2.236	0.865	-0.154
Kurtosis	6.357	0.107	0.604	5	-0.836	-1.507
D	0.149Ln	0.422Ln	0.308Ln	0.473Ln	0.274Ln	0.204Ln

N = number of measurement; Min = minimum value; Max = maximum value; D=Kolmogorov-Smirnov test with 1% of probability of error.

McIntosh is a simple index more complex since it considers the total number of individuals (N) and the U-value, which is the square root of the sum of squared individuals of each species. The diversity index McIntosh (D) is:

$$D = \frac{N - U}{N - \sqrt{N}} \tag{12}$$

Where: N is the total number of individuals (s) or sample (s); and U is calculated as follows:

$$U = \sqrt{\sum_{i=1}^{n} n_i^2} \tag{13}$$

Where i is the number of individuals belonging to the each species. Gleason diversity is a simple index of diversity considering only the species number (s) and the logarithm (base 10 or natural) of the individuals total number.

$$Dg = \frac{s}{Log_b N} \tag{14}$$

Where: s is the number of sampled species, N is the total number of individuals in all species and Logb = logarithm base b (2 or 10). Total diversity estimates a region diversity as:

$$TD = \sum_{i=1}^{n} w_{i[pi\,(1-pi)]} \tag{15}$$

Where: i is the weight given to the function, which expresses the importance that wants to give the species i in the global quantification of the regional diversity; pi is the relative frequency. The spatial variability was analyzed by constructing semivariograms according to Vieira (2000). The semivariogram, γ (h), a spatially distributed z variable (xi) is:

$$y(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2 \tag{16}$$

Where: N (h) is the number of observations separated by a distance h. The semivariograms were adjusted to a mathematical model according to the following parameters: nugget effect (C_0), level ($C_0 + C_1$), and range (a).

The equation 16 was determined by considering the intrinsic possibility of geostatistics, in which there is no requirement for the existence of a finite variance Var (z). It requires only the stationarity of averages and a second order stationarity for the differences [(Z (x) -Z (x + h)] (Journel and Huijbregts, 1978). The semivariogram behavior for small h values reveals important aspects of the spatial variability of the properties under which it can be used for comparison.

The semivariograms weeds were staggered, according to Vieira et al. (1997):

$$y^{sc} = (h) = \frac{y(h)}{Var(z)} \tag{17}$$

Where: y^{sc} (h) is the phased semivariogram, γ (h) is the original semivariogram, and Var (z) is the data variance.

Theoretically, this equation requires the existence of a finite variance, which can be ensured if the second order stationarity exists. However, the greatness that is used in this calculation is only the conveniently calculated number for the data variance, but not exactly the statistical magnitude of variance. The scale is used for designing various semivariograms on the same graph when otherwise have different scales on the axis of semivariances. When phased the semivariograms for clusters, it can be said that the properties involved have similar spatial variability (Vieira et al., 1997). The adjustment of the experimental semivariograms of weed was performed by adjusting the spherical models, exponential and gaussian, being chosen the best setting in the technical function of "*jack-knifing*", as presented by Carvalho et al. (2002). The spatial dependence ratio was calculated according to the equation below:

$$RD = \left(\frac{C_0}{C_0 + C_1}\right) x100 \tag{18}$$

As proposed by Cambardella et al. (1994), being 0.00 to 25% strong, 25 to 75% moderate and 75 to 100% weak.

RESULTS AND DISCUSSION

All the studied weeds species presented frequency distribution of lognormal type (Table 1). According to

Table 2. Density values, frequency, abundance and index values of relative importance found in the no-tillage system located at the Instituto Federal Goiano - Campus Urutaí.

Variable	Frequency		Density		Abundance		I.V.I (%)*
	Absolute	Relative	Absolute	Relative	Absolute	Relative	
C. echinatus	0.82	35.34	37.24	64.38	8.90	39.26	138.99
Chamaesyce sp	0.2	8.62	1.53	2.64	1.5	6.61	17.88
H. indicum	0.26	11.20	3.26	5.64	2.46	10.85	27.70
I. triloba	0.1	4.31	0.61	1.05	1.2	5.29	10.66
E. indica	0.4	17.24	8.26	14.28	4.05	17.86	49.39
B. pilosa	0.5	21.55	6.53	11.28	2.56	11.29	44.13
Total	2.28	98.27	57.44	100	20.672	91.17	300

*I.V.I=Importance Value Index.

Carvalho et al. (2002), skewness and kurtosis values near to 0 and 3 are indicative of a normal frequency distribution. In this case, the elevated values of skewness and kurtosis confirm the presence of log-normal distribution. However, *B. pilosa* and *E. indica* obtained skewness and kurtosis respectively below zero, in which case these two species probably have no log-normal distribution. According to Johnson et al. (1996) and Wiles et al. (1992), negative distributions as well as the aggregate behavior variables are typical weeds.

According to Warrick and Nielsen (1980), the number of rating individuals, *C. echinatus*, *H. indicum* and *E. indica* indicates a high coefficient of variation values (CV ≥ 60%), the other variables had moderate CV values. The species, *C. echinatus* was the most common weed in the area of study, occurring in 41 of the 50 sampling points, and *I. triloba* was the lower frequency species found only in 5 sampling points (Table 1).

It was found that three species of plants presented greater frequency, density, abundance and relative importance value. *C. echinatus* presented relative frequency (35.34), specific gravity (64.38), relative abundance (39.26) and relative importance index (138.99). *B. pilosa* was obtained for relative frequency (21.55), relative density (11.28), relative abundance (11:29) and relative importance index (44.13). Also, the grass *E. indica* indicated relative frequency (17.24), relative density (14.28), relative abundance (17.86) and relative importance index (49.39) (Table 2).

The coefficients of variation (CV%) for the diversity indexes are considered low, ranging from 0.293 to Menhinick index to 0.670 for McIntosh. The asymmetry parameter for the contents of D. Simpson, Simpson, Shannon, Menhinick, McIntosh and Margalef showed values lower than 0.5 which, according to Webster and Olivier (1990) who indicates normal distribution.

In this case, only the total diversity and Gleason index had values that did not follow a normal distribution (-0.851 and 1.592 respectively) (Table 3).

The linear correlation matrix (Table 4) demonstrate that among *H. indicum* x *E. indica* (*r* = 0.816), *H. indicum* x Shannon index (*r* = 0.797), Simpson index x Shannon index (*r*= 0.873), Simpson index x Menhinick index (*r* = 0.765), Simpson index x McIntosh index (*r*= 0.985), Simpson index x Margalef index (*r* = 0.827), Simpson index x Gleason index (*r*= 0.684), Shannon index x McIntosh index (*r* = 0.793), Shannon index x Margalef index (*r*= 0.824), index Menhinick x McIntosh index (*r* = 0.814), Menhinick index x Margalef index (*r*= 0.838), Menhinick index x Gleason index (*r* = 0.941), McIntosh index x Margalef index (*r*= 0.809), McIntosh index x Gleason index (*r* = 0.764) and index Margalef x Gleason index (*r*= 0.758) there is a high linear correlation according to Santos (2007) classification. The other correlations are considered low (| *r* | = 0.1-0.5) or zero (| *r* | = <0.1).

The presence of negative linear correlation for the vast majority of species with C. *echinatus* (*C. echinatus* x *Chamaesyce sp* = -0.162; *C. echinatus* x *I. triloba* = -0.783; *C. echinatus* x *E. indica* = -0.170; *C. echinatus* x *B. pilosa* = -0,371) indicating the superiority of the grass, *C. echinatus* in the colonization process of the area of study in relation to another weed species, this is confirmed when we analyze the occurrence of each weed species in 50 sampling points (Table 1).

The geostatistical analysis presented that the species, *H. indicum* and *I. triloba* showed pure nugget effect, as well as the Shannon diversity indexes, Menhinick, Margalef and Gleason (Table 5). According to Vieira (2000), the presence of nugget effect is mainly because of the spacing used, which was not enough to detect the spatial variability between the samples.

However, the presence of pure nugget effect for *H. indicum* and *I. triloba* is mainly because these two weed species are not common in the area of study, as evidenced by the number of sampled individuals (Table 1).

The spherical model was the most adjusted one to the weed plants data, corroborating to other studies that describe this model as the most adjusted one with soil and plant data (Cambardella et al., 1994; Vieira, 2000; Chiba et al., 2010; Siqueira et al., 2015), excepting the

Table 4. Linear correlation between species and levels of diversity of the weed plants presented in the area of study.

Variable	*C. echinatus*	*Chamaesyce sp*	*H. indicum*	*I. Triloba*	*E. indica*	*B. pilosa*	Div. total*	D. Si*	Si*	Sha*	Men*	Mc*	Mar*	Gleason
C. echinatus	1.000	-	-	-	-	-	-	-	-	-	-	-	-	-
Chamaesyce sp	-0.162	1.000	-	-	-	-	-	-	-	-	-	-		-
H. indicum	0.123	**	1.000	-	-	-	-	-	-	-	-	-	-	-
I. triloba	-0.783	**	**	1.000	-	-	-	-	-	-	-	-	-	-
E. indica	-0.170	0.478	0.816	**	1.000	-	-	-	-	-	-	-	-	-
B. pilosa	-0.371	0.522	0.168	**	-0.041	1.000	-	-	-	-	-	-	-	-
Div. Total	0.255	0.247	0.121	-0.848	-0.047	-0.511	1.000	-	-	-	-	-	-	-
D. Simpson	0.478	-0.475	-0.404	-0.339	-0.155	-0.230	-0.181	1.000	-	-	-	-	-	-
Simpson	-0.478	0.475	0.404	0.339	0.155	0.230	0.181	-1.000	1.000	-	-	-	-	-
Shannon	-0.259	0.452	0.797	-0.309	0.349	0.158	0.408	-0.873	0.873	1.000	-	-	-	-
Menhinick	-0.519	0.241	0.086	0.597	-0.400	-0.122	0.019	-0.765	0.765	0.581	1.000	--	-	-
McIntosh	-0.521	0.451	0.263	0.515	0.024	0.237	0.085	-0.985	0.985	0.793	0.814	1.000	-	-
Margalef	-0.235	0.286	0.387	-0.045	-0.225	-0.192	0.463	-0.827	0.827	0.824	0.838	0.809	1.000	-
Gleason	-0.491	0.260	0.051	0.617	-0.353	-0.234	-0.007	-0.684	0.684	0.441	0.941	0.764	0.758	1.000

Div. Total*= total diversity; D. Si*= D Simpson; Si*= Simpson; Sha*=Shannon; Men*=Menhinick; Mc*=McIntosh; Mar*= Margalef.

Table 3. Descriptive statistics of weeds diversity indexes in an Oxisol under the no-tillage system, Instituto Federal Goiano - Campus Urutaí.

Variable	Total diversity	D. Simpson	Simpson	Shannon	Menhinick	McIntosh	Margalef	Gleason
Numbers	48	48	48	48	48	48	48	48
Minimum	0	0	0	0	0.436	0	0	1,430.7
Maximum	0.965	1	1	0.626	1.414	1	3.419	6,643.9
Mean	0.631	0.575	0.424	0.258	0.794	0.337	1.452	2,712.0
Median	0.679	0.507	0.492	0.276	0.816	0.356	1.627	2,631.0
Variance	0.098	0.064	0.064	0.022	0.054	0.051	0.649	0.920
Standard deviation	0.314	0.253	0.253	0.150	0.232	0.226	0.805	0.959
Coefficient of variation	0.498	0.440	0.596	0.582	0.293	0.670	0.554	0.353
Skew	-0.851	0.218	-0.218	0.09	0.361	0.284	-0.086	1,592.0
Kurtosis	-0.377	-0.761	-0.761	0.06	-0.434	-0.042	0.128	4,714.0
Kolmogorov-Smirnov	0.157	0.117	0.117	0.142	0.115	0.08	0.11	0.138
Critical K-S stat, alpha=.05	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.192

grass, *C. echinatus* that set the gaussian model. In biodiversity, indexes were the exponential model (D Simpson, Simpson Diversity, McIntosh

Table 5. Adjustment parameters of the semivariogram for the studied weed species.

Variable		Model	C_0	C_1	A	RD
Species	*C. echinatus*	Gaussian	25.00	60.00	38.00	29.41
	Chamaesyce sp.	Spherical	0.00	0.60	28.00	0.00
	H. indicum	Pure nugget effect				
	I. triloba	Pure nugget effect				
	E. indica	Spherical	0.00	15.0	40.00	0.00
	B. pilosa	Spherical	0.10	1.30	20.00	7.14
Biodiversity indexes	Total diversity	Spherical	0.04	0.05	30.70	44.44
	Simpson (D)	Exponential	0.00	0.06	7.20	0.00
	Simpson diversity	Exponential	0.00	0.06	7.30	0.00
	Shannon diversity	Pure nugget effect				
	Menhinick diversity	Pure nugeet effect				
	McIntosh diversity	Exponential	0.00	0.05	7.70	0.00
	Margalef diversity	Pure nugget effect				
	Gleason diversity	Pure nugget effect				

diversity), with the exception of the total diversity that adjusted to the spherical model (Table 5).

Several studies have reported that some weed species are aggregated or occur in reboilers, so the infestation mapping of the agricultural area enables located management application. That's because when the areas are mapped with the occurrences, they also know other aspects of weeds, such as the degree of infestation, contagiousness, species present and edaphoclimatic relations (Wiles et al., 1992; Jonhnson et al., 1996; Schaffrath et al., 2007). *C. echinatus* had high values of nugget effect (C_0, Table 5). Siqueira et al. (2008) pointed out that the nugget effect values represent the spatial variation not detected in the sampling process, indicating that if the spacing was shorter it would be possible to detect other patterns of variability thanks to this attribute. The grass, *C. echinatus* had the higher range value (a = 38.00 m) and *B. pilosa* had the lowest range value (a = 20.00 m).

For the biodiversity reach indexes, the highest value was the total diversity (30.70 m). Siqueira et al. (2015) studying the variability of weed found a range between 40 and 210 m. The spatial dependence reason was calculated according to Cambardella et al. (1994), was high for *Chamaesyce sp*., *E. indica*, *B. pilosa*, Simpson (D), Simpson diversity and McIntosh Diversity (RD = 0.0 to 25%), medium for *C. echinatus* and Total diversity (RD = 25-75%).

Figure depicted the phased semivariogram. In Figure 1 A, the total diversity, as well as the Margalef index exhibit greater dispersion when compared to the other indexes. Similarly, *Chamaesyce sp, H. indicum* and *I. triloba* presents great dispersion in the semivariance pairs in a short distance and upon the increasing of the distance (Figure 1B). The other variables that presented spatial variability had the same spatial behavior.

The spatial variability map for the diversity of Simpson and Shannon full diversity which weeds species are present throughout the study area occurred with greater intensity on the left side of the sampled area (Figure 1 C). In general, all kinds of weeds present in the area showed spatial variability, with the exception of *I. triloba* and *H. indicum*.

The weed species presented distribution in “reboilers”. The range of values (a) showed that the space between the samples can be extended to all weed species. It was not possible to determine specific areas of management in the studied area since different species of weed infested plots of the area.

Conflict of Interests

The author has not declared any conflict of interest.

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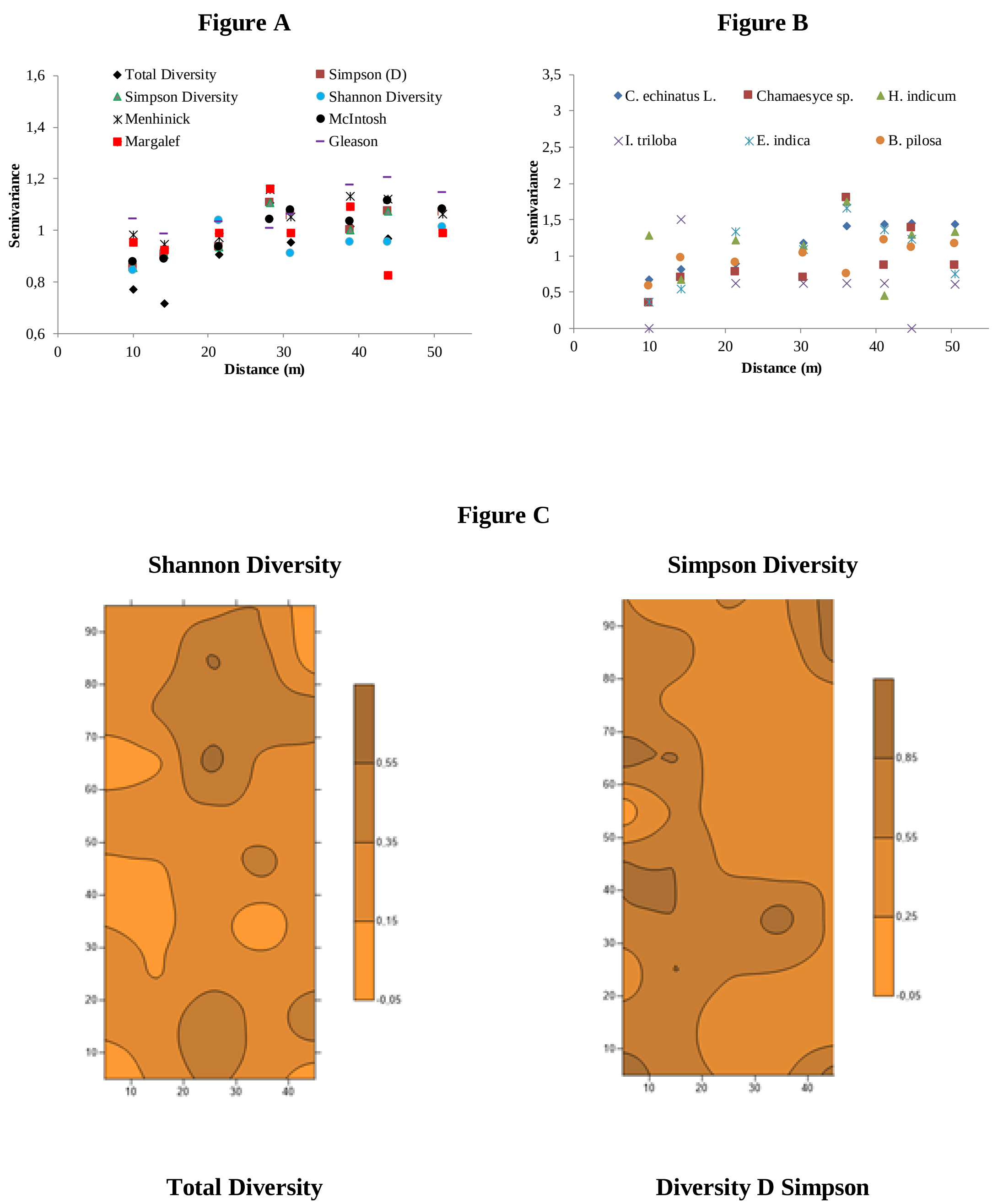

Figure 1. Semivariogram phased for the biodiversity indexes (Figure A), for the weed species (Figure B), and variability maps (Figure C) in the studied area.

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Changes in soil organic carbon fractions in response to sugarcane planting in the central-south region of Brazil

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Sugarcane planting area in Brazil has considerably increased during recent years by occupying areas used for pasture and grain crops production. Land use change (LUC) processes related to sugarcane expansion can affect the soil organic carbon (SOC) stocks, and the quality of the soil organic matter. Preliminary studies have shown that the land-use transition into sugarcane leadto a decrease in the SOC, however no studies have accessed the impact of LUC on the SOC distribution within soil particle-size classes. The study investigated the modifications on total SOC, particulate organic carbon (POM) and mineral-associated C (MOC) in response to conversion from native vegetation (NV), pastures and annual cropland (AC) to sugarcane crop (SCN). Soil samples were collected at 34 field-sites to 20 cm depth and POM fraction separated through 53-mm sieve after soil dispersion. The study results indicated that LUC affects both the labile as well as the more stable SOM fraction, with a mean C content decreasing of 40 and 30% in POM and MOC respectively, following the transition from NV to SCN. The replacement of pastures caused C depletion by 33% at POM and 30% at MOC; meanwhile C accumulation at MOC (5%) was detected for the conversion from AC to SCN. The impact of LUC on POM for AC-SCN transition could not be totally clarified, however the result could be an indication that the POM response to LUC varies as function of previous management in AC.

Key words: Soil organic carbon, C fractionation, particulate organic matter, mineral-associated C, land use change.

INTRODUCTION

Sugarcane (*Saccharum officinarum* sp.) is a perennial grass originating from New Guinea that has been cropped in Brazil since the colonial period, reaching a harvested area of 9.7 Mha which accounts for 40% of worldwide production (FAOSTAT, 2011).

Since sugar from sugarcane is the main feedstock used to produce ethanol in Brazil, the planted area with this energetic crop has meaningfully grown in the last decade, and the trend is to continue expanding for the next years in order to achieve the national goals of production (Conab, 2013; Brasil, 2009; Cerri et al., 2010).

As the soil is an important natural reservoir of carbon

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(C), land use changes (LUC) during the transition to sugarcane production can lead to a decrease in soil carbon stocks (Lal and Kimble, 1997; Six et al., 2002). Land use and land changes are widely recognized as key drivers of global C dynamics (Schimel, 1995; Houghton et al., 1999). Soil organic matter (SOM) has a very complex and heterogeneous composition, and it is generally mixed or associated with the mineral soil constituents to form soil aggregates (Del Galdo et al., 2003).

A recent study performed in Brazil demonstrated that the greenhouse gases (GHG) emissions due to LUC for conversions from native vegetation and pastures to sugarcane could result in net transitional soil C debt with payback times ranging from 17 years in native vegetation, and 5 to 6 years in pastures. Conversely, when sugarcane replaced annual cropland, the soil C stocks increased by 17% in the 0 to 30 cm depth layer (Mello et al., 2014).

In addition to the C quantity, land use practices affect the soil C quality varying the distribution between particulate organic matter and SOM associated to the mineral fraction (Cambardella and Elliott, 1992; Christensen, 2001). Christensen (2001) proposes that the main effects of land use management can be observed by changes in the distribution of soil organic carbon (SOC) within particle-size classes.

Several studies have shown the influence of soil tillage system on the C pools, suggesting that particulate organic matter can be used as an early indicator of changes in C dynamics (Cambardella and Elliott, 1992; Six et al., 1999; Bayer et al., 2002; Freixo et al., 2002). However, few studies have been carried out to determine the impact of LUC on particle-size fractions, and no studies have been performed involving the land-use transition into sugarcane.

The correct evaluation of soil C changes and SOM dynamics is the one which functionally distinguishes among different SOM fractions. The development of management systems for sustained production requires a better understanding of the impact of land-use and LUC in the SOM pools.

In this context, this study aimed to investigate the modifications on total soil organic C and C content of the particle-size fractions, as results of LUC due to sugarcane planting in Central-South region of Brazil. The study used the physical fractionation method, based on the premise that SOM associated with particles of different size and therefore also of different mineralogical composition differ in structure and function (Christensen, 1992).

MATERIALS AND METHODS

Study area

The study area involved seven counties distributed throughout the Central-South region of Brazil, covering the states of Minas Gerais, São Paulo, Goiás and Paraná. Comparative soil samples were collected from sugarcane fields and native vegetation, pastures and annual crops areas as indicated by Mello et al. (2014). The selection of the study areas considered historical land use information and existence of reference areas (pasture, annual cropping or native vegetation) with similar geomorphic characteristics (topography, soil type etc.) as the sugarcane sites. Consequently, 34 study sites forming 17 comparison pairs were selected for soil sampling: 17 sugarcane fields, 13 pastures, 2 annual crops and 2 native vegetation areas.

Soil sampling and C determination

In each evaluated site, three pits were opened and soil samples were taken from the layers of 0 to 10 cm and 10 to 20 cm depth, totalizing 6 soil samples per site and 204 for the study area. Soil samples were sieved (2 mm), ground and sieved at 150 µm for carbon determination by dry combustion on a LECO® CN elemental analyzer (furnace at 1350°C in pure oxygen).

Soil C fractions were determined according to the method described by Cambardella and Elliott (1992), and adjusted by Feller and Beare (1997) and Christensen (1997). Briefly, 20 g of 2 mm sieved soil was mixed with 80 mL of hexametaphosphate solution (0.5%), refrigerated and subsequently sonicated for 15 min with a maximum power output of 500 W. The dispersed soil was sieved through a 53 mm sieve, and the sand-sized organic material (POM) retained on the sieve was thoroughly rinsed, transferred to aluminum pans, oven-dried (60°C), and weighed. The dried samples were ground, and the total carbon content in the fraction was determined by dry combustion on a LECO® CN elemental analyzer. Mineral-associated organic C (MOC) was estimated by difference between total C (TOC) and POM C.

Soil texture was determined as described by Mello et al. (2014).

Statistical analyses

The study was arranged in a completely randomized design comprising four land use systems - with three pseudo replications. Data was checked for normality using the Shapiro test and for homoscedasticity by Bartlett test. Analysis of variance with the F-test was performed, and differences detected were compared using the Tukey tests at a significance level set at $P < 0.05$.

The comparison of the relative portion of the fractions (POM and MOC) was performed using the Student t test. Changes in the TOC and the C content in each fraction were estimated considering the means between comparison pairs, hence descriptive analysis is presented for those results since only two comparison pairs were studied for native vegetation and annual crops situations.

All statistical analysis was performed using R Statistical Software (R Development Core Team (2011).

RESULTS

Total soil organic C and relative fractions of POM and MOC

The TOC content was quantified for all the land use systems-involving both layers: 0 to10 and 10 to 20 cm depth (Figure 1). The mean comparison test performed, which included all the sugarcane sites without considering the previous management, indicated TOC higher in

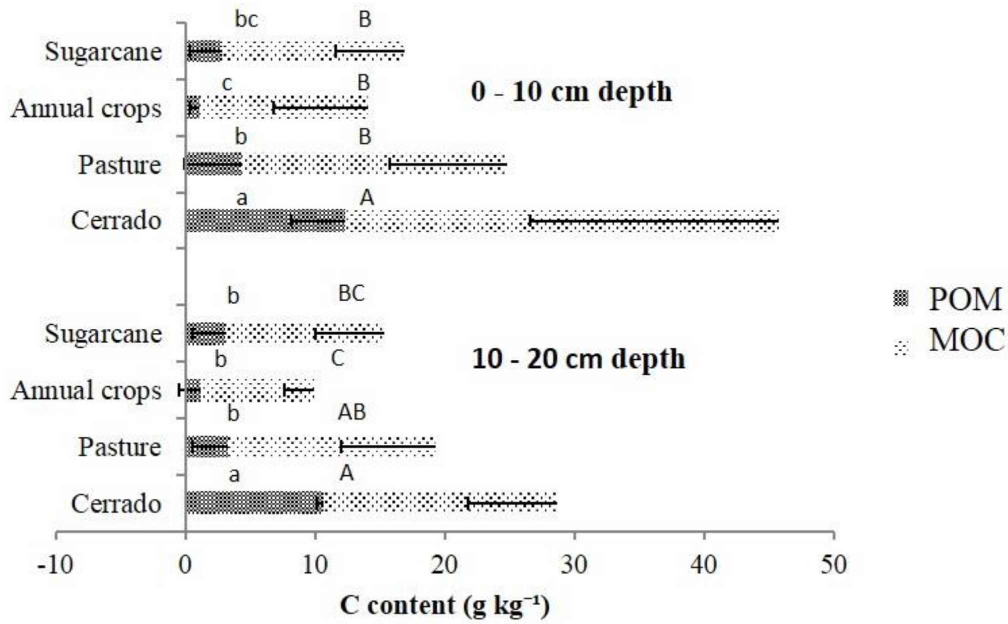

Figure 1. Total soil organic C (g kg-1) in each land use system for the 0 -10 cm and 10 – 20 cm depth. (* Means followed by the same capital letter within MOC and same lowercase letter within POM fraction in each layer are not significantly different according to Tukey's test at the 5% level).

native vegetation than in the other land use systems in both depths, with more contrasting differences in the top layer. The TOC in pastures was higher than quantified in sugarcane only in the 0 to 10 cm layer. No significant differences (P<0.05) were detected between sugarcane and annual cropping.

Carbon content in POM and MOC fractions was influenced by management at all sites and differences varied across depth. In the top layer (0 to 10 cm depth) the C in POM was more variable than in 10 to 20 cm layer, where no differences were estimated among the planted areas. Opposite pattern was found for the C content in the MOC fraction (Figure 1).

A trend of C declining with depth was observed in all land use systems. The TOC was 1.3 times lower in the deeper layer than in the top with changes more contrasting at MOC than in POM fraction. The mean relative amount (%) of POM and MOC as a portion of total soil C was estimated for the sugarcane sites and compared with the land use system used as reference (Figure 2A and 2B). According to the statistical analysis, the relative portion of both soil organic C fractions was not different between sugarcane, and the areas selected as reference of previous management for the two soil depth studied.

The proportion of POM in the condition accessed was affected by the soil clay and silt content. Relationships between POM relative fraction and silt plus clay content occurred in both layers, with higher coefficient of correlation in the 10 to 20 cm depth (R^2 = 0.59) than in the top layer (R^2 = 0.28).

Relative changes in soil C fractions

Carbon variation for each fraction due to the planting of sugarcane was quantified through the difference between the mean C content found in the sugarcane areas and their references. Subsequently the relative change in each C fraction was estimated (Table 1).

Greater C losses were found for the conversion from native vegetation to sugarcane crop. The LUC affected the labile organic carbon (POM) as well as the more stabilized C fraction (MOC). The impact of those processes was higher in the superficial layer than in the 10 to 20 cm layer.

In contrast, sugarcane planting over areas used for annual cropping production seems to increase the SOC, favoring the C accumulation in the mineral-associated fraction.

DISCUSSION

Soil organic carbon content reflects the long-term balance between additions and losses of organic carbon to the ecosystem. In this study, we compared the TOC content among the different land use systems accessed without considering the previous management of the sugarcane areas. Under this approach, higher C contents were found

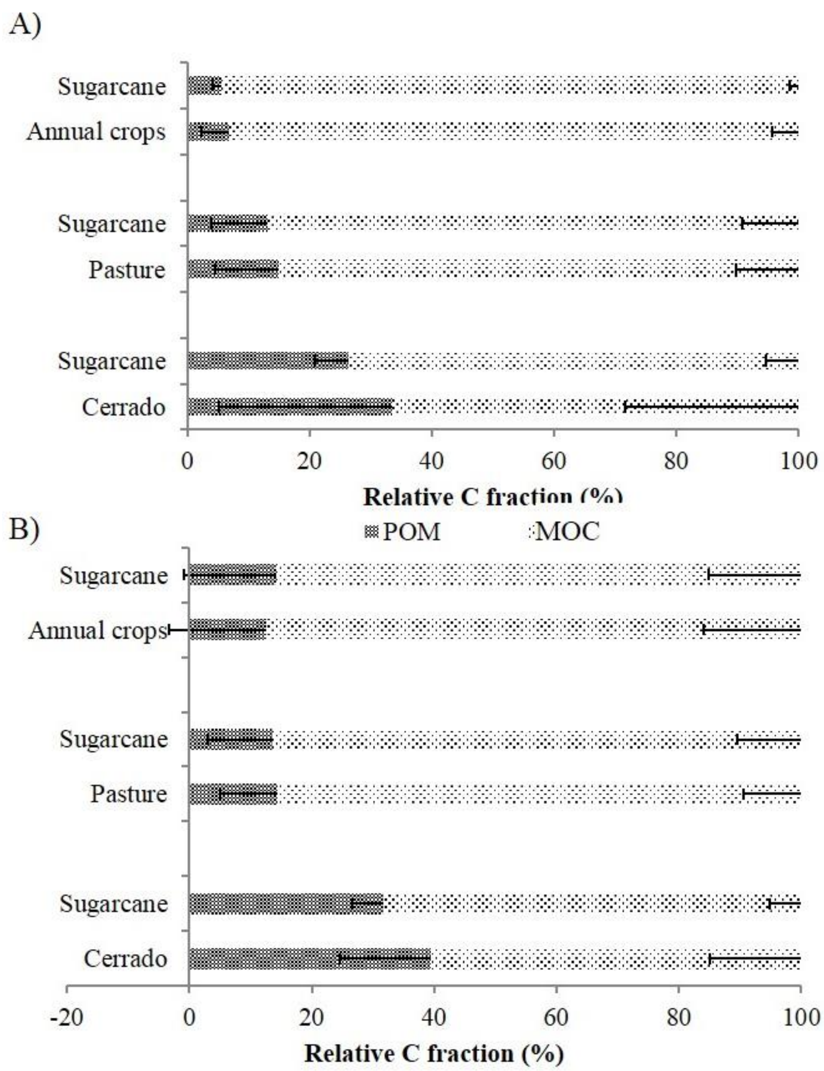

Figure 2. Relative portion (%) of particulate organic C (POM) and mineral-associated C fraction (MOC) for each conversion type involving sugarcane planting in 0 -10 cm depth (A) and 10 to 20 cm depth (B).

Table 1. Relative changes (%) in total C content (COT), particulate organic C (POM) and mineral associated C (MOC) due to the sugarcane planting in Central-South part of Brazil.

Reference	COT	POM	MOC
	0 - 10 cm depth		
Cerrado	-48.6	-50.82	-47.89
Pasture	-34.5	-37.11	-33.97
Annual crops	3.25	-23.13	5.53
Reference	**10 - 20 cm depth**		
Cerrado	-17.8	-29.15	-11.22
Pasture	-24	-20.18	-24.80
Annual crops	5.8	7.90	5.37

at native vegetation in both soil depth studied, in agreement with results reported in tropical soils. All studies that focused on the effects of land conversion from forest to cultivated land concluded that LUC induces a reduction of the available soil C and a decrease in its quality (Batlle-Aguilar et al., 2010). In tropical soils the soil C can be reduced by 50% in the first years of cultivation due to several processes, including microbial decomposition and erosion (Mielniczuk et al., 1999).

When compared with pastures, the study estimated that TOC under this land use system was 45 and 32% lower than native vegetation in 0 to 10 cm and 10 to 20 cm depth, respectively. Carvalho et al. (2010) studying different pastures areas in the Brazilian Cerrado region found C content varying as a function of the pastures management. Pasture lands can lead either to a positive or negative impact on the overall soil C. In this study, lower C content can be atributed to degradation process caused by the soil management. More than 70% of the total areas under cultivated pastures in Brazilian Cerrado region show some degree of degradation (Junior and Vilela, 2002; Batlle-Bayer et al., 2010) due to increasing stocking rates without maintenance fertilization, which results in a rapid decline of nutrients in the soil (Nair et al., 2011).

Even though no significant differences (P<0.05) were found in the C content of POM and MOC fractions among the planted areas, a trend of decreasing MOC (g kg^{-1}) from pasture to sugarcane areas (Figure 1) and in both fractions to annual crops sites was observed, pointing out the contrasting impact of the land use in SOC dynamic. Soil tillage has been indicated as a highly disturbing management practice (Silva-Olaya et al., 2013) which alters aggregate dynamics by enhancing the turnover time of SOM thus decreasing the formation of the more stabilized C fractions, such as POM C and mineral-associated C (Six et al., 1999).

Regarding the percentage of C presented for different fractions, the study found that POM fraction varied from 6 to 34% in the land use systems evidencing the differences in C inputs into the soil and the role of the historical use in the C dynamics, since the relative fractions in sugarcane crop followed the same pattern observed in the reference sites. Excepting for the annual crops areas, where less than 10% of POM was found, the values observed were within the range of values indicated by Feller and Beare (1997). C-turnover rates for the particulate organic matter associated with the sand-size fraction are higher, hence it is more depleted by annual cultivation than clay-bound organic matter (Chenu and Plante, 2006).

Despite the land use system affected the soil C content of all fractions; the sugarcane planting did not affect their proportional weight distribution when compared to the land use system reference (Figure 2A and 2B). The mean relative portion of POM and MOC does not vary significantly between sugarcane and its references in both layers, suggesting C losses in the labile fraction as well as in the stabilized pool, hypotheses corroborated later when estimated the relative change of each fraction as function of its reference (Table 1).

Mello et al. (2014) accessing the effects of LUC due to the sugarcane planting in South-Central part of Brazil on soil C stocks demonstrated soil C decrease following LUC from native vegetation and pastures, and increase where cropland is converted to sugarcane. In this study, using some of the areas sampled by Mello et al. (2014), the study estimated that the conversion from native vegetation to sugarcane crop affects both SOM pools. Particulate organic matter was the most sensitive fraction to LUC and declined by an average of 40% followed by the silt and clay sized fractions which had 30% less C in the surface soil horizon (0 to 20 cm depth) after the conversion. The same pattern was observed when sugarcane replaced pastures. Mean C losses of 33 and 30% in POM and MOC, respectively, were quantified for the same soil depth.

By analyzing each layer, the study found that the impact of LUC of SOC variation is greater in the top layer for both types of land use conversion, where wake relationship between POM and clay plus silt content was observed, evidencing that likely much of the POM found in this depth correspond to free particulate organic matter or unprotected SOM, which is relatively easily decomposable and are greatly depleted upon cultivation (Cambardella and Elliott, 1992; Six et al., 1999). At the 10 to 20 cm depth, the correlation between soil texture and relative fraction of POM suggests a protection of the labile C from decomposition in the inter-aggregates (occluded POM), which can influence the rate of C losses with soil perturbation. Even though SOC associated with mineral soil (MOC) is considered the more stable and recalcitrant fraction of SOM (Wiesenberg et al., 2010) these results imply that a significant proportion of the SOC at that pool is relatively labile, as suggested by Feller and Beaer (1997), and hence it is negatively affected by the LUC. Upon reviewing data obtained in literature Von Lützow et al. (2007) estimated that the generally higher allocation of SOC in smaller particles is not always congruent with a longer turnover time. In the sand fraction, ^{13}C turnover times ranged from 0.5 to 374 years, in the silt fraction from 115 to 676 years and in the clay fractions from 76 to190 years. The same trend was observed when used ^{14}C as indicator of mean residence time (MRT). Additionally, the authors found that the MRT and turnover time of fine clay is lower in than in the coarser clay fraction.

García-Oliva et al. (1994) reported that the C silt-associated fraction was subject to a lower loss rate than the clay-associated C fraction in a tropical deciduous forest after conversion to pasture. Similar results were found by Covaleda et al. (2011) comparing conserved and degraded forest soils. The clay-fraction is more enriched in new SOM because of microbial activity which decomposed faster than the silt-sized aggregate fraction (Gregorich et al., 1991). Since the study estimated the C associated with the mineral fraction as the difference between TOC and POM, the study believes that those differences between clay and silt association to SOM could explain the susceptibility of MOC to LUC in this study. Moreover, sugarcane crop maintains lower amounts of SOM when compared to pastures and native vegetation. Pre-harvest burning performed in this crop affects the potential mineralizable C of the SOM labile pool, soil microbial biomass and physical soil properties influencing the soil aggregation which is one of the main SOM stabilization mechanisms (Prieto-Fernández et al., 1998; Ceddia et al., 1999; Six et al., 2002; de Souza et al., 2005). Also, sugarcane fields pass through a cultivation cycle every five years which causes the emission of 3.5 Mg ha^{-1} C-CO_2 (Silva-Olaya et al., 2013); meanwhile either pastures or native vegetation remain for long periods without any soil perturbation.

In contrast to both conversions mentioned earlier, the LUC from annual cropland to sugarcane increased the TOC. According to Mello et al. (2014) the C stocks in sugarcane areas were 17% higher, leading to an accumulation of 36 Mg CO_2 ha^{-1} in the 0 to 30 cm of soil depth after 20 years of time span. In this study, using two of the comparison pairs reported by Mello et al. (2014) we estimated that 5% of that C increases occurred in the MOC fraction. Mean C depletion of 23% in POM fraction was observed at the 0 to 10 cm of soil depth and C accumulation at the deeper layer; however because of the high standard deviation of this parameter in both areas (sugarcane and annual crops) the POM dynamics cannot be conclusive. The two cropland areas had different management history and also different clay + silt content, variable that can influence the relative portion of the labile pool as indicated by the regressions performed for the 10 to 20 cm of soil depth. The combination of crop rotation and no-till at one of the reference sites resulted in higher C content at POM at the top layer which is depleted because of the conversion to sugarcane. In the 10 to 20 cm of soil depth the high clay + silt content of the same area seems to offer some degree of physical protection to labile fraction thus this is not strongly affected by the LUC. Conversely, management as well as soil attributes at the other reference site resulted in the opposite dynamic, thus C content in POM increased with the sugarcane planting in both layers. Even though the results of this pool cannot be conclusive, those could be an indication that the POM response to LUC varies as function of previous management in annual cropland, however a larger sample size of annual cropland to sugarcane conversion would be necessary to better evaluate soil C changes at the labile pool and to confirm that hypothesis. Sugarcane expansion at the Central-South region of Brazil is an important process that can affect the greenhouse gases (GHG) balance of this energy crop and consequently the overall sustainability of the biofuel production. In this sense, our results provide essential knowledge about the real SOC dynamic due to those LUC processes. With future expansion projected to involve mainly pastures and cropland areas it is important to develop management systems in sugarcane production that allow for the restoration of the stable C fraction (MOC) which has been considerably depleted because of the conversion from pastures, contributing to long-term soil C sequestration.

CONCLUSIONS

The proportion of particulate organic matter at 10 to 20 cm depth is affected by the clay plus silt content. Land-use conversion from native vegetation and pastures to sugarcane modifies the distribution of C within soil particle-size classes. Important C depletion in the stable fraction of SOM as well as in the labile fraction is caused by those kinds of transitions. In contrast, C accumulation in MOC is favored by the replacement of cropland areas by sugarcane.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Participatory evaluation cum demonstration of improved faba bean cultivars with inorganic and bio-fertilizers in West Gojam Zone, Amhara Region, Ethiopia

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The trail was conducted in Yilemana Densa district of the Amhara Region in 2013 with the objectives to evaluate the performance of improved faba bean cultivars with P-fertilizer and bio fertilizer under on-farm conditions, while demonstrating such a technology package to farmers thereby paving the way for wider adoption. The trial consisted of four treatments (two improved cultivars with and without fertilizers) was established on fifteen farmers' fields selected from three Kebeles of the district. The trial was laid out as a simple design, considering Kebeles as blocks and host farmers as replications. The fertilizer inputs applied for this trial were 25 kg DAP and inoculated seed with FB-EAL-110 bio fertilizer at rate of 500 g per hectare at planting. Host farmers were participated in the whole process of the trail from land preparation to harvesting. Field days were organized whereby three groups of farmers evaluated the four treatments with their own evaluation criteria by scoring. Agronomic data were also collected on plant height, biomass and grain yields, and analyzed using analysis of variance. CIMMYT partial budget analysis was used for economic analysis. The result of farmers' preference analysis showed that Wolki and Tumssa with fertilizers; Wolki and Tumssa without fertilizers were ranked 1st, 2nd, 3rd and 4th, respectively with the overall evaluation criteria. The result of ANOVA showed that there was significant difference in mean grain yield between Tumssa and Wolki cultivars, between treated and non-treated plots with fertilizers, between Tumssa with and without fertilizers, and between Wolki with and without fertilizers. The result of partial budget analysis indicated that Tumssa and Wolki cultivars with fertilizers can give marginal rates of return of 254% and 300%, respectively over their respective cultivars without fertilizers. The overall result showed that Wolki cultivar with fertilizers gave the highest grain yield and economic benefit and farmers' also ranked it first among the four treatments evaluated. Therefore, to increase the productivity of faba bean with low cost, Wolki cultivar with fertilizers should be promoted in the district and elsewhere in the Amhara region for wider adoption.

Key words: Faba bean improved cultivars, bio-fertilizer, inorganic fertilizer, farmers' evaluation criteria.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important cool season grain legumes in Ethiopia in terms of hectarage, total production, foreign exchange earnings and soil amelioration (Amare, 1990). Faba bean contributes to smallholder livelihoods in multiple ways. It can play a significant role in improving smallholders' food

Table 1. Treatment structure.

Treatment	Faba bean variety	DAP	Inoculants
1	Tumsa	-	-
2	Wolki	-	-
3	Tumsa	+	+
4	Wolki	+	+

security, as an affordable source of protein and other essential nutrients.

Faba bean can have an income benefit for smallholders as it yields a higher gross margin than cereals (IFPRI, 2010). Its crop residue is also widely used as animal feed In addition to improving food and nutritional well-being, faba bean can improve soil fertility through its ability of fixing atmospheric nitrogen to the soil. According to Somasegaran and Hoben (1994), faba bean is the efficient N fixer (240 to 325 kg ha^{-1} yr^{-1}) when inoculated with *Rhizobium leguminosarum* bv. *viciae.*

The most common mineral N fertilizer source employed in Ethiopia is urea. However, the price of mineral fertilizers has tremendously increased and reached to the level that a good proportion of the subsistence farmers often face difficulty to purchase and utilize it so that the productivity of faba bean and cereal crops are generally far below the potential. Biological N fixation, on the other hand, the major means of recycling of N in the biosphere, is an economically justifiable and ecologically safe N source to agriculture. It is a relatively low-cost source of N for small-holder farmers in Ethiopia where chemical N input is not affordable for most farmers (Amanuel et al., 2000).

Research on cropping systems in Ethiopia indicated that the improvements in soil fertility from planting wheat after faba bean in rotation can improve grain yield of wheat by more than one ton per hectare and can reduce fertilizer usage for cereals in the next season by up to 60% (Amanuel and Daba, 2006). Different research works made in recent years revealed that inoculation of faba bean with *R. leguminosarum* can *increase* yield by 10 to 50% (Abere et al., 2009).

However, as most of the research works on faba bean with inoculants were conducted in the controlled conditions in green houses, farmers have no awareness about the existence of such technology to utilize. Therefore, this study was conducted with the objectives to evaluate the performance of improved faba bean varieties with P-fertilizer and bio fertilizer under on-farm conditions while introducing such a technology package to farmers thereby paving the way for wider adoption.

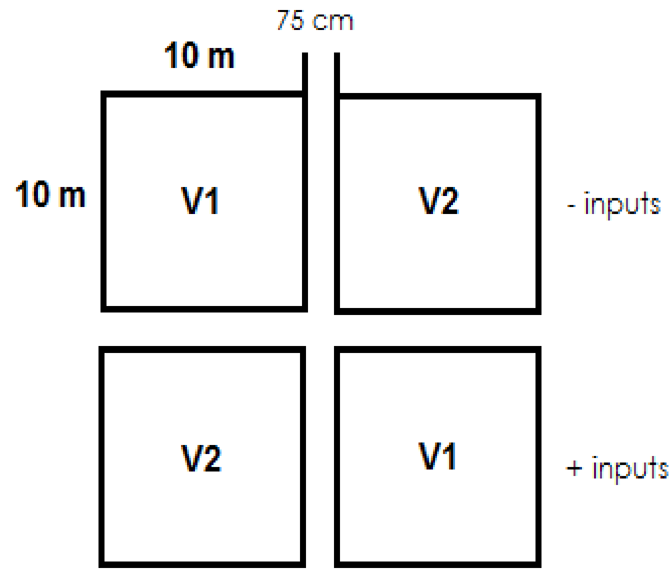

Figure 1. Trial design (*Note: V1=Tumsa; V2=Wolki).*

MATERIALS AND METHODS

The trail was conducted in Yilemana Densa district, West Gojam Zone of the Amhara Region in the 2013 main cropping season. From the district three representative Kebeles and from each Kebele five host farmers were selected. The trial consisted of four treatments (two improved faba bean cultivars that is, Tumsa and Wolki, each treated with and without fertilizer inputs) was established on each host farmer's field (Table 1 and Figure 1). The trial was laid out as a simple design, considering Kebeles as blocks and host farmers as replications.

The fertilizer inputs applied for this trial were 25 kg DAP per hectare banded, 10 cm away from the planting line, in a 2-cm deep trench, and inoculated seed with FB-EAL-110 bio fertilizer at rate of 500 g per hectare at planting with a planting density of 30 cm between rows and 10 cm between plants. All plots were weeded by hand two times as recommended. Host farmers were participated in the whole process of the trail from land preparation to harvesting.

Field days were organized at podding stage of the crop to collect farmers' opinion about the treatments under evaluation. During the field days, three groups farmers by site were asked to evaluate the four treatments with their own evaluation criteria by scoring 1 to 4

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Table 2. Farmers' preference scores and ranking.

Treatments of cultivars	Adet Hana site rank (n=11)	Debre Mewi site rank (n=9)	Geregera site rank (n=8)	Location mean score (Rank)
Tumssa with fertilizers	2	3	1	2 (2nd)
Tumssa without fertilizers	4	4	4	4 (4th)
Wolki with fertilizers	1	1	2	1.33 (1st)
Wolki without fertilizers	3	2	3	2.67(3rd)

Source: Own computation.

(1= the best). Farmers' scores given to each treatment by three groups of farmers independently were analyze by summing the scores given by all the three groups and divided by the number of groups and the lowest sum was ranked first. Agronomic data were also collected on plant height**,** biomass and grain yields. For agronomic data analysis, simple statistics, mean and percentage were used to compare mean grain yields and analysis of variance (ANOVA) was used to see whether there was significant difference or not among the four treatments in plant height**,** biomass and grain yield susing SPSS 16 Soft ware.

CIMMYT partial budget analysis was used for cost-benefit analysis. For partial budget analysis, fertilizers prices used were 14.97 Ethiopian birr (ETB) per kg of DAP and 160 ETB per 500 g for bio fertilizer. Farm gate price of faba bean at harvesting and improved seeds at planting were 11.75 and 15.00 ETB/kg respectively.

Daily wage was set at 30 ETB/day. Estimated labor for planting, fertilizers application, hand weeding, harvesting and threshing were 50, 36, 25 and 20 man days/ha respectively. Grain yield was adjusted down by 10% to more accurately reflect yields obtained under farmers' conditions.

RESULT AND DISCUSSION

Field days were organized at full podding stage of the crop where, three groups farmers, from three kebeles (Adet Hana, n=11; Debre Mewi, n=9 and Geregera, n=8) were asked to evaluate the four treatments (without labels to avoid bias) with their own evaluation criteria by scoring 1 to 4 (1= the best).

Farmers' evaluation criteria for faba bean were found number of pods per plant, pod length and plant height and ultimately grain yield. The result of farmers' preference analysis showed that Wolki with fertilizers, Tumssa with fertilizers, Wolki cultivar without fertilizers and Tumssa cultivar without fertilizers were ranked 1st, 2nd, 3rd and 4th, respectively across locations and with the overall evaluation criteria (Table 1). The result of mean score ranking predicted by farmers before harvest by seeing different parameters for grain yield is found exactly the same as the actual mean grain yield obtained after harvest (Table 2). However, farmers suggested that their own local faba bean variety with and without fertilizers inputs should have been included in the trail.

Out of 15 farmer's fields, 14 were successfully harvested and data were analyzed for grain yield parameter using simple statistics, mean and percentage, to compare mean grain yield among the four treatments (Table, 3). In addition, Wolki faba bean cultivar with fertilizers gave the highest mean grain yield with mean grain advantage of 48.1% over without fertilizers. Tumssa cultivar with fertilizers gave the second highest mean grain yield with mean grain advantage of 51.8% over without fertilizers.

To see whether there was significant difference or not among the four treatments in plant height**,** biomass and grain yield (these parameters were used to capture farmers' evaluation criteria: number of pods per plant, pod length and plant height and ultimately grain yield), ANOVA was used. The result of ANOVA showed that there was significant difference in mean grain yield between Tumsa and Wolki varieties, but there was no significant difference in biomass and in plant height between the two cultivars. This implies that by using Wolki faba bean cultivar regardless of fertilizers, a mean grain yield advantage of 20. 12 % (2.64qt/ha) can be obtained over using Tumsa cultivar.

There was significant difference between treated and non-treated plots with fertilizers in all of the three parameters regardless of varieties. This implies that, by using fertilizers alone, regardless of varieties, an additional mean grain yield advantage of 49.83% (5.76qt/ha) can be obtained over non-using fertilizers (Tables 1 and 4). This result is in agreement with the result of (Abere et al., 2009) which revealed that inoculation of faba bean with *R. leguminosarum* can increase yield by 10 to 50%.

Regarding cultivars and fertilizers, there was significant difference in grain yield between Tumsa cv. with and without fertilizers, and between Wolki cultivar with and without fertilizers. But there was no significant difference between Tumsa and Wolki with fertilizers and Tumsa and Wolki without fertilizers (Table 4).

Partial budget analysis

Using fertilizers on faba bean gave higher net benefits and higher Marginal rates of return over not using fertilizers. Growing improved faba bean varieties, Tumsa

Table 3. Mean grain yield and yield advantage of treated over non-treated plots by cvs.

Tumssa with fertilizers (qt/ha)	Tumssa without fertilizers (qt/ha)	Yield advantage of Tumsa with fertilizers over with out (%)	Wolki with fertilizers (qt/ha)	Wolki without fertilizers (qt/ha)	Yield advantage of Wolki with fertilizer over with out (%)
15.82 (2)	10.42 (4)	51.8	18.81(1)	12.70 (3)	48.1

Source: Own Computation; Figure in ( ) is rank.

Table 4. Analysis of variance.

Block	**Bio mass yield (qt/ha)**	**Grain yield (qt/ha)**	**Plant height (cm)**
Debre Mewi	53.63a	16.13a	97.44a
Adet Hana	42.88a	13.70a	98.24a
Geregera	42.81a	13.49a	76.38b
Cultivars	-	-	-
Tumssa	45.38a	13.12b	90.15a
Wolki	47.50a	15.76a	91.22a
Fertilizers			
With	51.58a	17.32a	94.51a
with out	41.29b	11.56b	86.86b
Cultivar x fertilizers			
Tumssa x Fertilizers	49.58a	15.82ab	93.18a
Tumssa x without Fertilizers	41.17a	10.42c	87.12a
Wolki x Fertilizes	53.58a	18.81a	95.84a
Wolki x without Fertilizers	41.42a	12.70bc	86.61a
CV %	12.6	10.4	4.8

Source: Own Computation; Means with the different letter are significantly different at the 0.05 level of significance.

and Wolki, with fertilizers can give a marginal net benefit of 4,096.25 and 4,848.25 ETB, over their respective varieties without fertilizers.

The marginal rates of return (MRR) for Tumsa and Wolkie cultivars with fertilizers were 254 and 300%, respectively (Table 5). This implies that for one birr additional cost incurred on the use fertilizers for example, on Wolkie variety, an additional birr of 3.00 can be obtained after paying the input cost.

CONCLUSIONS AND RECOMMENDATIONS

The result of farmer's preference analysis showed that Wolki and Tumssa cultivars with and without fertilizers ranked 1st, 2nd, 3rd and 4th, respectively across locations and with the overall evaluation criteria. The result of mean score ranking predicted by farmers before harvest by seeing different parameters for grain yield is found exactly the same as the actual mean grain yield obtained after harvest .

The result of ANOVA showed that there was significant difference in mean grain yield between Tumsa and Wolki cultivars. This implies that by using Wolki faba bean cultivar regardless of fertilizers, a mean grain yield advantage of 20. 12% (2.64qt/ha) can be obtained over using Tumsa variety. The result of ANOVA also indicated that there was significant difference between treated and non-treated plots with fertilizers in all of the three parameters. This implies that, by using fertilizers alone, regardless of cultivars, an additional mean grain yield advantage of 49.83% (5.76qt/ha) can be obtained over non-using fertilizers. This result is in agreement with the result of Abere et al. (2009) which revealed that inoculation of faba bean with *R. leguminosarum* can increase yield by 10 to 50%. Regarding varieties and fertilizers, there was significant difference in grain yield between Tumsa variety with and without fertilizers, and between Wolki cultivar with and without fertilizers. But there was no significant difference between Tumsa and Wolki cultivars with fertilizers and Tumsa and Wolki cultivars without fertilizers.

Table 5. Partial budget analysis.

Variable	Treatments of the tested cultivars			
	Tumsa without fertilizers	Tumsa with fertilizers	Wolki without fertilizers	Wolki with fertilizers
Mean grain yield (kg/ha)	1.042	1.582	1.270	1.881
Adjusted yield (kg/ha)	938	1424	1.143	1.693
Gross field benefit (ETB/ha)	11.021.50	16.732.00	13.430.25	19.892.75
Cost of seed (ETB/ha	3.000	3.000	3.000	3.000
Labor cost for planting (ETB/ha)	1.500	1.500	1.500	1.500
Cost of P-fertilizer (ETB/ha)	0	374.25	0	374.25
Cost of bio-fertilizer (ETB/ha)	0	160	0	160
Labor cost for P-fertilizer application (ETB/ha)	0	1.050	0	1.050
Labor cost for bio-fertilizer application (ETB/ha)	0	30	0	30
Labor cost for weeding ETB/ha)	750	750	750	750
Labor cost for harvesting and threshing ETB/ha)	600	600	600	600
Total cost that vary (ETB/ha)	5.850	7,464.25	5.850	7.464.25
Net benefit (ETB/ha)	5.171.50	9.267.75	7.580.25	12.428.50
Marginal cost (ETB/ha)	-	1.614.25	-	1.614.25
Marginal net benefit (ETB/ha)	-	4.096.25	-	4.848.25
Marginal rate of return (%)	-	254	-	300

Source: Own computation.

The result of partial budget analysis indicated that growing improved faba bean varieties, Tumsa and Wolki, with fertilizers can give a marginal net benefit of 4,096.25 and 4,848.25 ETB, over their respective varieties without fertilizers. The marginal rates of return (MRR) for Tumsa and Wolkie cultivars with fertilizers were 254 and 300 %, respectively. This implies that for one birr additional cost incurred on the use fertilizers on Tumssa and Wolkie cultivars an additional birr of 2.54 and 3.00 can be obtained, respectively after paying the input cost.

All the analysis showed that Wolkie variety with fertilizers can give the highest grain yield and economic benefit and farmers' also ranked it first among the four treatments evaluated. Therefore, to increase the productivity of faba bean with low cost, Wolkie cultivar with fertilizers should be promoted, while evaluating Wolkie cultivar and farmers own local faba bean cultivar with and without fertilizers to fine-tune this recommendation as farmers suggested.

Conflict of interests

The author has not declared any conflict of interests.

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Full Length Research Paper

Evaluation of DSSAT model for sprinkler irrigated potato: A case study of Northeast Algeria

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This study was conducted in order to evaluate the performance of potato crop in two major irrigation schemes situated in the Northeast of Algeria. This area is characterized by alluvial soil texture, very fine clay, a very flat topography, and a typical Mediterranean climate. This was done to guide farmers on any changes to their crop management to improve the yields of potato crops. The model SUBSTOR-potato (Decision Support System for Agrotechnology Transfer, DSSAT 4.5) was calibrated and validated for two cropping seasons 2008-2009 and 2009-2010, in order to estimate yields. The calibration of the model required the combination of genetic coefficients that characterize the phenology and morphology of this culture. The model performance was evaluated from statistical coefficients (R^2, root mean square error (RMSE), and BIAS). The results appear to be satisfactory (R^2=0.715, RMSE=34.52 qx/ha, BIAS=-7.34). The observed yield is 254.33 qx/ha and the estimated one is 238.77 qx/ha. There is a positive impact of climate change generated by the model weatherman on yields of potato particular for 2050. This indicates the model using possibility because it improves the results of farmer's strategies over multiple years.

Key words: SUBSTOR-potato, calibration, simulation, statistical coefficients, Algeria.

INTRODUCTION

The Mediterranean region seems to be particularly affected by climate change. The warming is projected to be greater than the global average, with also a large percent reduction of precipitation and an increase in its inter-annual variability (Giorgi, 2006). A pronounced decrease in precipitation over the Mediterranean is expected (Tanasijevic et al., 2014). Algeria, who belongs to the southern areas, faces a water stress which is a threat to the sustainable development of irrigated agriculture.

Potatoes have a dominant place in the diets of people in Algeria and according to the local statistics, this crop is in the fifth place in the agricultural production with two leading varieties Desiree and Spunta (MADR, 2010). However, the large area that is put into potato production each year requires full irrigation in order to meet the crop water requirements, especially during the most sensitive growth stages. The annual production approaches 2.8 million tons and the cultivated area was 161873 hectares with a resulting yield of 17.3 tons per hectare (MADR,

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2010). This value is clearly too low if compared to those of the developed countries or South American's (40 and 25 tons per hectare, respectively).

In these conditions, optimal scheduling of water application in the framework of "future climate change impacts on freshwater resources and their management" should be improved (Kundzewicz et al., 2008). Progress in understanding is conditioned by adequate availability of observation data, which calls for enhancement of monitoring endeavors worldwide, addressing the challenges posed by projected climate change. One way to meet this objective is to use a crop-model that simulates crop yield under different soil-climate conditions and crop management practices (Stastna et al., 2010).

Crop models can be useful tools for managers in the irrigated areas with different agroecological conditions to improve the sustainability of agricultural systems. Many authors, namely Hoogenboom (2003) and Ritchie et al. (1995) applied Decision Support System for Agrotechnology Transfer (DSSAT) model to optimize land use and water allocation to crops at plot level. Moreover, Medany (2006) and Abdrobbo et al. (2010) applied it in Egypt and stated that DSSAT predicts increases in yields. However, no similar work has been conducted elsewhere in the south Mediterranean.

The objectives of this study were to calibrate and validate the DSSAT model for potato (variety Desiree) in the Bounamoussa and Guelma irrigation district, Northern Eastern Algeria. The conclusions should provide valuable information to water managers and potato producers on the future challenges due to climate change.

MATERIALS AND METHODS

Description of the study area

The study area is located in the Northeast of Algeria (Figure 1) and includes two irrigation districts: the Bounamoussa irrigation district and Guelma irrigation district (36°46´and 36°49´ N; 7°43´and 7°49´ E, respectively). The total equipped areas of the Bounamoussa irrigation district is about 16500 ha, while in Guelma irrigation district, it is about 6000 ha (ONID, 2010). The irrigation water is supplied by the Cheffia and Hammam Dabbagh dams, respectively. The climate is Mediterranean characterized by two periods; a rainy period from September to April and a dry and hot period from May to August. The minimum and maximum daily air temperatures range between 22.6 and 12.2°C, respectively, while the annual average precipitation, relative humidity, and wind speed are 686 mm, 75%, and 3 km day^{-1}, respectively (FAO, 2006). The soil has a sedimentary formation which is dominated by clay.

Both irrigation districts are equipped with sprinkler irrigation system to irrigate mainly vegetable crops with dominance of tomato in Bounamoussa and potatoes in Guelma. For performing their crop irrigation management, the majority of farmers appreciate the soil moisture by touching the soil and its color appearance. This technique is very well controlled by farmers especially for clay soil texture. Some farmers use corn (*Zea maize*) as an indicating plant for hydric-stress to adequately assess the irrigation requirements. The average seasonal irrigation dose is about 6940 m^3 ha^{-1} in Bounamoussa and 7000 m^3 ha^{-1} in Guelma (Guemraoui and Chabaca, 2005). The potato yield recorded in the study areas (with different types of soils) varied between 15.8 and 30.0 t ha^{-1} in 2009.

Model description

For this study, the SUBSTOR potato model (Simulate Underground Bulking Storage Organs) included in DSSAT 4.5 was used to evaluate its performance for the variety Desiree in the study area using data from six representative types of soils (Table 1). The model describes daily phonological development and growth in response to environmental factors (soils, weather, and management). DSSAT uses the Ritchie model to calculate crop evaporation and transpiration and to update the soil water balance on a daily basis as a function of the water transfer processes affecting the soil profile (precipitation, irrigation, transpiration, soil evaporation, runoff, and drainage) (Ritchie, 1998). The growth stages simulated by the DSSAT SUBSTOR include sprout germination, emergence, tuber initiation, maturity, and harvest. The model includes five cultivar specific coefficients (G2, G3, P2, TC, and PD) that control the tuber initialisation, the leaf area development, and the tuber growth rate. These coefficients determine the crop responses to the management and the environmental factors, and their level of performance (Hunt et al., 1993). G2 (cm^2 m^{-2} d^{-1}) is the leaf area development rate and G3 (g m^{-2} d^{-1}) is the tuber growth rate. P2 and TC unitless coefficients correspond to the effect of photoperiod and temperature sensitiveness. P2 takes values between 0 and 1, where lower values are assigned for late cultivations, those developing with fewer hours of daily radiation. TC is the temperature value above which the tuber initiation is inhibited. PD is a dimensionless coefficient that describes to what extent the cultivar is determinate. Output data files provide a detailed description of tuber yield and the above ground biomass as well as information about soil reserves of available water and nitrogen. The model simulates physiological crop responses (phonology and growth throughout the season). More additional information about the model is provided by using the DSSAT model.

Model input data

The required input data includes daily weather data (maximum and minimum air temperature, solar radiation, precipitation, relative humidity, and wind speed), soil data (field capacity, wilting point, depth, bulk density, rooting preference coefficients, runoff, radiation reflection coefficients, initial soil water, and nitrogen content for each soil layer), and crop management practices (sowing date and dates and amounts of irrigation and N fertilization). The meteorological input data considered in the study (2009 and 2010), were recorded by two meteorological stations located in Bounamousa (36°49´N, 7°49´W, 4 m altitude) and Guelma (36°28´N, 7°28´W, 227 m altitude).

Some of the soil's data belonging to the six representative types of soils considered were taken from the National Agency of Hydraulic Resource database (Table 1). The total nitrogen was estimated from the carbon/Nitrogen (C/N) ratio. The drained upper limit (field capacity), lower limit (wilting point), soil water content at saturation, and soil rooting preference function for each layer were calculated by the algorithm included in DSSAT, based on input of measured soil characteristics (layer thickness and depth, clay, silt, coarse, and organic fractions C). Permanent wilting point (PWP) and field capacity (FC) were determined from soil texture by DSSAT 4.5 model. The soil albedo, drainage rate, and soil run-off curve number were set as 0.09, 0.4, and 76 for Guelma and 0.09, 0.05, and 61 for Bounamoussa, respectively. The potato management data were the same for all soils types, including planting date (February 01), harvest date (July 01), planting density (6.5 plants m^{-2}), row spacing

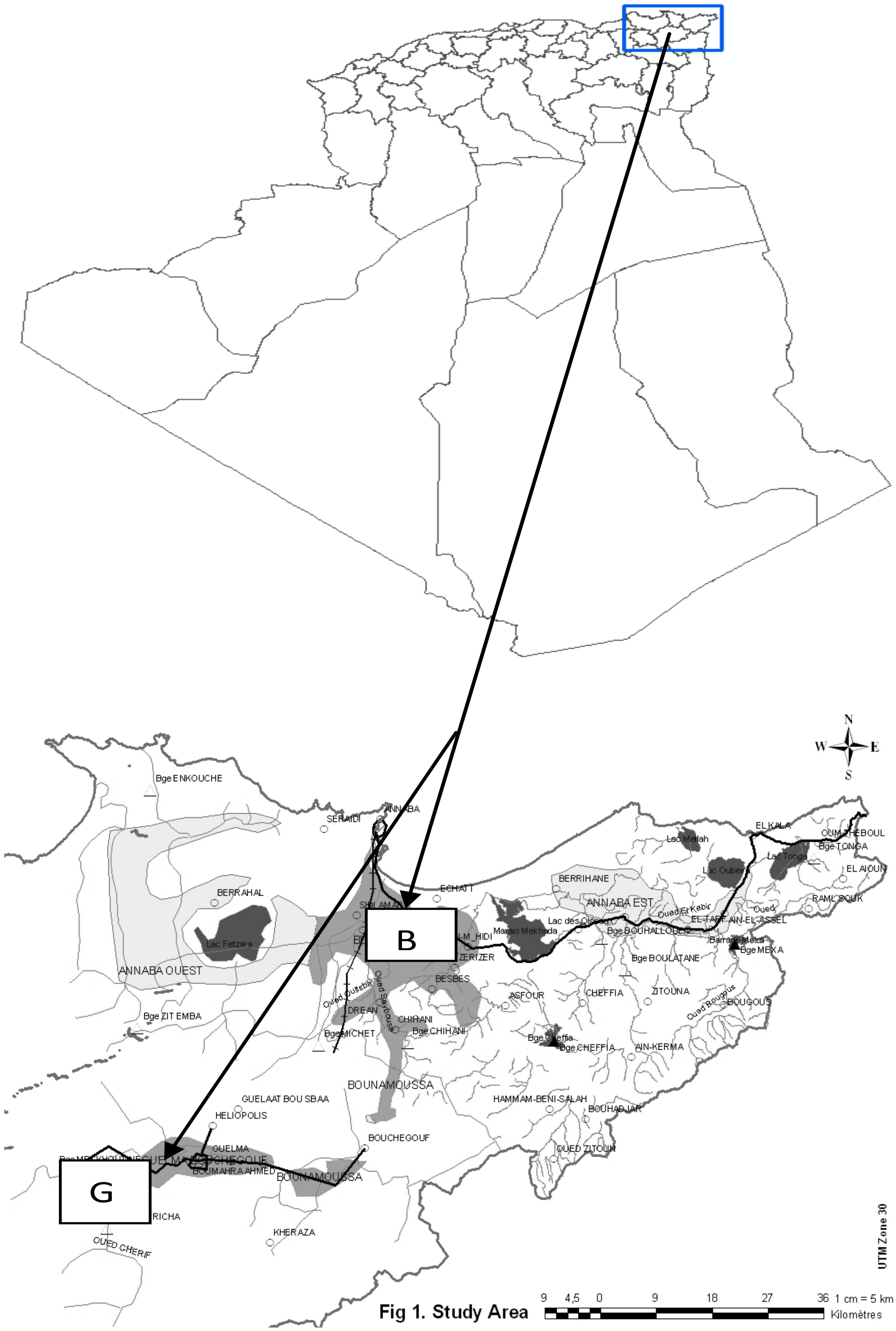


Figure 1. Bounamoussa (B) and Guelma (G) irrigation districts localization.

(75 cm), irrigation (100 mm in Guelma and 80 mm in Bounamoussa), fertilizer application before planting (15.15.15, 18 quintals ha^{-1}), and chemical applications (various products).

Model calibration and validation

The SUBSTOR model calibration and validation processes were

Table 1. Soil characteristics of the study areas.

Plot	Irrigation district	Soil depth (m)	pH	Clay (%)	Silt (%)	Sand (%)	Bulk density (g cm^{-3})	Organic carbon (%)
7	Guelma	0.70	8.3	37.1	39.5	23.3	1.30	0.60
8	Guelma	0.87	8.0	39.9	50.4	9.6	1.24	0.70
9	Guelma	0.66	7.9	38	50	12	1.29	0.81
10	Guelma	0.95	7.6	35.4	52.6	11.9	1.29	0.75
1	Bounamoussa	0.86	8.3	30.5	55.1	14.3	1.31	0.54
4	Bounamoussa	0.60	8.1	55.9	38.4	5.6	1.19	0.65

performed for the Desiree potato variety. The calibration process consists of adjusting the genetic coefficient values (G2, G3, P2, PD, and TC) considering the six types of soils and the 2009 input data. The validation process consists of a comparison between observed and simulated tuber yield using the observed meteorological data of 2010. For both processes, the model runs were performed with water and nitrogen balance simulation switched "on," using FAO 56 method for the evapotranspiration calculation. In this study, only the potato tuber yield was used as an evaluation parameter using the following statistical criteria: (i) linear regression and coefficient of determination (R^2) between simulated and observed potato tuber yield; (ii) the root mean square error (RMSE) of these variables, and (iii) BIAS. The R^2 represents the percentage of the variance in the measured data explained by the simulated data. The BIAS measures the average difference between measured and simulated values. If on the 0 average the model under-estimates, then the BIAS is positive and conversely if the model over-estimates. The optimal value of BIAS is 0.0. The RMSE is equal to the sum of the variance of the modeled values and the square of the BIAS. The smaller the RMSE, the better the performance of the model. A RMSE value of 0.0 represents a perfect simulation of the observed potato tuber yield. The calibration objectives for potato tuber yield were to maximize R^2 and to minimize the absolute value of BIAS and RMSE.

Climate change

The daily observed climate parameters (2006-2011), needed to manage a future climate by applying a weatherman model that is integrated in DSSAT, were collected nearby the National Office of Meteorology (Figure 2). The lack of solar radiation data was filled by the estimation of this parameter by applying the CROPWAT 8.0 model.

RESULTS AND DISCUSSION

Model evaluation

After the iterative process of calibration, the best derived genetic coefficients were G2=2000, G3=22.5, G4=0.2, PD=0.7, P2=0.4, and TC=17. These coefficients are constants in the model and are used to quantify differences in development's responses between potato cultivars. The comparison between simulated and observed yield is presented in Figure 3. Results indicated a significant correlation between observed and simulated values (R^2 = 0.71). The measured yield was 23.5 t ha^{-1} and the simulated one was 22.8 t ha^{-1} with a coefficient of variation (CV) of 0.21 and 0.30, respectively. The statistics evaluation associated with the average error between observed and simulated values of the six plots presents RMSE and BIAS values of 3.45 t ha^{-1} and -7.34, respectively. These results are analogous to those indicated by Pereira et al. (2008) in Brazil (Itararé IAC-5986 cultivar), Medany (2006) Valour cultivar, Abdrabbo et al. (2010) in Egypt (Valour and Desiree cultivar), and Daccache et al. (2011) in England's humid climate (Bintje cultivar). For this author, the differences expressed as a percentage between the simulated and observed mean yields were very small (1 to 3%). Medany (2006) found that there is no difference between observed and predicted data and concluded that SUBSTOR-Potato crop model can be used successfully in Egypt.

The simulation performed with the adjusted genetic parameter using the 2008 data indicated that the Plot 7 was the only plot that presents the lowest crop yield because of water stresses observed during the growing season (Table 2). This was due to the inadequate irrigation management by the farmer. Simulation results indicate also a nitrogen deficit for all plots throughout the vegetative stages and especially during tuber formation until maturity. This had got a negative effect on potato tuber yield that are close to the national average production of potato (22.6 t/ha). Tuber fresh weight was peaked at 150th day after planting for all plots except plot 7 which the tuber fresh yield was reached at 130th day due to water deficit. The tops weight is obtained for the six plots between 60 and 90th day of the vegetative stages. The maximum simulated leaf area index (LAI) is 0.75 and 2.5, corresponding respectively to plot number 7 and 10 for the 60 and 80th day of the vegetative stages (Figure 4). These results were derived by the SUBSTOR model after its calibration.

The validation process was based on independent sets of field data corresponding to 2009 (Figure 5). Results indicate that the correlation between measured and simulated yield was better during the validation process (R^2 = 0.73) than during the calibration (R^2 = 0.71). The comparison between the measured and calculated yield of fresh tubers showed a significant correlation with average values of BIAS and RMSE of -7.34/23.33 and

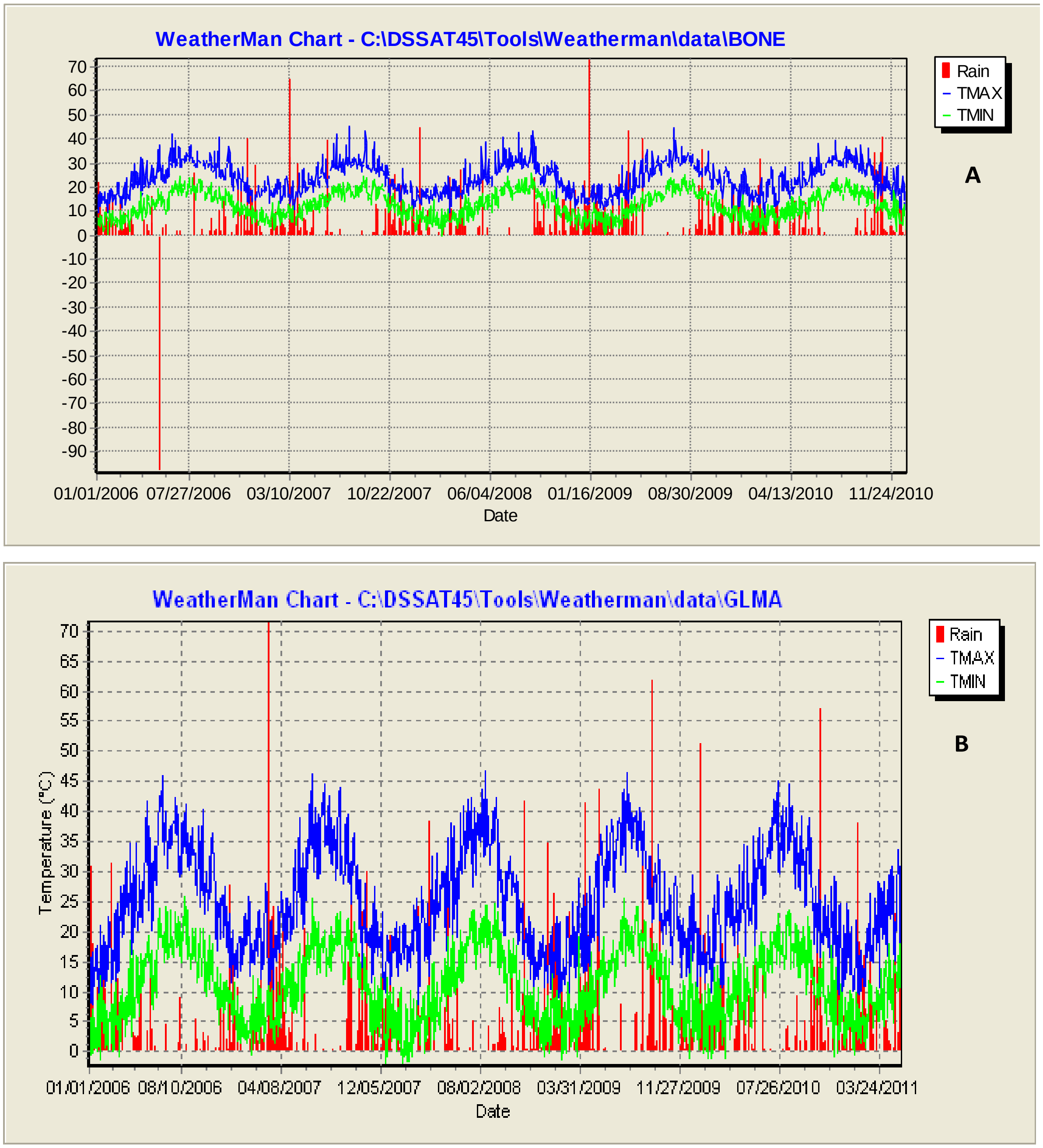


Figure 2. Daily variation of rainfall and minimum and maximum temperature for (A) Bone=annaba and (B) glma=Guelma climatic stations. (2006-2011).

34.52/43.59 quintals ha^{-1}, respectively for model's calibration and validation.

Climate change

Table 3 provides information about the future yields evolution (2020-2050-2080) simulated with DSSAT4.5 according to climatic changes (minimum and maximum temperature and average cumulative rainfall) (Figure 6) and water stress during the vegetative stages for all the plots in the study area, without taking into account the evolution of the CO_2 and maintaining a constant agricultural crop management.

The results show essentially:

(1) Increased yields as compared to 2009.
(2) Falling yields in 2080 compared to 2020 for all plots

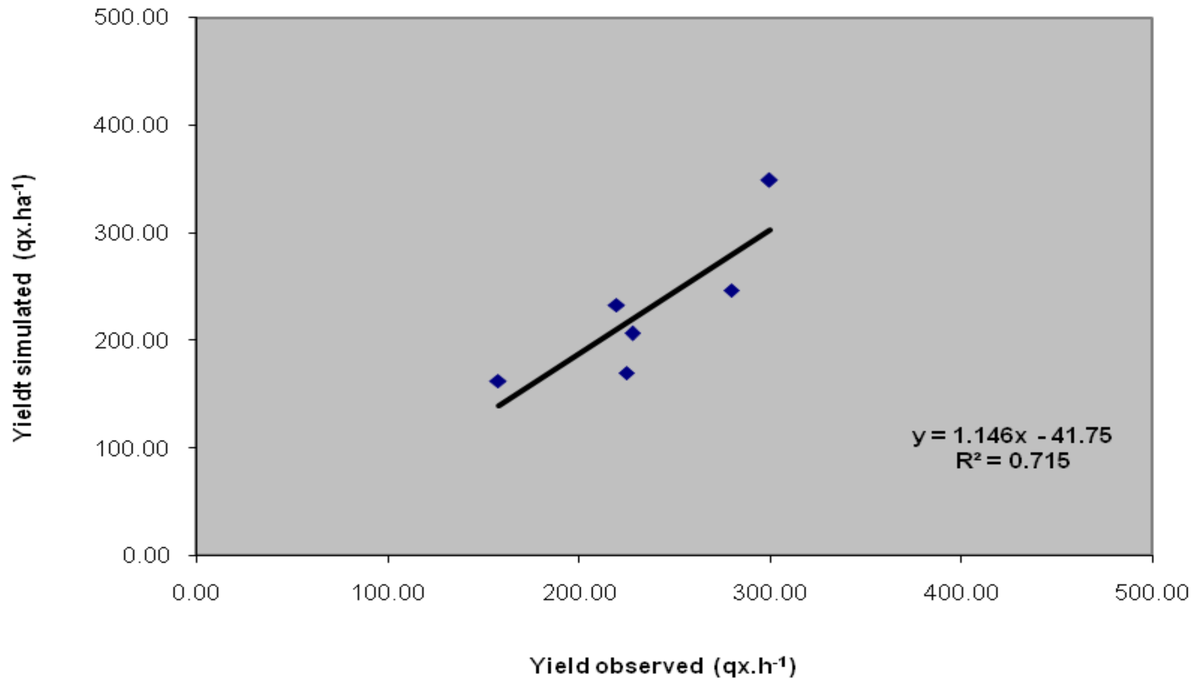


Figure 3. Simulated versus observed tuber fresh yield and linear regression during calibration process.

Table 2. Effect of the climatic parameters on water and nitrogen stress during vegetative stages of potato.

Plot	Vegetative stage	Timespan days	Rain (mm)	Tuber yield observed (t/ha)	Water stress	Nitrogen stress
	I	28	67.1		0.218	0.063
7	II	82	319.8	15.8	0.389	0.529
	III	180	548.9		0.283	0.399
	I	29	67.1		0.000	0.026
8	II	84	319.9	22.0	0.000	0.559
	III	180	548.9		0.000	0.414
	I	29	67.1		0.000	0.029
9	II	80	319.8	22.5	0.000	0.596
	III	180	548.9		0.000	0.438
	I	29	67.1		0.000	0.003
10	II	85	320.0	28.0	0.000	0.544
	III	180	548.9		0.000	0.397
	I	25	61.4		0.026	0.000
1	II	84	206.5	30.0	0.000	0.452
	III	185	354.8		0.003	0.371
	I	25	82.9		0.000	0.085
4	II	91	309.3	22.8	0.000	0.496
	III	185	610.1		0.000	0.384

I: Stage Emergence-Begin Tuber; II: Stage Begin Tuber-Maturity; III: Planting to Harvest.

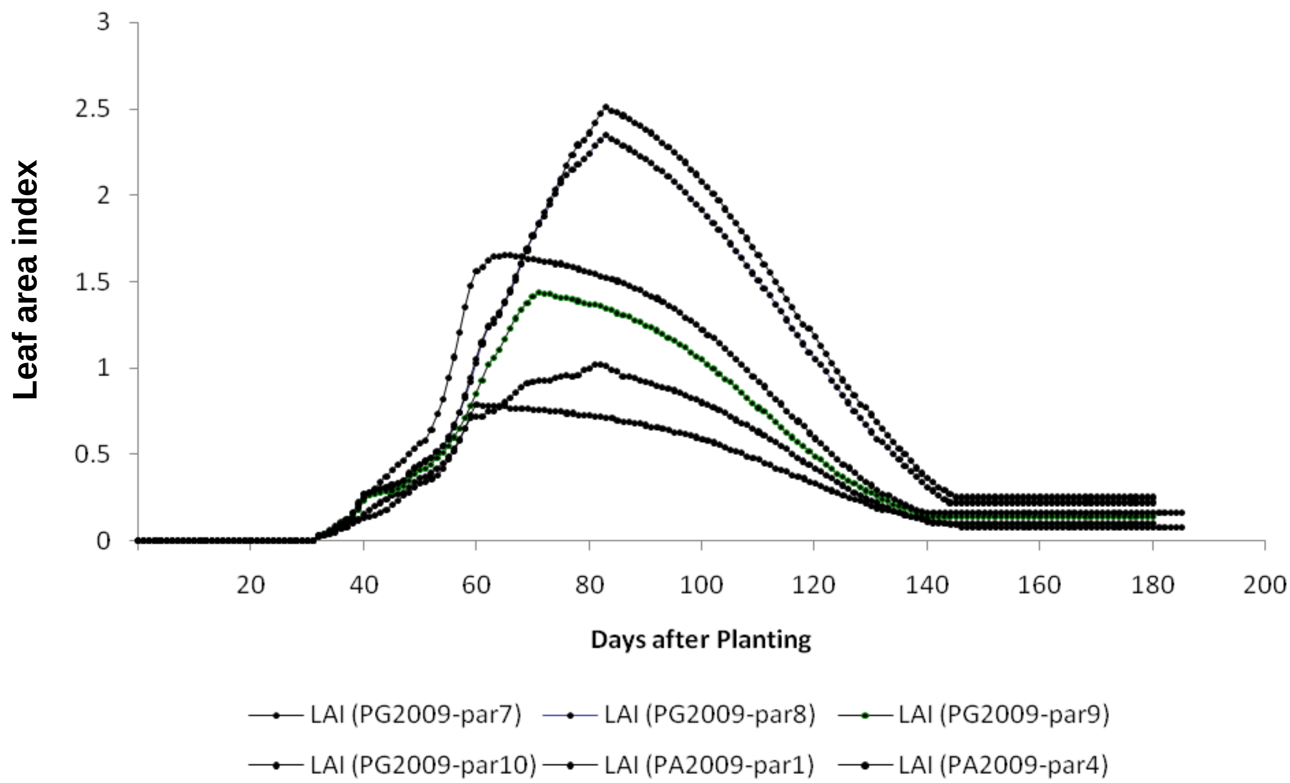


Figure 4. Simulated potato LAI (Leaf Area Index) for the six plots during the crop cycle.

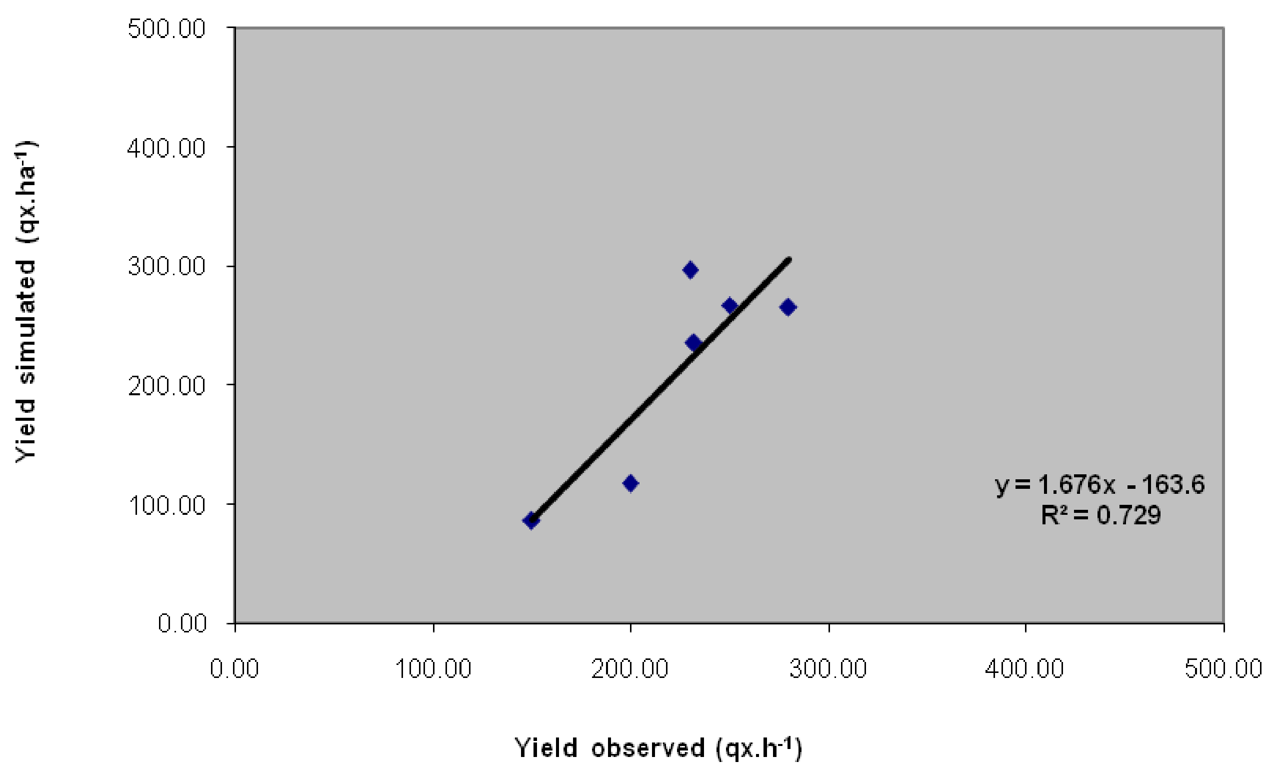
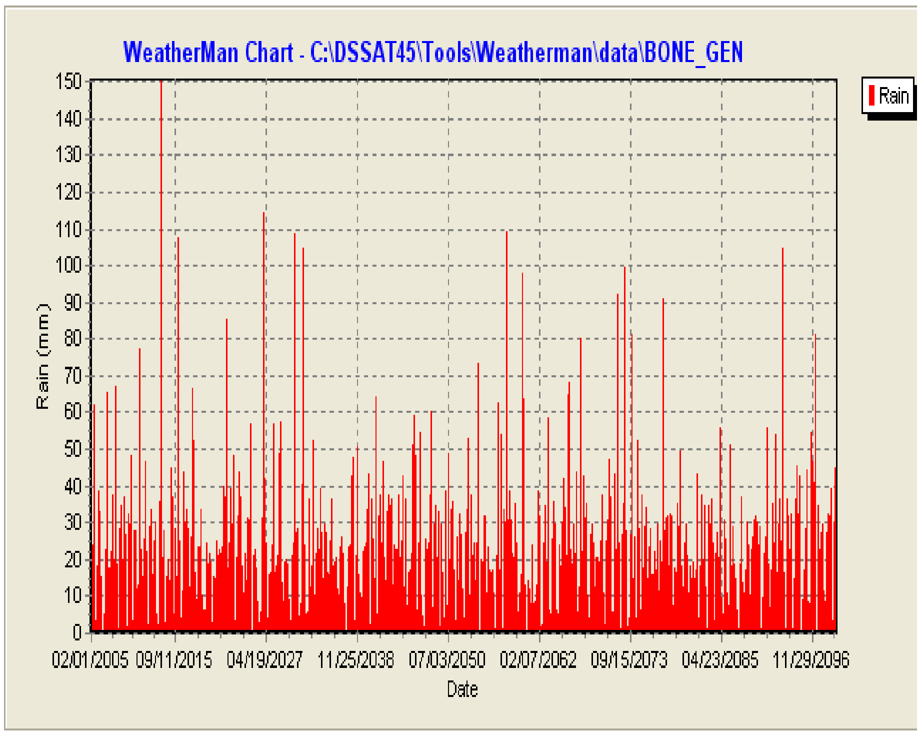


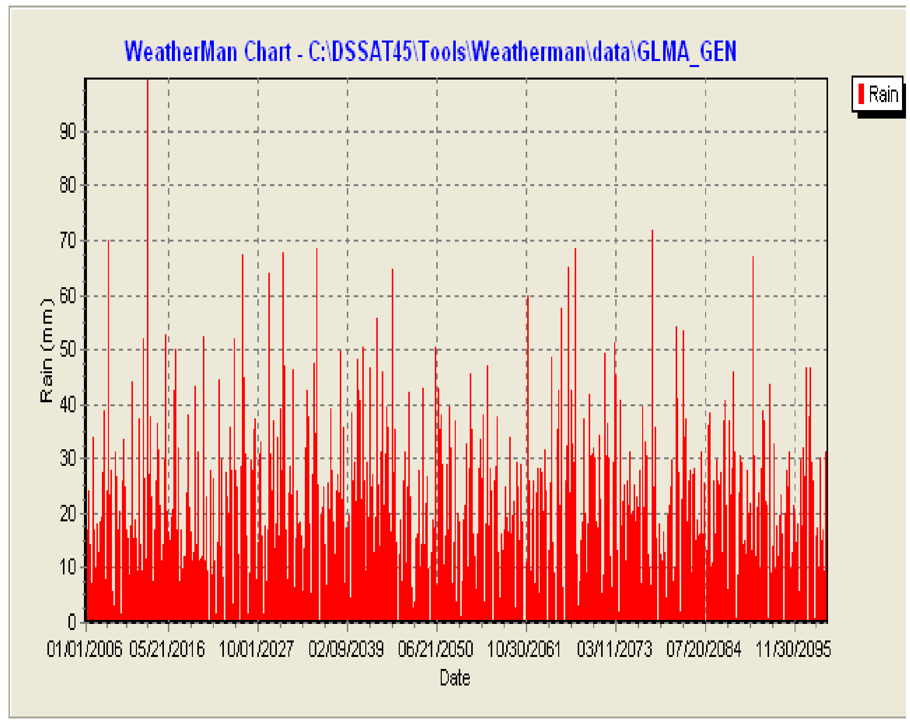
Figure 5. Comparison between simulated and observed yields of potato plots during year 2010.

Table 3. Climatic parameters, water stress and yield of potato in the different plots for projected periods 2020-2050-2080.

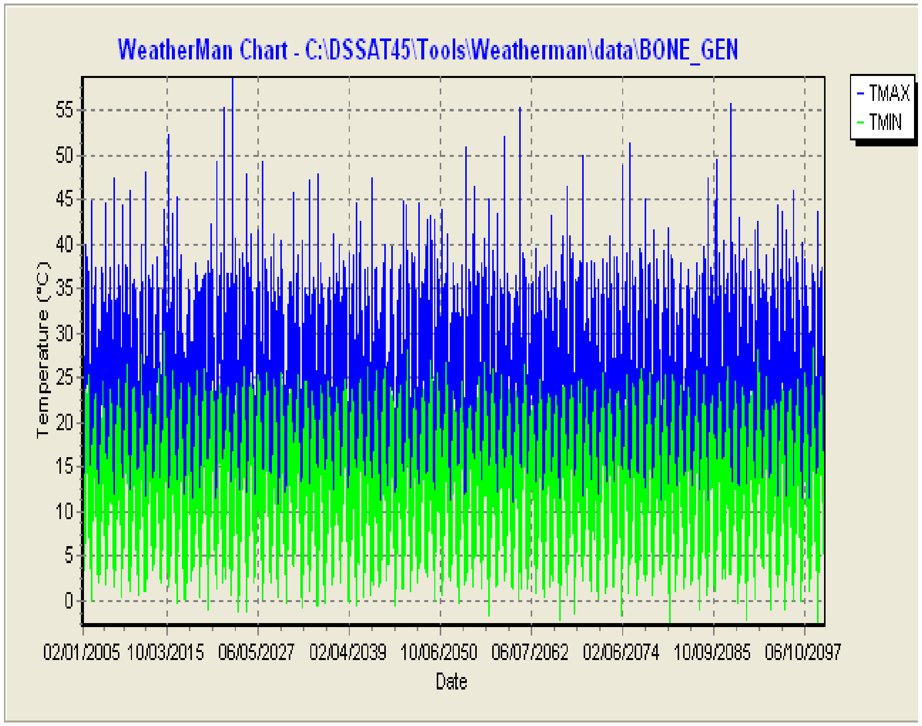
Plot	Yield (T/ha cycle days)	2020 Water stress (%)	Tmax (°C)	Tmin (°C)	Rain (mm)	Yield (T/ha cycle days)	2050 Water stress (%)	Tmax (°C)	Tmin (°C)	Rain (mm)	Yield (T/ha cycle days)	2080 Water stress (%)	Tmax (°C)	Tmin (°C)	Rain (mm)
7	34.65 (113)	22	23.4	3.9	269.8	23.88 (106)	2.4	21.7	7.8	136.6	23.26 (104)	15	21.8	5.4	169.2
8	30.72 (116)	29.9	23.6	3.7	269.7	25.47 (102)	7.6	21.6	7.7	130.5	26.58 (106)	15.2	21.9	5.5	169.2
9	35.34 (112)	29.5	20.8	8.8	232.3	29.76 (106)	21.3	19.9	7.6	169.9	24.21 (106)	44.2	21.4	8.0	174.4
10	27.51 (130)	25.9	22.0	8.8	367.1	34.14 (118)	3.5	20.6	7.6	220.3	22.31 (114)	22.6	21.6	7.9	175.9
1	28.77 (115)	4.	21.0	8.8	246.8	29.49 (104)	17.5	19.7	7.6	169.9	22.43 (117)	37.5	21.4	7.9	174.4
4	27.59 (102)	0.0	20.3	8.8	318.3	32.20 (106)	0.0	19.9	7.6	169.9	34.72 (101)	23.7	21.2	8.0	174.4



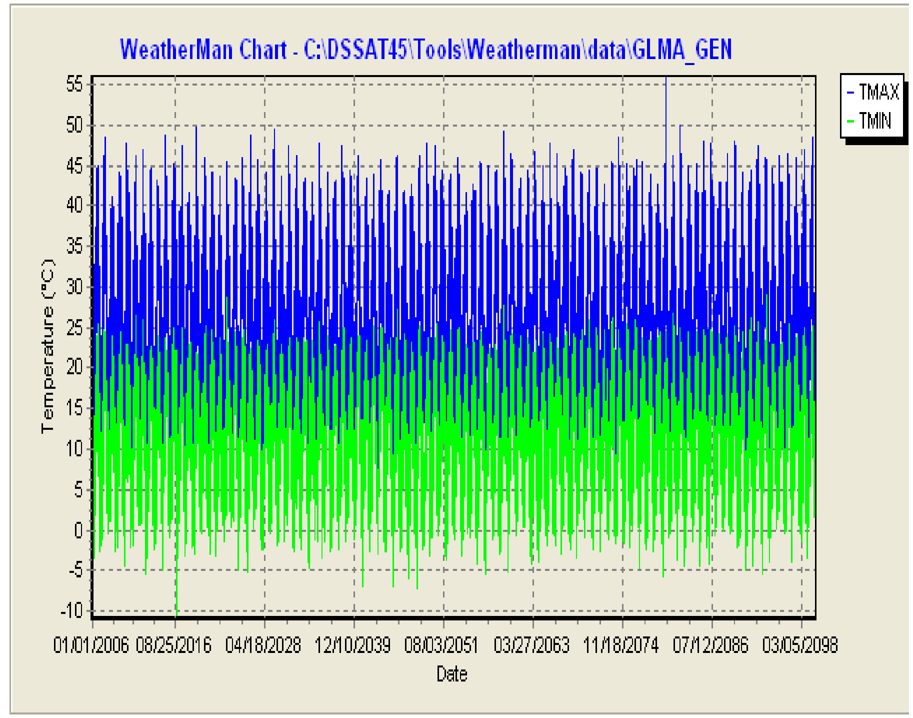
(a) Rainfall



(b) Rainfall



(c) Minimum and maximum temperature



(d) Minimum and maximum temperature

Figure 6. Daily rainfall and minimum and maximum temperature generated for BONE=Annaba and GLMA=Guelma stations.

except plot 5, which recorded an increase due essentially to the lack of water stress and probably to the spring warmed climate.

(3) 2050 appears to be the most stable year from the point of view of the climatic parameters and yields.

(4) A shortening of the growth period related to the increase of the minimum and maximum temperatures.

The results obtained for 2080 appear to be encouraging in terms of yield estimation, despite longer vegetation period and slightly higher temperatures. Many explanations are possible to discuss the results. In Algeria, potato is cultivated during three seasons (autumn, winter, spring/ summer). The main problem in the production of potatoes during the second season is the germination's delay. The minimum temperature after planting is the major factor for the success of germination (Table 3). The change of planting date is necessary to avoid low temperature period and reduce the growth cycle ensuring good performance.

Conclusion

In this study, an adjustment of the genetic co-efficient of SUBSTOR-potato model was proposed for Desiree potato variety under Mediterranean climate. The comparison between measured and estimated tuber fresh yield showed a significant correlation with average values of BIAS and RMSE of -7.34/-12.42 and 34.52/51.41, respectively for both calibration and validation processes and enables interesting applications for potato production. Since the amount of water during irrigation was the same for the different types of soil, the model indicated an irrigation water deficit in one plot. The analysis of the performed simulations indicated also some nitrogen management deficit. Possible potato management improvements are presented. The results suggest that it is necessary to change the date of planting to avoid low temperatures and increase yield.

This study highlights the impact of climate change on future yields of potato in the North of Algeria, after integration of daily data linked to climate over a period of more than five years in the model DSSAT 4.5. It is also important to note that taking into account other factors affecting performance, including weeds, diseases, pests, and soil salinity would be very useful in the improvement of the reliability of the results.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Screening of some rice varieties and landraces cultivated in Nigeria for drought tolerance based on phenotypic traits and their association with SSR polymorphisms

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Breeding for drought tolerance based on direct selection for high grain yield under drought has been hindered by the complex nature of drought tolerance mechanisms and the approaches used. Molecular marker-based approaches are a promising alternative. In this study, 30 rice (*Oryza sativa* L.) accessions cultivated in Nigeria were screened in a greenhouse for drought tolerance based on morpho-physiological traits and assessed for DNA polymorphisms using SSR markers for possible marker-trait associations. Our results showed that five Nigerian rice landraces (IJS-02, IJS-09, IK-PS, IK-FS and Lad-f) and three improved varieties (FARO-44, IR-119 and IWA-8) were highly drought tolerant. Sixteen of 20 markers tested yielded amplified products and generated 221 alleles (4 to 5 alleles per marker) with PIC values ranging from 0.24 to 0.95 per marker. Although, none of the markers were present in all the accessions that were found to be highly drought tolerant with respect to any particular morph-physiological trait, some of the markers (RM252, RM331, RM432, RM36, RM525, RM260 and RM318) amplified alleles unique to nearly all the tolerant Nigerian landraces (IJS-02, IJS-09, IK-PS, IK-FS) and FARO-11, a drought tolerant control. These markers may be usefully exploited for molecular breeding of rice for drought tolerance.

Key words: Nigeria, climate change, rice, drought stress, drought tolerance, SSR markers, molecular breeding.

INTRODUCTION

Rice is recognized as one of the most important staple food crop, accounting for more than half of human caloric intake globally. It is generally valued for its high nutritional benefits apart from being rich in calories, it is high in fibre, vitamins and minerals and low in cholesterol and sodium, suggesting it is a healthy source of energy. Asia is the largest producer and consumer of rice (Khush, 2005; Sellamuthu et al., 2011). In 2009, Nigeria was ranked 12th in the world's list of rice-consuming countries, while it is ranked 17th globally, third in Africa and first in West Africa, as producers of rice (FAO, 2011). However, Nigerian rice production does not meet current demand or have the capacity to cope with an expanding population. Production is also suggested to be declining due to effects of climate change particularly through drought, heat, flooding and pests and diseases (Rosenzweig et al., 2000).

Drought is recognized as a major abiotic stress that limits rice productivity and adversely affects grain quality in rain-fed and upland ecosystems (Bimpong et al., 2011; Yang, 2008). Rice is most sensitive to drought stress during reproductive development at which time moderate water shortages can result in a significant reduction in grain yield (O'Toole, 1982; Venuprasad et al., 2008). The extent to which drought affects yield varies depending on the intensity and the time of occurrence of the stress within the crop growth cycle (Srividhya et al., 2011). Yield losses ranging from 15 to 50% have been reported (Pandey and Bhandari, 2009; Srividhya et al., 2011). The situation becomes more serious with increasing global climate change. Hence, the development of high-yielding and drought- tolerant varieties for rain-fed regions is a major goal of rice breeding.

Plant responses to drought are well known and believed to be complex involving numerous changes at the physiological, biochemical and molecular levels (Atkinson and Urwin, 2012, Bargaz et al., 2015). Tolerance to drought stress is therefore the result of expression of a number of traits over the stress time period. Thus, no single trait is likely to improve crop productivity, in response to water- deficits (Farooq et al., 2009; Kamoshita et al., 2008). Various traits associated with rice performance under drought stress, including root morphology, root penetrability and distribution, leaf rolling, reduced leaf area, early flowering and early seed maturity, osmotic adjustment (accumulation of compatible solutes such as proline and soluble sugars), and increased production of ABA and stomatal closure, have been reported (Bimpong et al., 2011; Price et al., 2000). Selection and use of these traits in breeding programmes could lead to sustainable production in drought prone regions (Nguyen et al., 1997). The wild species of rice, though phenotypically inferior in agronomic traits, are important reservoirs of many useful genes, especially genes for tolerance to major biotic and abiotic stresses, and can be used to improve the cultivated species for these desired traits through breeding (Ali et al., 2010; Sanchez et al., 2014). Genes from *O. glaberrima* were used to develop NERICA lines with improved yield, earliness, weed competitive ability and tolerance to abiotic stresses, by interspecific hybridization with *O. sativa* (Sanchez et al., 2014).

Complex responses to drought coupled with often unreliable and labour-intensive conventional phenotyping have made it difficult to breed rice varieties with improved drought tolerance (Ingram et al., 1994). To overcome this problem, molecular markers have been utilized to identify genotypes having traits directly related to drought tolerance and the strategy is already well developed and known to be more efficient than conventional variety improvement. Development of molecular markers and their use for the genetic dissection of agronomically-important traits has become a powerful approach for studying the inheritance of complex plant traits such as drought tolerance (Suji et al., 2011). The use of molecular markers for the selection of complex breeding traits offers greater selection accuracy with less labour and time inputs, and enables assemblage of different target traits into a single cultivar. Hence, use of molecular markers to detect QTLs controlling drought tolerance related traits has the potential to accelerate breeding for drought tolerance and will ultimately contribute to reducing the problem of food security aggravated by changing climatic conditions.

Substantial efforts have been made towards the identification of QTLs underlying traits associated with drought tolerance in rice chromosomes using molecular markers. Zheng et al. (2000) identified two QTLs for root penetration ability and root thickness that co-localizes with rice SSR markers RM252 on rice chromosome 4 and RM60 on chromosome 3. Rice QTLs for root growth rate and root penetration ability have also been mapped using RFLP and AFLP markers (Price et al., 2000; Price and Tomas, 1997). The co-location of QTLs for root traits with those of yield under drought, has allowed combined selection of both traits (Salunkhe et al., 2011). Vasant (2012) found 12 SSR markers that were strongly associated with root traits under drought and 14 SSR markers that were significantly associated with yield and its components under drought. Several other studies of molecular markers associated with drought related traits have also been reported in the literature, indicating that these markers could be usefully utilized in the molecular breeding of rice for improved drought tolerance.

The objectives of this study were to evaluate some of the SSR markers reportedly linked to drought tolerance traits in rice varieties and landraces cultivated in Nigeria as part of the development of a protocol for marker-assisted breeding for drought tolerance for sustainable rice production in the face of increasing climate change.

MATERIALS AND METHODS

Plant

Thirty *O. sativa* accessions from different regions of Nigeria

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Table 1. *Oryza sativa* L. accessions used in the development of drought response traits and markers.

S/N	Name	Source	Status	Code	Remark
1	Nwadende*	Ebonyi	Landrace	Nwad	-
2	Ihenkiri	Ebonyi	Landrace	Ihek	-
3	Lady's finger	Ebonyi	Landrace	Lad-f	-
4	Agreement*	Ebonyi	Landrace	Agre	-
5	Arubus*	Ebonyi	Landrace	Arub	-
6	Room and palour*	Ebonyi	Landrace	R-P	-
7	$Mass^{L}$	Ebonyi	Landrace	Mass	-
8	Ogbese*	Ebonyi	Landrace	Ogbe	-
9	$IJ_{SLLW}FS$-02	Ekiti	Landrace	IJS-02	Faluyi and Nwokocha
10	IJ_{SLLW} FS-09	Ekiti	Landrace	IJS-09	Faluyi and Nwokocha
11	$IKph^{+}PS$	Ekiti	Landrace	IK-PS	Faluyi and Nwokocha
12	$IKph^{+}FS$-217	Ekiti	Landrace	IK-FS	Faluyi and Nwokocha
13	AWGU I $Pr^{++}PS$	Enugu	Landrace	AGW-PS	-
14	AWGU I FS-116	Enugu	Landrace	AGW-116	-
15	AWGU II FS-55	Enugu	Landrace	AGW-55	-
16	AWGU III FS-102	Enugu	Landrace	AGW-102	-
17	IFW $Pr^{++}FS$-55^{*}	Osun	Landrace	IFW-55	Faluyi and Nwokocha
18	IFW FS-07	Osun	Landrace	IFW-07	Faluyi and Nwokocha
19	IFW FS-13	Osun	Landrace	IFW-13	Faluyi and Nwokocha
20	FARO 11 (or OS6)	AfricaRice	Improved variety	FARO-11	Tolerant check
21	FARO 19	AfricaRice	Improved variety	FARO-19	Susceptible check
22	FARO 44	AfricaRice	Improved variety	FARO-44	-
23	FARO 57	AfricaRice	Improved variety	FARO-57	-
24	IWA 8	AGRA	Improved variety	IWA-8	-
25	IWA 10	AGRA	Improved variety	IWA-10	-
26	UPIA 1	WARDA	Improved variety	UPIA-1	-
27	UPIA 2	WARDA	Improved variety	UPIA-2	-
28	NERICA 34	WARDA	Improved variety	NERI-34	Interspecific hybrid
29	IR06N 184	WARDA	Improved variety	IR-184	-
30	IR06A 119	WARDA	Improved variety	IR-119	-

*Accessions lost during the experiment.

including a drought tolerant control and a susceptible control as well as some improved varieties were used (Table 1). Seeds of the local rice accessions selected based on popularity were obtained from farmers in the Nigeria States of Ebonyi, Enugu, Ekiti and Osun, while the improved varieties were obtained from the AfricaRice and the Alliance for Green Revolution in Africa (AGRA) through the Biotechnology Research and Development Centre, Ebonyi State University, Abakaliki, Nigeria.

Drought screening

Drought screening was conducted in a greenhouse of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria where temperature ranged between 23 and 39°C during April and October, 2015. Rice seeds were germinated in plastic pots containing sandy-loam soil. At the 3-leaf stage corresponding to around 2-weeks after sowing, seedlings were transplanted (one plant per pot) into polyethylene bag growing pots measuring 30 × 20 cm with a volume of about 9,420 cm^3. Drainage holes were provided at the bottom and the pots arranged in a completely randomized block design with ten replicates. The plants were irrigated for 45 days after sowing (DAS) by daily watering to slightly above soil saturation and thereafter five replicates each were assigned to one of two treatments – the control (well-watered) and those exposed to drought by withholding of water.

Adequate irrigation was maintained for the control treatment, while irrigation was withheld for 8 days in the drought stressed treatment during which the soil volumetric moisture content (SVMC measured using ASTM D-2216, 2014) declined from 19.5 ± 0.7 to 2.2 ± <0.1%.

The physical and chemical properties of the soil used are shown in Table 2. Compound fertilizer (NPK 15–15–15) was applied at the rate of 4.4 g pot^{-1} corresponding to 200 kg ha^{-1} in two applications (2 weeks after transplanting and at panicle initiation stage). The plants were kept weed-free throughout the period of the experiment by regular hand weeding. Irrigation was resumed on the drought-stressed group after the 8 days and continued till maturity at the

Table 2. Properties of the soil (sandy loam) used to grow *O. sativa* L. accessions to assess phenotypic responses during withholding water.

Property	Value
pH in water	6.70
pH in $CaCl_2$	6.40
Phosphorus concentration (µg/kg)	17.6
Potassium concentration (mg/kg)	0.30
Nitrogen concentration (%)	1.26
Organic carbon content (%)	0.94
Organic matter content (%)	1.61
Sand (%)	73.7
Silt (%)	9.3
Clay (%)	17.0

same rate as that in the control treatment (Ndjiondjop et al., 2010).

Agro-botanical traits measurements

Data on plant height, leaf length and width, panicle length, number of primary branches, number of spikelet per panicle, spikelet fertility, panicle density, grain weight per plant and 1000 seed weight (adjusted to 14% moisture content) were collected using the procedures specified in Standard Evaluation System for Rice (SES) (IRRI, 1996) and those of Vasant (2012). After grain harvest, plants were harvested and the soil was washed off and the shoots and roots separated and wrapped in aluminium foil for oven drying at 80°C to a constant weight. Shoot and root dry weights were recorded and used to calculate root/shoot ratios (Vasant, 2012). Grain length and breadth were measured using Vernier callipers. Days to 50% booting and heading or flowering and days to maturity were also recorded using the SES procedures (IRRI, 1996).

Measurement of leaf water potential (Ψ)

Leaf water potential (LWP) was measured on the youngest fully developed leaf on the main tiller using WP4C Dewpoint psychrometer (Decagon Devices, Inc., USA) following the procedures used by Xiong et al. (2015).

Genotyping using SSR markers

Genomic DNA was extracted from two week old leaves of the rice accessions grown in greenhouse using Zymo Research plant/seed DNA extraction kit (Dixit et al., 2005). Twenty SSR primers were tested to find polymorphisms among the rice accessions. The SSR markers were chosen based on previous reports of their association with drought tolerant traits in rice (Vasant, 2012; Temnykh et al., 2001; Zheng et al., 2000). The list of SSR primers used for the study is shown in Table 3.

The PCR mixture composed of 2.0 µl of DNA template (50 ng 25 μl^{-1}), 1.0 µl each of the forward and reverse primers (5 µM), 1.5 µl of $MgCl_2$ (50 mM), 2.0 µl of 10 X Taq buffer, 0.4 µl of 2.5 mM dNTP mix, 1.0 µl DMSO (dimethyl sulfoxide), 0.1 µl of 5 units Taq DNA polymerase and made up to 25.0 µl with nuclease-free water. The PCR profile was 94°C for 2 min followed by 30 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 45 s with a final extension at 72°C for 5 min. The amplification products were resolved on 8% denaturing polyacrylamide gel and the DNA fragments were revealed by silver staining and captured using a gel imager. The presence or absence of specific amplification bands were scored and used to determine number of alleles per primer and polymorphism information content (PIC) value of each microsatellite locus and to generate a dendrogram of the 30 rice accessions.

Statistical analysis

Analysis of variance (ANOVA) was performed using SAS software version 9.0, on the morpho-physiological trait values to compare the performances of the accessions under the imposed drought; percentage changes in the mean values of the traits due to stress or drought tolerance index (DI) and stress index (I_s) based on a combination of traits values were also computed using the formulae shown subsequently and were used to rank the performances of the accessions under the drought; principal components analysis was also performed on the drought tolerance index values to determine traits that contributed most to the observed differences in drought performance among the rice accessions. Population genetic structure was determined using a dendrogram of the SSR data by the UPGMA method using Numerical Taxonomy System (NTSYSpc) version 2.02, while association analysis was carried out by physically comparing the allelic pattern of individual SSR markers and the pattern of phenotypic traits depressions. Number of alleles per primer and polymorphism information content (PIC) of each SSR locus were also recorded.

Drought tolerance index (DI) = $(X_{control} - X_{drought})/X_{control} \times 100$ (Reyniers et al., 1982)

where $X_{control}$ is the measured trait mean value under well-watered conditions and $X_{drought}$ the mean values under drought stress induced by 8 days of withholding water, while

Stress index (I_s) = $1 - Y_{drought}/Y_{control,}$

where $Y_{drought}$ is the sum of means of trait values under drought stress condition and $Y_{control}$ the sum of means under irrigated condition (Fischer and Maurer, 1978). The closer to 1 the greater the negative effect of drought on yield, while the closer to zero the greater the tolerance to drought.

RESULTS

Effect of 8 days drought on the rice accessions

Water withholding for 8 days decreased SVMC from 19.5 to 2.2% (≈ 88.6% reduction) and Figure 1 is a photo showing the effect on the rice plants. Almost all the measured growth, yield and physiological parameters were significantly affected and the accessions responded differently to the drought treatment (Tables 4).

For growth and yield, only 2 of the rice accessions (AGW-PS and UPIA-1) did not show reductions in plant heights whereas around 65% showed significant (P<0.05) height reduction between 4.5 and 23.4%, with the largest reduction observed in AGW-55 and FARO-11. Around 44% of the accessions had significant reductions in leaf

Table 3. List of primer sequences used to study marker-trait associations in *Oryza sativa* L. accessions induced by withholding water.

S/N	Primer name	Primer Sequence	Associated traits	Source
1	RM38	F: ACGAGCTCTCGATCAGCCTA R: TCGGTCTCCATGTCCCAC	DRDW, GYP, NPT, SF	Srividhya et al., 2011
2	RM331	F: GAACCAGAGGACAAAAATGC R: CATCATACATTTGCAGCCAG	RL, NOT, NPT	Srividhya et al., 2011
3	RM60	F: AGTCCCATGTTCCACTTCCG R: ATGGCTACTGCCTGTACTAC	NPT, DS, RL	Vikram et al., 2011
4	RM252	F: TTCGCTGACGTGATAGGTTG R: ATGACTTGATCCCGAGAACG	RL, RT	McCouch et al., 2002
5	RM170	F: TCGCGCTTCTTCCTCGTCGACG R: CCCGCTTGCAGAGGAAGCAGCC	GYP, NPT, PL, DS, SF, SY	Yue et al., 2005
6	RM318	F: GTACGGAAAACATGGTAGGAAG R: TCGAGGGAAGGATCTGGTC	DRDW, GYP, SY	Srividhya et al., 2011
7	RM279	F: GCGGGAGAGGGATCTCCT R: GGCTAGGAGTTAACCTCGCG	NOG	Samuel et al., 2010
8	RM7390	F: CTGGTTAACGTGAGAGCTCG R: GCAGATCAATTGGGGAGTAC	GYP, PL, DS, SY	McCouch et al., 2002
9	RM432	F: TTCTGTCTCACGCTGGATTG R: AGCTGCGTACGTGATGAATG	NOG, SF	Vikram et al., 2011
10	RM5367	F: AGTACCTCTCACTCGCCTGC R: TGTCAGCTGTGAGTGAAGTCG	GYP, DS, SY	McCouch et al., 2002
11	RM5423	F: ATCCCACTTGCAGACGTAGG R: ACAGCAGCAAGGTGCCTC	DFF, PL, DS	McCouch et al., 2002
12	RM5850	F: ATACACAGATGACGCACACG R: TTAGGTGTGTGAGCGTGGC	DFF, GYP, SY	McCouch et al., 2002
13	RM36	F: CAACTATGCACCATTGTCGC R: GTACTCCACAAGACCGTAC	DRDW, GYP, RT, DS	Brondani et al., 2002
14	RM3558	F: ACGAGAGATCTTCTTTGCAG R: CCTCTATTTATGCCTCTACGC	DFF	McCouch et al., 2002
15	RM517	F: GGCTTACTGGCTTCGATTTG R: CGTCTCCTTTGGTTAGTGCC	DFF	Hong *et al*., 2005
16	RM6130	F: GGCAGAGAGAGCTGCATCTC R: GACGACGACGAACCCAAC	DFF, NOC, NOG, NOT, RT, PH, TBM	McCouch et al., 2002
17	RM583	F: AGATCCATCCCTGTGGAGAG R: GCGAACTCGCGTTGTAATC	DFF, NOC, NOG, PL, RT	Swamy et al., 2011
18	RM1141	F: TGCATTGCAGAGAGCTCTTG R: CAGGGCTTTGTAAGAGGTGC	DRDW, GYP, DS, SF, SY	McCouch et al., 2002
19	RM260	F: ACTCCACTATGACCCAGAG R: GAACAATCCCTTCTACGATCG	DRDW, GYP	McCouch et al., 2002
20	RM525	F: GGCCCGTCCAAGAAATATTG R: CGGTGAGACAGAATCCTTACG	DRDW, GYP, NPT, DS	McCouch et al., 2002

DRDW = deep root dry weight, GYP = grain yield per plot, DS = drought score, NPT = number of productive tillers, PL = panicle length, SF = spikelet fertility, SY = straw yield, NOG = number of grains per panicle, DFF = days to 50% flowering, RT = root thickness, NOC = number of chaffs per panicle, NOT = number of tillers, PH = plant height, TBM = total biomass, RL = root length.

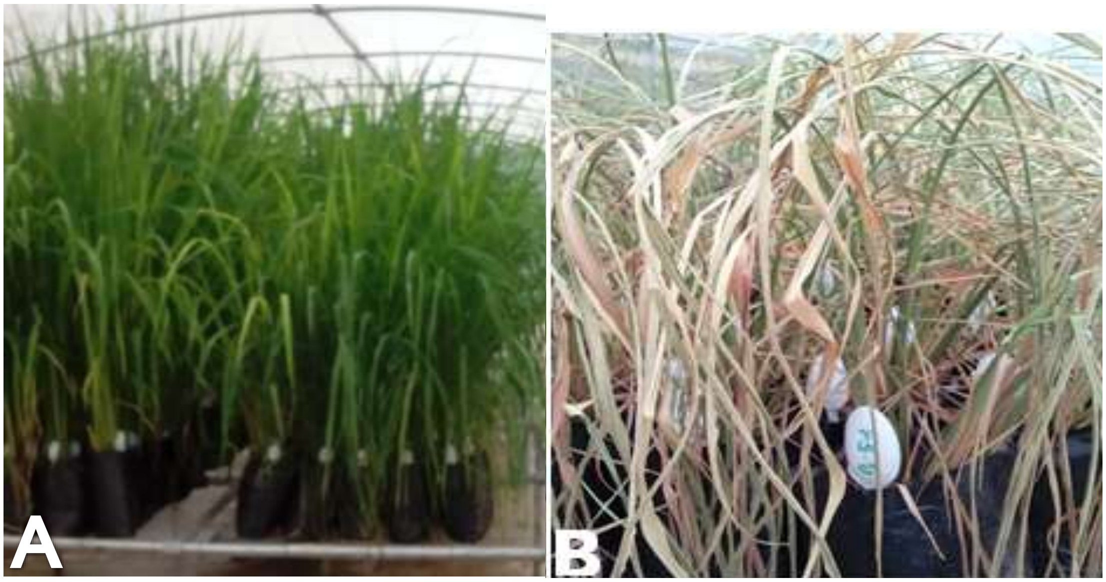

Figure 1. *O. sativa* L. plants subjected to withholding of water for 8 days in a greenhouse. A shows the control plants and B shows the plants at eight days drought stress.

Table 4. Percentage depressions in trait values in *O. sativa* accessions induced by 8 days of withholding water.

Accession	Height	LL	LW	PL	NPBPP	NSPP	SF	PD	SWPP	1000 SW	SDW	RDW	RSDWR	GL	GW
AGW-102	19.2	10.2	3.3	5.4	3.2	24.8	2.6	19.8	29.4	8.8	11.3	-8.4	-22.3	5.0	1.5
AGW-116	2.4	6.6	12.4	3.9	15.0	20.0	5.2	16.6	37.2	4.7	40.2	43.6	5.8	-1.1	8.9
AGW-55	23.4	2.2	20.7	8.5	3.7	27.1	6.1	20.3	11.8	2.6	39.9	6.5	-55.6	0.6	0.5
AGW-PS	-4.3	16.9	29.4	3.3	32.4	18.8	0.2	16.6	17.0	3.9	27.2	-28.5	-76.4	-1.1	-6.0
FARO-11	22.0	8.8	0.0	9.6	23.9	22.2	6.5	13.8	17.7	7.7	13.9	12.3	-1.8	-1.7	0.8
FARO-19	2.3	-7.4	-10.8	18.7	-14.6	14.6	0.3	-5.1	3.3	-0.7	25.6	40.2	19.5	-2.6	-1.5
FARO-44	1.6	-26.2	3.5	-9.9	0.0	-27.8	2.2	-16.3	25.2	2.1	21.4	-28.7	-63.6	6.8	1.4
FARO-57	11.4	12.4	14.0	5.1	0.0	34.0	10.1	30.5	13.9	3.0	3.7	26.6	23.8	-8.0	2.5
IFW-07	11.5	16.1	15.2	12.2	31.7	37.3	6.3	28.4	40.0	5.8	5.8	10.1	4.6	-5.7	3.7
IFW-13	5.6	18.2	20.4	9.2	14.1	36.8	2.8	29.9	9.4	0.8	11.0	5.0	-6.8	2.4	-1.0
IHEK	17.4	9.6	11.4	28.6	7.1	40.1	13.9	14.5	43.7	14.7	36.0	37.0	1.5	-1.9	11.3
IJS-02	3.8	-1.7	12.0	-9.0	10.9	1.9	3.7	10.2	-1.7	1.7	15.0	16.0	1.2	3.0	6.5
IJS-09	2.0	4.7	1.1	-6.3	-4.3	4.0	7.4	9.5	0.4	2.1	3.4	-34.4	-39.2	-2.7	-3.9
IK-FS	3.2	6.6	3.0	7.2	9.1	6.3	4.4	-1.1	9.5	2.7	29.0	27.6	-1.9	-7.7	-2.5
IK-PS	6.2	-2.6	1.0	5.3	5.7	10.1	5.6	5.8	18.3	1.8	22.1	7.2	-19.2	1.6	2.5
IR-119	12.6	-20.2	22.4	-5.2	8.1	1.9	20.4	6.5	14.7	5.8	10.5	14.5	4.5	2.1	-2.7
IR-184	20.8	4.6	21.4	-1.2	10.3	9.9	13.6	11.3	18.9	3.4	11.3	12.8	1.7	1.5	-1.6
IWA-10	11.7	-27.7	8.1	3.8	15.9	40.0	-0.3	37.9	2.8	7.1	27.9	2.3	-35.5	2.9	-1.0
IWA-8	8.7	2.3	3.5	-7.6	7.6	9.3	8.0	15.2	2.1	14.2	-7.2	-15.7	-7.9	2.0	5.9
Lad-f	4.5	12.2	9.8	9.7	-3.7	15.6	10.1	6.5	10.3	3.1	-47.4	-7.9	26.8	7.6	3.3
NERI-34	13.4	-27.6	-1.2	11.1	-1.7	7.8	15.1	-3.5	17.1	9.1	32.0	33.2	1.7	6.5	7.9
UPIA-1	-0.4	23.1	11.7	9.5	1.8	21.9	12.9	13.4	14.9	6.4	19.0	-14.2	-40.9	-1.1	-7.4
UPIA-2	10.9	-7.1	15.1	0.6	8.3	21.6	1.7	20.8	25.1	10.8	45.7	36.5	-16.8	9.4	-0.5

*LL = leaf length, LW = leaf width, PL = panicle length, NPBPP = number of primary branches per panicle, NSPP = number of spikelet per panicle, SF = spikelet fertility, PD = panicle density, SWPP = seed weight per plant, 1000 SW = 1000 seed weight, SDW = shoot dry weight, DRW = root dry weight, RSDWR = root-shoot dry weight ratio, GL = grain length, GW = grain width.

length (LL) on the main tillers between 6.6 and 23.1% with the largest reduction occurring in UPIA-1, IFW-13 and AGW-PS, while IWA-10, NERI-34, FARO-44, IR-119, FARO-19, UPIA-2, IK-PS, and IJS-02 were not affected. 61% of the accessions showed significant reductions in leaf width (LW) between 8 and 29% with, AGW-PS and IR-119 having the largest reductions but some accessions such as FARO-19, NERI-34 and FARO-11 were not affected. Panicle length (PL) was significantly reduced from 5 to 28% in 56% of the accessions, IHEK and FARO-19 showed the largest reductions. Sixty one percent of the accessions showed significant reductions of between 5.6 and 32% in the number of primary branches per panicle (NPBPP) on the main tillers, the largest effect was on AGW-PS, IFW-07 and FARO-11, while 26% of the accessions including FARO-19, IJS-09, Lad-f, NERI-34 and FARO-44 did not show any effect. The drought treatment significantly reduced the number of spikelet per panicle on the main tillers in 78% of the accessions, but had no effect on FARO-44. Reduction ranged from 1.9 to 40.1%. IJS-02 and IR-119 exhibited the lowest reductions while IHEK, IWA-10, IFW-07 and IFW-13 had the largest reductions. Reductions in spikelet fertility (SF) between 3.7 and 20.4% were observed in 70% of the accessions. The largest reduction occurred in IR-119 and NERI-34 whereas in IWA-10, AGW-PS and FARO-19 there were no effects. Panicle density (PD) was depressed in all the accessions, ranging from 5.8 to 37.9% except in FARO-44, FARO-19, NERI-34 and IK-FS. Reductions were significant ($P<0.05$) in 70% of the accessions from 9.5 to 37.9% in PD. PD was most depressed in IWA-10, FARO-57, IFW-13 and IFW-07. With the exception of IJS-02, seed weight per plant (SWPP) decreased in all the accessions with the reduction ranging from 0.4% in IJS-09 to 43.7% in IHEK, reductions (9.4 to 44%) were significant in 78% of the accessions. The reduction in 1000 seed weight (1000SW) varied from 0.8% in IFW-13 to 14.7% in IHEK, while there was no reduction for FARO-19. Shoot dry weight (SDW) decreased significantly in 78% of the accessions, with reductions ranging from 10.5% in IR-119 to 45.7% in UPIA-2, but no reduction was observed with Lad-f and IWA-8. Root dry weight (RDW) depression occurred in almost all accessions varying from 2.3% in IWA-10 to 43.6% in AGWU-116, reductions were significant in 56.5% of the accessions (7 to 43.6%), the exceptions were IJS-09, FARO-44, AGW-PS, Lad-f and IWA-8. Root shoot dry weight ratio was significantly depressed (from 1.5 to 26.8%) in about 39% of the accessions. Significant increases in RSDWR of around 6.8 to 76% were apparent in many accessions (47%), but there were no effects in 13% of the accessions. The greatest reduction was seen with Lad-f, FARO-57 and FARO-19 while the largest increase was with AGW-PS, AGW-55, FARO-44, IJS-09, IWA-10 and UPIA-1. The effect of drought on grain lengths and widths showed that only about 30% of the accessions had significant reductions ($p<0.05$) in grain lengths between 3 and 9.4%, while significant reductions in grain width between 5.9 and 11.3% occurred in 22% of the accessions. The most reduced growth and yield traits were shoot dry weight, seed weight per plant and root dry weight.

The effects of withholding water, on all of the traits, were combined in Stress Index (Is) for each accession and the values were used to rank the accessions in order of drought tolerance. Based on this, FARO-44, the Nigerian landraces (Lad-f, IJS-02, IJS-09, IK-FS and IK-PS) and IR-119 showed lower depressions in growth and yield traits due to withholding water, while IHEK and IFW-07 exhibited the largest growth depressions (Figure 2).

For delays, booting, flowering and maturity dates were significantly delayed in almost all accessions. Delays ranged from 0 to 21 days for days to 50% booting, 1 to 21 days for days to 50% flowering and 1 to 22 days for days to maturity were observed (Table 5). Accessions IK-FS, FARO-44, IK-PS and IHEK delayed the least, while FARO-11 followed by IFW-13 and FARO-19 had the longest delays to booting and flowering dates. Stress index based on earliness traits indicated that IK-PS, FARO-44 and IK-FS were more drought tolerant while FARO-11 followed by IFW-13 and FARO-19 exhibited higher sensitivity to drought (Figure 3).

For leaf water status, leaf water potential (LWP) was the most significant drought affected metric (Table 6). Reduction ranged from 145 to >4,000%. The Nigerian landraces (IJS-09, IJS-02, IK-FS and IK-PS) and the landraces IJS-09, IJS-02, IK-FS followed by IR-119, IK-PS and FARO-44 to have lower reductions in LWP, while accessions AGW-102, AGW-PS and AGW-116 were reduced the most (Figure 4).

Principal components analysis of trait depression values

The percentage depressions in trait values were subjected to principal components analysis to determine those most responsible for the observed differences in the accessions responses to withholding water. Eight (8) components were used but only 4 were significant (Table7) and explained about 62% of the total variations. The result of the PCA indicates that reductions in NSPP, PD and PL, as well as, the delay in maturation (MD) provided the greatest contributions to the observed differences in the performance of the accessions under drought. The second set of agro-morphological traits whose reductions contributed noticeably to the observed drought response differences among the accessions include grain weight, root dry weight, root-shoot dry weight ratio and spikelet fertility; the third set of traits were 50% BD, 50%HD, number of primary branches per panicle, leaf width and seed weight per plant; whereas

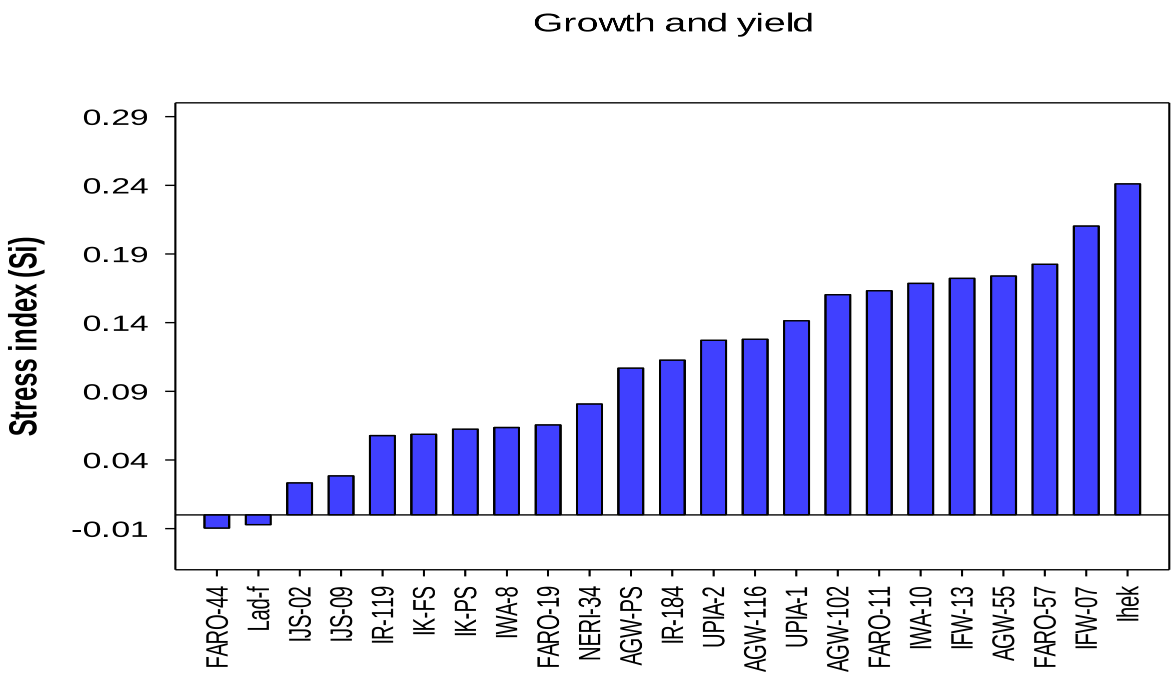

Figure 2. Ranking of rice accessions using drought stress index based on growth and yield traits. Bars represent Is values. The closer the value is to zero, the more drought resistant is the rice accession.

Table 5. Delays in booting, heading and maturity dates in *Oryza sativa* L. accessions induced by 8 days of withholding water (drought stress) in a greenhouse.

Accession	Delay in days to 50% booting	Delay in days to 50% heading date	Delay in days to maturity
AGW-102	10	13	10
AGW-116	11	14	19
AGW-55	10	16	17
AGW-PS	8	9	9
FARO-11 (OS6)	14	20	18
FARO-19	17	17	14
FARO-44	1	2	3
FARO-57	3	2	18
IFW-07	11	11	19
IFW-13	21	21	18
IHEK	4	4	5
IJS-02	7	8	4
IJS-09	9	4	3
IK-FS	1	2	2
IK-PS	0	1	1
IR-119	9	6	8
IR-184	8	7	6
IWA-10	11	12	16
IWA-8	7	7	3
Lad-f	11	16	11
NERI-34	9	14	12
UPIA-1	6	4	22
UPIA-2	4	10	6

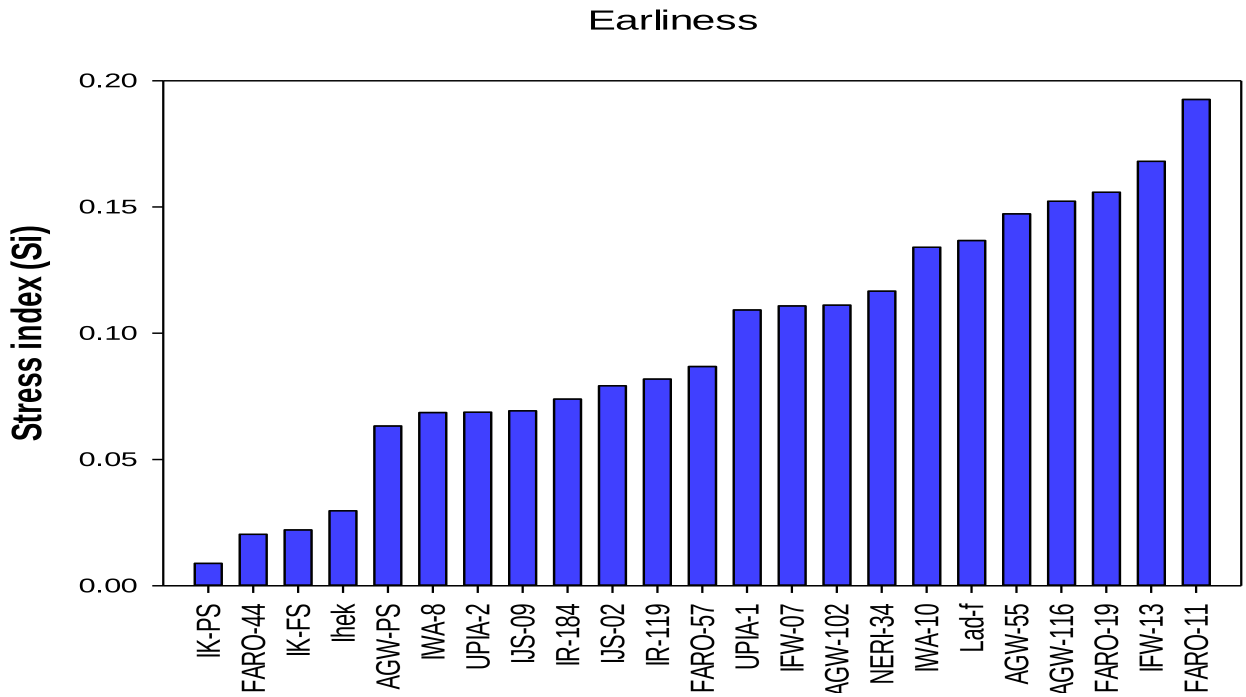

Figure 3. Ranking of rice accessions using drought stress index based on earliness traits (booting, heading and maturity dates). Bars represent Is values. The closer the value is to zero, the more drought resistant.

Table 6. Effect of 8 days of withholding water on leaf water potential (LWP) in *O. sativa* accessions.

Accession	Unstressed (Mpa)	Stressed (Mpa)	% reduction in LWP
AGW-102	-0.70	-33.12	-4631
AGW-116	-0.88	-26.19	-2876
AGW-55	-1.12	-26.66	-2280
AGW-PS	-0.76	-25.37	-3238
FARO-11	-0.78	-16.26	-1985
FARO-19	-0.88	-19.97	-2169
FARO-44	-0.87	-3.20	-268
FARO-57	-1.15	-21.60	-1778
IFW-07	-0.71	-3.53	-397
IFW-13	-0.97	-26.70	-2653
IHEK	-1.16	-4.38	-278
IJS-02	-0.70	-1.74	-149
IJS-09	-0.69	-1.69	-145
IK-FS	-0.19	-0.49	-158
IK-PS	-0.43	-1.53	-256
IR-119	-1.39	-3.95	-184
IR-184	-1.17	-21.66	-1751
IWA-10	-1.18	-10.92	-825
IWA-8	-0.87	-4.26	-390
Lad-f	-0.71	-4.93	-594
NERI-34	-1.03	-21.95	-2031
UPIA-1	-1.22	-16.53	-1255
UPIA-2	-0.93	-16.27	-165

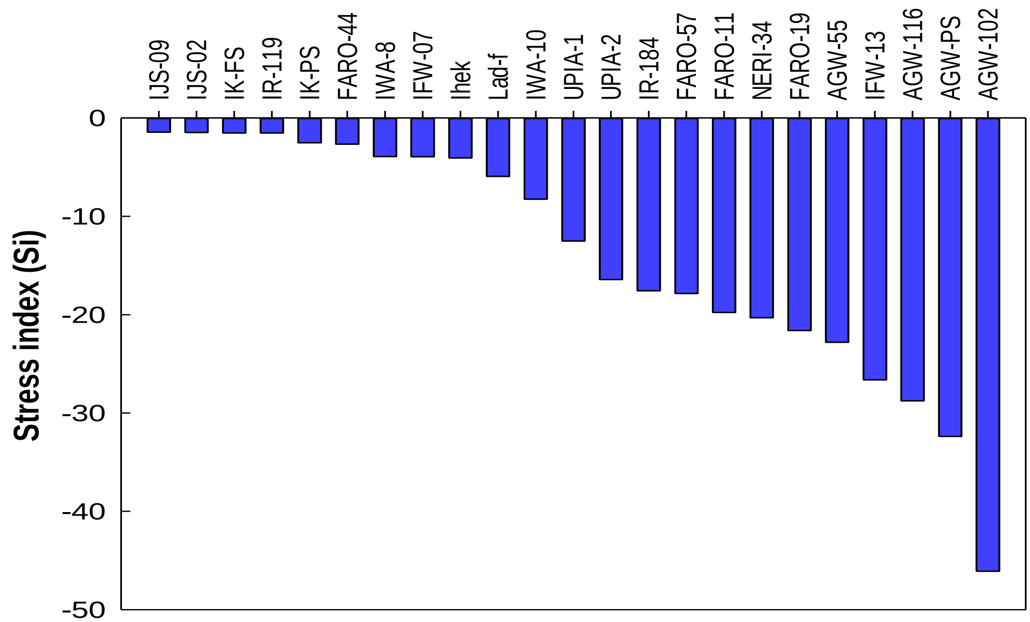

Figure 4. Ranking of rice accessions using drought stress index based on leaf water potential (LWP). The closer the value is to zero, the more drought resistant is the rice accession.

Table 7. Eigenvectors and eigenvalues of the phenotypic traits of *O. sativa* accessions induced by 8 days of withholding water.

Phenotypic trait	PC1	PC2	PC3	PC4
Height	0.199	0.245	0.045	0.174
LL	0.221	-0.178	0.147	*-0.394*
LW	0.141	-0.204	*0.365*	-0.011
PL	*0.299*	0.206	-0.077	-0.107
NPBPP	0.196	-0.172	*0.366*	0.074
NSPP	0.*421*	-0.003	0.097	-0.091
SF	-0.021	*0.293*	0.092	-0.269
LWP	-0.262	0.168	0.110	*-0.297*
PD	0.*335*	-0.156	0.202	-0.039
SWPP	0.177	0.241	*0.313*	0.137
1000 SW	0.099	0.288	0.260	0.192
SDW	0.061	0.085	0.187	*0.414*
RDW	0.189	*0.387*	-0.107	0.022
RSDWR	0.129	*0.342*	-0.266	-0.339
GL	-0.117	0.030	-0.140	*0.471*
GW	0.075	*0.424*	-0.012	0.083
50% BD	0.275	-0.185	*-0.398*	0.043
50% HD	0.298	-0.102	*-0.392*	0.226
MD	0.*354*	-0.129	-0.132	-0.057
Eigenvalue	24	16	12	10
Cumulative proportion	24	40	52	62

*LL, leaf length; LW, leaf width; PL, panicle length; NPBPP, number of primary branches per panicle; NSPP, number of spikelet per panicle; SF, spikelet fertility; LWP, leaf water potential; PD, panicle density; SWPP, seed weight per plant; 1000 SW, 1000 seed weight; SDW, shoot dry weight; RDW, root dry weight; RSDWR, root to shoot dry weight ratio; GL, grain length; GW, grain width; BD, date to 50% booting; 50% HD, date to 50% heading; MD, maturity date; PC1, 2, 3 and 4, principal components 1, 2, 3, and 4.

Table 8. Detected genetic diversity indices in rice accessions using SSR markers.

S/N	SSR marker	Major allele frequency	Allele number	PIC
1	RM170	0.10	22	0.94
2	RM60	0.20	11	0.86
3	RM38	0.17	20	0.92
4	RM36	0.13	17	0.92
5	RM279	0.10	25	0.95
6	RM260	0.60	4	0.52
7	RM318	0.10	21	0.94
8	RM331	0.60	7	0.57
9	RM432	0.63	5	0.52
10	RM517	0.07	25	0.95
11	RM525	0.57	7	0.61
12	RM583	0.87	5	0.24
13	RM1141	0.20	18	0.90
14	RM5423	0.30	9	0.80
15	RM5850	0.33	15	0.84
16	RM6130	0.40	10	0.76
	Mean	0.34	13.8	0.77

the last set of traits were grain length, shoot dry weight, leaf length and leaf water potential.

SSR polymorphism and population structure of the accessions

To access the level of genetic diversity in the population studied, a total of 20 SSR primers were used to study DNA polymorphism among the rice accessions. Table 8 shows the major allele frequency, number of alleles and PIC of each of the microsatellite loci in the studied accessions. Of the 20 primers, 16 produced scorable amplification bands used in the analysis, while 4 primers failed to amplify any of the rice DNA. The 16 SSR primers amplified a total of 221 alleles. Number of alleles per primer ranged from 4 to 25 with a mean of 13.8 while the PIC values spanned from 0.24 to 0.95 with an average value of 0.77.

A dendrogram of the 30 rice accessions using UPGMA procedure clustered the accessions into 6 major groups almost in accordance with their source locations (Figure 5). Nwad, a landrace from Ebonyi State, formed a distinct group (Group 1) suggesting that it is distantly related from the rest of the accessions. Group 2 included all accessions from Ebonyi State with only 2 accessions (Nwad and Lad-f) falling outside this group. Group 3 was a large cluster with distinct sub-groups. Accessions from Enugu State (AGW-PS, AGW-116 and AGW-102) are clustered together with only 1 (AGW-55) outside the sub-group but still showing a significant relationship. Accessions from Ife in Osun State (IFW-55, IFW-07 and IFW-13) are clustered together; the FARO lines (FARO-19, FARO-44 and FARO-57) except FARO-11 are grouped together while the improved varieties (UPIA-2, NERI-34, IR-119 and IR-184) are clustered together. Group 4 contained the single accession (Lad-f) also from Ebonyi State. Group 5 was comprised of accessions from Ekiti State (IJS-02, IJS-09, IK-PS and IK-FS) and FARO-11, whereas group 6 was another cluster of improved varieties including IWA-8, IWA-10 and UPIA-1).

Analysis of marker-trait association under drought-stress

The pattern in which each of the SSR markers clustered the accessions was compared with the pattern of individual trait depression due to the drought. Our result show that none of the markers clearly grouped the accessions according to the pattern of trait depression but few of the markers amplified alleles common only to accessions IJS-02, IJS-09, IK-PS, IK-FS and FARO-11. With the exception of FARO-11, these accessions are among the first five accessions that exhibited lower depressions in LWP (Figure 4) and also among the first seven accessions that showed lower drought depressions in overall growth and yield traits (Figure 2). Furthermore, they are among the first ten accessions that exhibited the least delay in flowering and maturation. FARO-11(OS6) is a known drought tolerant cultivar and is used here as a drought tolerant control. RM252 amplified about 100 bp fragment, RM331 amplified about 80 bp, RM432 amplified about 90 bp, RM36 produced about 80 bp,

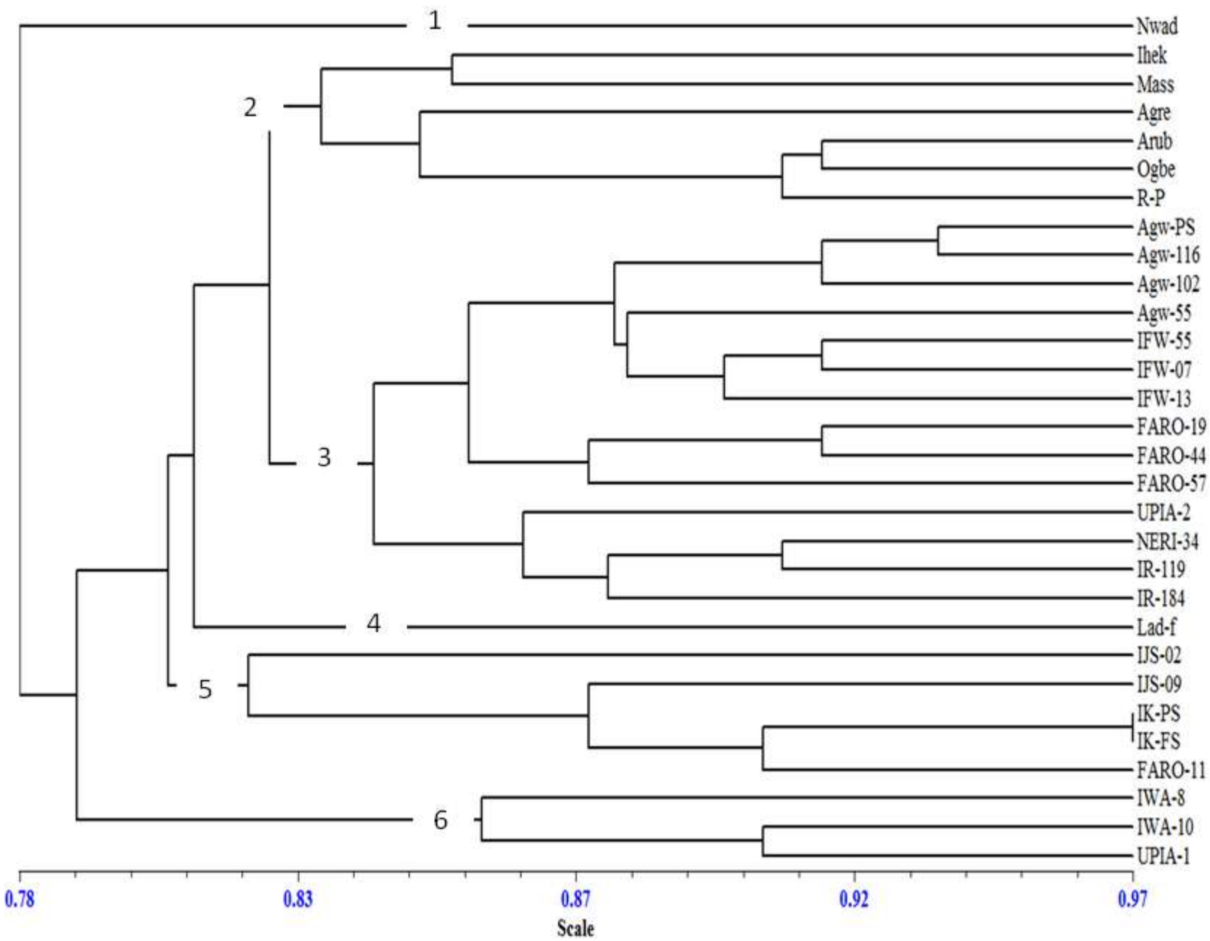

Figure 5. A dendrogram of 30 rice accessions from 20 SSR markers based on UPGMA. Numbers 1 to 6 represent separate clusters.

RM525 produced about 70, 72 and 75 bp fragments in these accessions (IJS-02, IJS-09, IK-PS, IK-FS and FARO-11), whereas RM260 amplified as short 30 bp fragment in the accessions including ARUB. RM318 amplified about 70 bp fragment in the landraces IJS-02, IJS-09, IK-PS, IK-FS and ARUB but not in FARO-11 (Figure 6).

DISCUSSION

Screening of rice for growth and yield performance under drought stress

Drought tolerance generally denotes the ability of a crop plant to survive, grow and yield satisfactorily under water-limited conditions (Fleury et al., 2010; Turner, 1979). In this study, some rice varieties cultivated in Nigeria, including landraces and improved varieties, were screened in the greenhouse for their growth and yield performance under complete withholding of water for 8 days when at a late vegetative stage in their development (45 DAS). A number of vegetative and reproductive traits were used to characterize the accessions under drought. The rice accessions exhibited large differences in their responses to withholding water. Although the duration of withholding of water was short, the adverse effects (Figure 1) were actually severe probably due to the low water retention capacity of the soil used (74% sand; Table 2), with SVMC declining by 88% of field capacity and drought stress developing more rapidly due to the soil's low water holding capacity. A SVMC of 2% measured in a sandy loam soil, means that all the available moisture would have been used with the soil at or close to the permanent wilting point (Kramer, 1969). However, it is suitable to assess tolerance to drought within a short period of the stress since genotypic differentiation of leaf elongation rates is greatest under moderate short stress (Cal et al., 2013). Leaf elongation rate is a useful indicator of drought tolerance and is

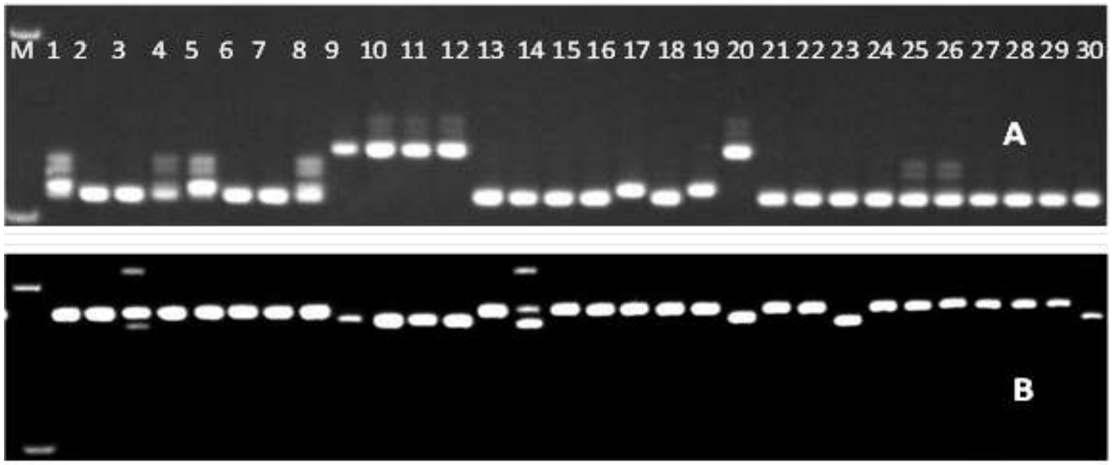

Figure 6. Gel photos of some SSR markers that amplified fragments common in size to rice accessions IJS-02, IJS-09, IK-PS, IK-FS and FARO-11 corresponding to lanes 9, 10, 11, 12 and 20. Numbers 1 – 30 represent each of the rice accessions, A = RM525, B = RM432, while M = 50bp DNA ladder.

closely correlated with a subsequent loss of yield due to drought. Under severe drought, leaf differences in elongation rate are very small between sensitive and tolerant cultivars. Serraj and Sinclair (2002) asserted that comparison of genotypic growth responses to drought is more appropriate under short and mild stress before the drought survival phase become apparent. The observation of physiological responses (reduction in LWP) implies that biochemical and molecular changes occurred in the rice accessions under the mild drought stress. It is also very relevant to the study environment where rice growing season in much of Nigeria is characterized by periods of short droughts rather than terminal drought.

It was observed that no single accession showed either the greatest or least depressions in all the traits measured. For instance, AGW-PS followed by UPIA-1, FARO-44 and IJS-09 were the most drought tolerant accessions based on reductions in plant height, whereas FARO-44, IJS-02, IWA-8 and IJS-09, respectively, were the most tolerant using panicle length depression. Similarly, FARO-44 followed by IJS-02, IR-119, IJS-09 and IK-FS were the most tolerant in terms of depression in spikelet number on the main panicle while IJS-02 followed by IJS-09, IWA-8 and IWA-10 was the most drought tolerant in terms of grain yield per plant (SWPP) (Table 4). This type of response can be linked to the complex nature of drought tolerance involving mechanistic interactions between an array of morphological, physiological, biochemical and genes and their expression (Li and Xu, 2007; Mitra, 2001; Price et al., 2002) and the differential responses of different rice accessions to drought (Vasant, 2012). It also suggests that tolerant plants have various pathways of exercising trade-offs to ensure survival.

To unambiguously rank the rice accessions based on their overall growth and yield performance under the imposed water withholding conditions and to select the most drought tolerant accessions, a stress index (Is) was used which relies on a combination of trait values under withholding water and well-watered (Fischer and Maurer 1978). Based on this procedure, FARO-44, Lad-f, IJS-02, IJS-09, IR-119, IK-FS and IK-PS, in decreasing order, were the most tolerant of the 23 accessions screened in relation to growth and yield, while IHEK followed by IFW-07, FARO-57, AGW-55, IFW-13 and IWA-10 were the most susceptible. It is worth noting that 5 of the 7 most tolerant accessions here are Nigerian landraces (Lad-f, IJS-02, IJS-09, IK-FS and IK-PS). These accessions, especially IJS-02, IJS-09, IK-FS and IK-PS, were found to be the earliest maturing of the 23 accessions (91 to 104 days; data not shown), which is an important late season drought avoidance strategy (Araus et al., 2002; Jongdee et al., 2002). These landraces also recorded the highest 1000 seed weight (39 to 42 g). However, they have a very low tiller number (3 to 5) which requires improvement to fully exploit their drought avoidance potential. Alternatively, increasing the sowing density of these accessions may adequately compensate for the lower tiller number, considering their high seed quality (1000 seed weight). Furthermore, planting them in this way may not lead to an unacceptable level of competition for photosynthetically-active radiation, but would amount to effective utilization of space and soil resources, since the accessions are not of an ‘open plant’ type. These accessions can be promising breeding material for improvement of higher yielding genotypes for enhanced drought tolerance in Nigeria and other similar situations and locations. However these genotypes have been somewhat neglected, by farmers, owing to their low tillering and yields, but as landraces, they appear better adapted to the Nigerian environment and are potential reservoirs of adaptability genes including those for drought and other abiotic stress tolerance (Villa et al., 2005; Friis-Hansen and Sthapit, 2000). Furthermore, these landraces performed better than FARO-11 which was used here as drought tolerant control (Ubi et al., 2011), while sharing several similar phenotypic features such as tiller number, height, culm morphology, grain shape and size with FARO-11. Principal components analysis revealed depressions in panicle lengths, number of grains per panicle, panicle density and delays in maturity date as the most important traits determining variations in rice performance under drought.

Screening for ability to maintain leaf water status

Analysis based on LWP also indicated that same landraces (IJS-09, IJS-02, IK-FS and IK-PS) among the 5 most drought tolerant accessions by their ability to maintain higher LWP under water withholding conditions. This suggests that these accessions may be using drought avoidance mechanism to cope with the stress of a water shortage. Drought tolerance is frequently apparent as increased capacity to maintain a higher LWP relative to a reduction in SVMC (Fukai et al., 1999; Kato et al., 2001, 2006; Mitra, 2001). By so doing they are able to extract water from the soil as its water potential falls thereby minimizing the yield losses (Singh et al., 2012). Of the 23 accessions screened, these accessions (IJS-09, IJS-02, IK-FS and IK-PS) also maintained the highest root to shoot ratio (0.23 to 0.28; data not shown) which can enhance root soil exploitation to extract more of the available soil moisture to maintain root and leaf tissue turgor and therefore growth under drought (Blum et al., 1989; Samson et al., 2002; Wang et al., 2006).

Screening for SSR polymorphism and their association with phenotypic drought traits

Genetic improvement of rice for drought tolerance through conventional breeding is slow due to the spatial and seasonal variations in drought timing and severity, the complex nature of drought tolerance itself and the difficulty in selecting for combinations of traits which best suit combating drought induced yield reductions (Courtois et al., 2003; Khush, 2001). Among the factors accounting for the slow progress in developing drought tolerant rice is the low heritability, multiple gene control, epistatic gene interaction, high incidence of genotype x environment interactions, etc. which could seriously influence ‘actual’ yields (Atlin and Lafitte, 2002; Cattivelli et al., 2008). The use of molecular markers to select accessions possessing genes and genomic regions that control target traits can fast-track the progress in breeding for drought tolerant rice, because molecular markers are transmitted faithfully from generation to generation and are not subject to environmental influences (Crouch and Ortiz, 2004; Gupta et al., 1999; Korzun et al., 2001; Senior et al., 1998). SSRs are a DNA marker system of choice for genetic analysis in rice because of their abundance in the rice genome, high level of polymorphism and high but simple reproducible assays involved (Powell et al., 1996; Singh et al., 2010). The 16 SSR primers used here generated 4 to 25 alleles per primer with PIC values ranging from 0.24 to 0.95. As high as 11 out of the 16 markers (≈69%) produced PIC values between 0.76 and 0.95 reflecting the high discriminating powers of the markers used.

The patterns of accession clustering of individual SSR markers when compared with that of the individual trait depressions caused by drought, was used to determine marker-trait associations for drought tolerance. Although none of the markers typically clustered the accessions absolutely in accordance with the pattern of trait depressions, some of the markers (RM252, RM331, RM432, RM36, RM525, RM260 and RM318) each amplified alleles unique to accessions IJS-02, IJS-09, IK-PS, IK-FS and FARO-11. Four of these accessions (IJS-02, IJS-09, IK-PS and IK-FS) are landraces from Ekiti State and are among the most drought tolerant accessions found here based on their capacity to maintain LWP, and grow and yield satisfactorily under the imposed drought.

Dendrogram analysis provided supporting evidence of which plant characteristics facilitate drought tolerance and how these characteristics are linked to apparent geographical origin, but equally important, it has capacity to illuminate linkages between trait groupings and functional aspects of tolerance. Here rice accessions in group 5 of the dendrogram contains all the drought tolerance accession with respect to little fall in leaf water potential, and most of these show little change in leaf length reductions. One of the first and well described responses to drought is a reduction in leaf growth (Cutler et al., 1980). Reductions in leaf area, particularly during canopy establishment can reduce yield (Cal et al., 2013). Here we see the greatest reduction in leaf length with UPIA-1, IFW-13 and AGW-PS. Conversely, there was little effect of drought on leaf length for IWA-10, NERI-34, FARO-44, FARO-19, UPIA-2, IR-119, IK-PS and IJS-02. The latter two accessions are in Group 5, where leaf water potentials showed the least reduction in response to drought of all the accessions. The accession AGW-PS also showed the largest reduction in leaf width and is in Group 3, where accessions lie which show the greatest decline in leaf water potential on exposure to drought. AGW-PS however does show the least change in height growth and root growth. The most likely explanation for this apparent paradox is that by maintaining root growth, maximal access and uptake of water can be achieved. This accession is able to utilise much of the available water to grow in the absence of conservative drought responses, such as stomatal closure which restricts water use and maintains leaf water potential (as with isohydric species). Reports suggest that rice under severe drought shows anisohydric behaviour (Boonjung and Fukai, 1996; Jongdee et al., 2002; Sibounheuang et al., 2006) keeping their stomata open and photosynthetic rates high for longer periods, even in the presence of decreasing leaf water potential. Root growth was also maintained in IJS-09 and FARO-44, Groups 5 and 3 accessions respectively, where limited drought induced little reductions in leaf water potential, while the opposite was true for AGWU-116. Comparative experiments, elsewhere, using deeper rooting rice Dro1-NIL, suggest that drought avoidance is achieved through root access to water deeper in the soil profile (Arai-Sanoh et al., 2014). Root growth potential has been the focus of trait exploration for rice drought avoidance (Price et al., 2002; Uphoff et al., 2015). Parent et al. (2010) observed lower sensitivity of drought in upland rice than lowland genotypes and suggested that drought sensitivity in rice maybe due to poor root system growth. FARO-44 and IJS-02, in groups 3 and 5 respectively, show little response to drought, results here strongly support the idea that root growth and water uptake provide a means of drought avoidance, at least for intermittent drought stress.

The genetic dendrogram was highly effective in reflecting the source locations of the accessions. The accessions from Ebonyi State (IHEK, Mass, AGRE, Arub, Ogbe and R-P) were grouped almost entirely together with only 2 accessions (Nwad and Lad-f) outside the cluster. This analysis also separately grouped accessions from Enugu State (AGW-PS, AGW-116,AGW-102 and AGW-55) and accessions from Osun State (IFW-55, IFW-07 and IFW-13) and showed that these 2 groups were closely related, which was supported by their poor performances under water withholding. The dendrogram also revealed a close linkage of the Enugu (AGW-) and Osun (IFW-) accessions with the FARO lines except FARO-11. It was noted that all accessions from Ekiti State (IJS-02, IJS-09, IK-PS and IK-FS) were grouped with FARO-11 and that all members of this group exhibited highly similar phenotypic features, but the landraces were much more drought tolerant than FARO-11 (a drought tolerant control). The improved varieties other than the FARO lines (UPIA-2, NERI-34, IR-119, IR-184, IWA-8, IWA-10 and UPIA-1) were clustered into two separate groups. It can be deduced from this study that the markers were able to partition the accessions in line with their source locations reflecting the robustness of SSR markers to dissect the population genetic structure and demographic history of domestication (Akkaya et al., 1992; Cho et al., 2000; Garris et al., 2005).

It is important to note that though FARO-11(OS6) is a known drought tolerant cultivar (Ubi et al., 2011) and used as a drought tolerance validation, it did not really perform in this study as expected.

Conflict of interests

The authors have not declared any conflict of interest.

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Chemical properties of soils in agroforestry homegardens and other land use systems in Eastern Amazon, Brazil

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Homegardens are considered as an alternative to preserve and/or restore the fertility and productivity of degraded soils. The assessment of changes in soil chemical properties resulting from land use and management is important to understand these changes and allow a rational intervention to ensure production on a sustainable basis. Thus, the aim of this study was to evaluate the soil chemical properties of homegardens and other systems of land use, as well as to identify the factors that influence fertility in areas of family farming in the municipality of Bonito, Eastern Amazon. Three soil samples were collected from secondary forest, cassava (*Manihot esculenta*) monoculture, silvopastoral systems and homegardens, from the depth range of 0-20 cm, and were evaluated in a completely randomized design with 12 treatments and three replications. The data were subjected to analysis of variance with the Kruskal-Wallis test, correlation analysis and principal component analysis. The variables studied, except Mg and Ca, were influenced by the soil cover. The homegardens, agricultural monoculture and silvopastoral systems were similar to the secondary forest in terms of nutrient cycling, with the exception of one 35-year-old homegarden, where the levels of P and K were higher. The soil fertility was explained by three factors: soil nutrients and salinity (P, K and Na); soil acidity and aluminum toxicity (Ca, Al, Mg, and pH); and by soil organic matter (SOM).

Key words: Soil cover, agroforestry systems, tropical soils, family agriculture, Amazon region.

INTRODUCTION

The sustainable use of natural resources has become an increasingly relevant issue due to the intensification of human activities and the negative impacts they have (Kittur et al., 2014). The removal of natural vegetation and the implementation of agricultural activities disturb the balance of the ecosystem, particularly of the soil,

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since the management influences the physical, chemical and biological processes and consequently, modifies the soil chemical and physical properties (Costa et al., 2008).

Since the soil is the basis for agriculture and forestry production, concerns about the sustainable use and soil quality have increased (Araújo et al., 2007). The degree of alterations caused by human intervention in the natural system can be measured, among other means, by changes in some soil properties. These modifications of soil properties can be positive or negative, depending on the nature of the soil, the plant species, the management system, and period of agricultural exploitation (Salton et al., 2008; Carneiro et al., 2009).

In the case of agricultural crops, resulting from the migrating or shifting cultivation/agriculture, soil fertility can be recovered if the fallow period, in which the secondary forest grows, is long (Bargali et al., 2009; Joshi et al., 1997). Thus, agroforestry systems are recommended as alternatives for sustainable family farming in the Amazon region, for the rehabilitation of degraded areas (Rosa et al., 2009), and for having a number of advantages over the traditional systems of land use.

Homegardens are considered as an alternative to preserve and restore the fertility and productivity of degraded areas (Altieri, 2002; Rosa et al., 2007), since these agroecosystems normally host several perennial food, medicinal and ornamental species, they are considered as repositories of genetic diversity, aside from providing environmental comfort, locations of family aggregation and, above all, food security (Bargali, 2015; Bargali et al., 2015a).

The silvopastoral systems also have a series of advantages, as they raise the productive capacity of pastures and animals, improve fertility, decrease soil compaction, conserve the soil, minimize the environmental stress on livestock, improve the environmental conditions and increase the property value (Oliveira et al., 2003).

When comparing the conditions of the soil under original cover with soil from which it was removed or where a crop was planted, the resulting modifications and damage, especially with regard to fertility are generally visible, with greater or lesser evidence. Thus, the evaluation of fertility is critical for the diagnosis of soil nutrients from which specific recommendations and corrections can then be inferred, according to the crop requirements, or management practices that conserve or restore the soil fertility and productivity can be adopted.

The assessment of changes in soil chemical properties resulting from land use and management is important to understand these changes and allow a rational intervention to ensure production on a sustainable basis. Given the above, the objective of this study was to evaluate the chemical properties of soils of homegardens and different land use systems, and to identify the factors that influence their fertility in areas of subsistence farming in Eastern Amazon, State of Pará, Brazil.

MATERIALS AND METHODS

This study was conducted on seven family farms in four rural communities in the municipality of Bonito (Sumaúma, Cumaruzinho, Pau Amarelo and São Benedito), in the microregion Bragantina, northeast of the State of Pará, Brazil (01° 21’ 48” latitude S, 47° 18’ 21” longitude W).

Soil samples were collected in four land use systems, namely: secondary forest (capoeira); cultivation of *Manihot esculenta* Crantz - cassava (plantation); silvopastoral system (*Brachiaria* spp. and *Attalaea maripa* (Aubl) Mart.); and homegardens of different ages, not necessarily contiguous areas.

The three cassava fields were manually prepared with slash-burning of the secondary forest and were 5, 6 and 7 years old at the time of the research. The three silvopastoral systems were formed by a consortium of *Brachiaria* spp. with scattered adult *Attalea maripa* (inajá) trees in pastures. The Inajá palms grew spontaneously after burning and cleaning of pasture (natural regeneration), in areas with 10, 15 and 35 years of management. The three homegardens had been managed for 7, 12 and 35 years, for as long as these areas were occupied by the farmer families.

The capoeira areas, targets of this research, were the result of the ecological succession that took place when agricultural fields (farm) were left fallow. These cassava fields had previously been used for an average period of 5 years, and were abandoned after monoculture to regain soil fertility for future reuse and had been left fallow for 10, 12 and 15 years.

The secondary forest was used as a control to demonstrate that homegardens can have satisfactory conditions of environment conservation (Sena et al., 2007), with regard to the chemical properties, aside from serving as comparison with the farm and pasture systems.

The soil was collected as recommended by Silva Jr et al. (2006), using a auger, from the 0-20 cm depth range of each area. Three composite soil samples were collected in each treatments, consisting of 20 simple samples collected in a zig-zag line. The chemical variables soil organic matter (SOM); pH in H_2O; levels of exchangeable Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Al^{3+} and available P (Mehlich-1) were evaluated as described by Embrapa (1997).

The experimental design was completely randomized, with 12 treatments and three replications. The treatments consisted of four land use systems, which combined with their age resulted in 12 treatments: T1- secondary forest 10 years, T2: secondary forest 12 years, T3- secondary forest 15 years, T4- cassava monoculture 5 years, T5- cassava monoculture 6 years; T6- cassava monoculture 7 years, T7- pasture 10 years; T8- pasture 15 years; T9- pasture 35; T10- homegarden 7 years; T11- homegarden 12 years; T12- homegarden 35 years.

The variables were subjected to the Kolmogorov-Smirnov test to check data normality. Treatment effects on soil chemical properties were determined by analysis of variance (ANOVA), using the program Assistat 7.6 and the averages compared by Tukey’s test at 5% for pH, OM, Al, Na, Ca, Mg, with a normal distribution. The variables P and K, with non-normal distribution, were analyzed by the Kruskal- Wallis test at 5%.

Pearson’s correlations were established between the chemical properties studied and the data analyzed by multivariate factor analysis to find how the variables are grouped to explain soil fertility. The analysis was carried out with the Statistical Package for Social Sciences (SPSS ® 15.0).

RESULTS AND DISCUSSION

There was a significant difference ($p < 0.05$) between treatments for the variables pH, P, K, Na, Al and SOM.

Table 1. Means of chemical variables of soils (0 - 20cm) under different land use on family farms in Bonito, Eastern Amazon, Brazil.

T	pH_{H2O}	SD	P	SD	K	SD	Na	SD	Ca^{2+}	SD	Mg^{2+}	SD	Al^{3+}	SD	SOM	SD
	mg dm^{-3}				cmol$_c$ dm^{-3}										g kg^{-1}	
T1	5.53ab	0.12	1.00f	0.00	0.05e	0.00	0.06b	0.01	2.20a	0.30	0.60a	0.10	0.20bc	0.10	9.82ab	1.06
T2	5.43ab	0.15	8.33de	12.70	0.06d	0.00	0.08b	0.01	1.73a	0.21	0.57a	0.12	0.23abc	0.06	10.53ab	0.59
T3	5.40ab	0.40	1.00f	0.00	0.09b	0.01	0.10b	0.01	2.23a	1.23	0.77a	0.15	0.27abc	0.15	12.04ab	2.70
T4	5.17b	0.21	2.00c	0.00	0.07c	0.02	0.08b	0.00	1.47a	0.15	0.53a	0.15	0.53a	0.12	12.55a	0.76
T5	5.30ab	0.20	1.67e	1.15	0.06d	0.00	0.06b	0.01	1.93a	0.32	0.67a	0.12	0.20bc	0.10	8.05ab	1.15
T6	5.33ab	0.06	1.33e	0.58	0.07c	0.01	0.08b	0.01	1.73a	0.25	0.63a	0.06	0.20bc	0.00	8.64ab	0.96
T7	5.40ab	0.17	3.00cd	2.65	0.06d	0.01	0.09b	0.01	1.40a	0.30	0.53a	0.21	0.47ab	0.21	10.46ab	2.13
T8	5.37ab	0.06	1.00f	0.00	0.11b	0.02	0.13ab	0.01	1.63a	0.21	0.63a	0.21	0.37abc	0.06	12.07ab	2.41
T9	5.37ab	0.06	1.33e	0.58	0.06d	0.00	0.08b	0.00	1.53a	0.15	0.53a	0.26	0.37abc	0.06	11.61ab	1.01
T10	5.40ab	0.10	12.00b	1.00	0.07c	0.01	0.08b	0.01	1.57a	0.35	0.40a	0.06	0.30abc	0.00	8.77ab	2.73
T11	5.80ab	0.30	4.33cd	4.93	0.11d	0.11	0.11b	0.04	1.70a	0.36	0.60a	0.12	0.13c	0.06	6.77b	2.06
T12	5.83a	0.38	75.00a	21.52	0.26a	0.04	0.19a	0.01	2.57a	1.04	0.77a	0.12	0.17bc	0.12	10.31ab	3.91
M	5.44		9.33		0.09		0.10		1.81		0.60		0.29		10.13	
CV	3.38		40.29		22.96		10.08		22.48		23.11		29.40		16.85	

T= Treatments; SD=standard deviation; M=mean; CV= coefficient of variation (%). Means followed by the same letter in columns are statistically not different by Tukey's test at $p < 0.05$ (pH_{H2O}; Na^+; Ca^{2+}; Mg^{2+}; Al^{3+}; SOM- soil organic matter) and by the Kruskal-Wallis test at $p < 0.05$ (P and K). (T1- secondary forest 10 years ; T2: secondary forest 12 years ; T3- secondary forest 15 years ; T4- cassava monoculture 5 years ; T5- cassava monoculture 6 years; T6- cassava monoculture 7 years; T7- pasture 10 years; T8- pasture 15 years; T9- pasture 35 years; T10- homegarden 7 years; T11- homegarden 12 years; T12- homegarden 35 years).

Regarding pH, the soils studied were moderately acid (Table 1). Only T4 and T12 differed, the soil in T4 had a lower pH than in T12. This can be explained by the higher SOM content in T4, which increases the active acidity of the soil, favoring the development of active H^+ ions in the soil solution.

The average pH values observed in this study were higher than those found by Sena et al. (2007) in different systems of land use in Marituba (Brazil), including capoeira, agroforestry and açaí (*Euterpe oleracea* Mart.) monoculture. Oliveira et al. (2008) found high acidity levels in soils under forest plantations and degraded grassland in the State of Espírito Santo (Brazil). In T12, nutrient cycling was more efficient (Table 1), with higher mean values of soil P (75.00 mg dm^{-1}) and K (0.26 cmol$_c$ dm^{-3}) than in the other agroecosystems. Phosphorus is fundamental for the plant physiology. According to Fraga and Salcedo (2004), soils with low levels of available P, together with the water limitations, can be a serious combination that would severely restrict the recovery rate of degraded soils or may even impair their recovery, due to the drawback in plant growth.

The values of available P can be explained not only by the high weathering degree of these soils, culminating in the predominance of Fe and Al oxides in the clay fraction of Oxisols, but also by the high soil moisture, which increases the redox potential, leading to a probable reduction of ferric compounds and higher levels of available P to plants (Fernandez et al., 2008; Pandey and Srivastava, 2009). For being elements of low mobility in soil, the P highest level measured in the layer can be explained by the accumulation resulting from cycling promoted by the roots, which absorb it from deeper layers and after metabolism are deposited on the soil surface through the deposition of plant residues. Another factor that could justify the highest level of available P in T12 is the lower value of exchangeable acidity. Therefore, less Al^{3+} is available to adsorb the P in the soil solution.

In T12, the highest content and concentration of

Table 2. Correlation matrix of chemical variables of soils under different land use systems on family farms, Eastern Amazon, Pará, Brazil.

	pH	Ca	Mg	Al	P	K	Na	SOM
pH	1							
Ca	0.620	1						
Mg	0.456	0.726	1					
Al	-0.712	-0.624	-0.455	1				
P	0.518	0.468	0.348	-0.285	1			
K	0.608	0.344	0.316	-0.310	0.839	1		
Na	0.577	0.286	0.341	-0.233	0.732	0.931	1	
SOM	-0.003	0.349	0.257	0.272	0.079	0.113	0.216	1

P and K (75.00 mg dm^{-1} and 0.26 $cmol_c$ dm^{-3}, respectively) in the soil can be explained by the release of these nutrients from the manure of pigs reared in a semi-confined system in this area. According to Merten and Minella (2002), pig feces are rich in N, P and K, and a high P supply can cause serious impacts on ecosystems. The residue management should take the soil nutrient uptake capacity in waste into consideration to prevent the contamination of water resources.

In Bonito, Eastern Amazon, P levels were higher than the value reported by Gama-Rodrigues et al. (2008), in soils of forest monoculture, pasture and capoeira, and by Sena et al. (2007) in an agroforestry system and capoeira.

Menezes et al. (2008) studied the soil chemical properties of different agroforestry systems and forest fragments in Rondônia and found similar mean levels of available P as in Bonito, Eastern Amazon. However, the soils of the homegardens in Pará had higher levels of available P than in Rondonia (Brazil), due to the soil management (organic fertilization with pig manure).

The K values in the different land use systems in Bonito were generally low. Statistical differences were found only for T12, with 0.26 $cmol_c$ dm^{-3} K. This value was similar to that found by Gama-Rodrigues et al. (2008) for planted forests and higher than in the capoeira area in this study. Portugal et al. (2010) found K concentrations (0.061 $cmol_c$ dm^{-3}) in the Zona da Mata of Minas Gerais (Brazil), that were very close to those observed in this study in the secondary forest areas in Bonito, Eastern Amazon (0.065 $cmol_c$ dm^{-3}). The values of K were lower than those of other nutrients because K is actively leachable (Bargali et al., 1993, 2015b).

The Ca and Mg levels did not differ between the agroecosystems (Table 1). With regard to the Al^{3+} contents, most treatments were not statistically different; only T4 and T11 showed highly significant differences at 5% probability.

The Al^{3+} contents found in the agroecosystems under different land uses were low as compared to those reported by Portugal et al. (2010) and Sena et al. (2007). The latter authors found an average level of 1.44 $cmol_c$ dm^{-3} in land use systems evaluated in Marituba (Brazil), and inferred that, regardless of the systems, the Al^{3+} soil concentrations were high and toxic to plants.

The SOM level was lower in the 12-year-old homegarden than in the field cultivated for 5 years, showing that the practice of removing the litter from these systems tends to reduce sustainability. The values for organic matter in the evaluated systems and secondary forest areas were about 10.1 g, and 10.8 g kg^{-1}, respectively.. According to Gliessman (2005), SOM levels in the A horizon of natural ecosystems can reach 150-200 g kg^{-1}, but the content of this variable is on average around 10-50 g kg^{-1}.

In a comparative study of the chemical properties of native forest soils with an orange (*Citrus sinensis* (L.) Osbech) orchard and pasture in the Zona da Mata, Minas Gerais (Brazil), Portugal et al. (2010) identified significant SOM values. However, the values obtained by these authors were lower than those found in Bonito, Eastern Amazon. In contrast, Silva et al. (2006) reported higher SOM values in secondary forests (24.1 g kg^{-1}) and degraded pastures (33.7 g kg^{-1}) in Marituba, Pará. They stated that the high SOM levels in degraded pastures are due to the soil regeneration and death of grass root system.

The correlation matrix of the chemical variables analyzed in the soil under different agrosystems (Table 2) showed that the pH was positively but moderately correlated with the variables Ca, Na and K. This indicates that the removal of the basic cations, particularly of Ca^{2+} and K^{+} led to a decrease in the pH of the system and that their addition, mainly by liming or fertilization, induced Ph increase. In regions with high rainfall, the tendency for soil acidification by displacement of basic cations, such as Ca, Mg, K, and Na, from the exchange complex is greater (Sousa et al., 2007).

The degree of association between pH and Al^{3+} was negative and relatively high (-0.712), indicating that the lower the pH, the greater the amount of Al^{3+} in the soil, which according to Fernandéz et al. (2008) may occur due to the higher solubility of Al complexes in these pH ranges.

The pH reduction favored an increase of the Al^{3+}

Table 3. Results of the eigenvalues for the extraction of factors and the total variance explained by the factors.

Component	Eigenvalues (λ) and initial variances			Variance after rotation		
	Total variance	Variance (%)	Total variance	Variance (%)	Total variance	Variance (%)
1	4.12	51.50	51.50	2.91	36.42	36.42
2	1.48	18.47	69.97	2.66	33.31	69.74
3	1.30	16.29	86.26	1.32	16.53	86.26
4	0.45	5.69	91.95			
5	0.35	4.43	96.39			
6	0.18	2.21	98.60			
7	0.07	0.85	99.45			
8	0.04	0.54	100			

Table 4. Matrix of factor loadings (α) after orthogonal rotation by the Varimax method for the chemical properties of soils of different land use systems in Eastern Amazon, Pará, Brazil.

Variables	Factors			Commonalities
	F1	F2	F3	
pH	0.528	0.679	-0.217	0.787
SOM	0.088	0.112	0.941	0.906
P	0.855	0.247	0.033	0.794
K	0.965	0.182	0.009	0.964
Na	0.936	0.143	0.116	0.910
Ca	0.190	0.894	0.257	0.902
Mg	0.166	0.787	0.295	0.734
Al	-0.158	-0.811	0.470	0.904

concentration in the medium, raising P fixation, mainly by the formation of insoluble Al-P compounds, which explains the higher level of available P by the pH increase.

After the analysis of linear correlation, the variables were grouped by the method of principal component analysis and reduced to factors that can help explain soil fertility in the studied agroecosystems (Table 3). The factor extraction was performed from the eight variables in the soil analysis.

Three components (factors) with Eigenvalues greater than 1 were extracted. The value of the Kaiser-Meyer-Olkin (KMO) test was 0.641. According to Hair Jr. et al. (2009), KMO values above 0.50 allow the use of this tool to explain a phenomenon.

The data of the initial solution are rotated for the three possible factors and the explanatory power expressed by the Eigenvalues obtained from the spectral decomposition of the correlation matrix is shown in Table 3. The three factors together explain 86.26% of the data cloud related to the chemical properties of different land use systems in Bonito. In order of importance, Factor 1 explained most of the variance (36.42%), Factor 2 explained 33.31% of the variance and Factor 3 accounted for 16.53%.

According to Santana (2007), a meaning must be assigned to the factor solution. This author suggests that the significant factor loadings should be used in the process of interpretation and also recommends that the selection of names or labels to represent the factors be based on the variables with highest load. Thus, the appointment of factors, from the factor loadings, is presented in Table 4, which also shows the values of commonalities. According to Santana (2007), these values indicate the degree to which each variable can be explained by factors.

The variables P, K and Na were grouped in Factor 1. The combination of these variables generated the factor nutrients and soil salinity. The source variables had positive and high factor load, which shows a strong inter-relationship between them. In soils with predominance of variable loads, the K content in the solution is influenced mainly by the electrostatic adsorption of K to negative charges (Neves et al., 2009). Potassium reserves are very important for agricultural systems with low input (Rangel, 2008) and a dynamic element, with high mobility, which can move Na by the cation exchange capacity of the soil

(Melo et al*.,* 2000).

Factor 2 was formed by the inter-relationship between pH, Ca, Mg and Al. This factor was named soil acidity and aluminum toxicity, since the variables that compose it are related to soil acidity. The Al^{3+} content had a negative factor loading, showing that higher values of pH, Ca and Mg tend to produce smaller amounts of Al^{3+}. This result is in agreement with Leite et al. (2010), who studied the characteristics of soils under *Eucalyptus* sp. and found a reduction in the exchangeable Ca, Mg and K levels. Moreover, Lopes et al. (1991) stated that the removal of basic elements such as K, Ca, Mg and Na can lead to soil acidity.

Factor 3 was named soil organic matter, and consists only of the variable organic matter, showing the great importance of this compartment for soil fertility in the systems evaluated in Bonito, influencing the soil physical, chemical as well as biological properties. The mineralization of organic matter leads to, primarily, release of nitrogen to plants. According to Gliessman (2005), SOM has a close relationship with the soil N content and can influence the levels of this nutrient in the soil by up to 80%.

The soil organic matter supplies N to plants, and therefore its presence constitutes a great benefit to the soil. However, this required processes of microbial decomposition, accompanied by mineralization and humification of their constituents (Silva, 2008). It is considered to be a major soil component for maintaining the chemical, physical and biological quality. According to Silva and Mendonça (2007), its growth and maintenance in tropical soils has proved more difficult than in subtropical soils. For the authors, the adoption of conservation practices (no tillage, green manure, rotation, etc.) has achieved better results, indicating the importance of higher residue inputs and organic N in the maintenance of this property.

Conclusions

The variables studied, with the exception of Mg and Ca, are influenced by the soil cover. The homegardens, except for the 35-year-old in which P and K levels were higher, and the monoculture, agricultural and silvopastoral systems were similar to the secondary forest in terms of nutrient cycling.

The homegardens preserved some soil chemical properties, with similar values as the secondary forest, and shows that these systems conserve the fertility of tropical soils. The soil fertility in the four land use systems was explained by three factors: soil salinity and nutrients (P, K and Na), soil acidity and aluminum toxicity (Ca, Al, Mg and pH), and soil organic matter (SOM).

Conflict of interests

The authors have not declared any conflict of interest.

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Agroindustrial yield of sugarcane grown under different levels of water replacement and nitrogen fertilization

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The sugar and alcohol sector has invested heavily in technologies to increase the productivity of sugarcane and consequently the gross income of sugar and alcohol; among these practices irrigation and fertilization stands out. The objective of this study is to evaluate the agro industrial and sugarcane yield (plant cane and first ratoon cycle) grown in the Sudoeste Goiano, Brazil, as affected by water replacement and nitrogen (N) rates; and to evaluate the residual effect from N rates on sugarcane ratoon grown under water replacement levels. The study was carried out with pots filled with a mixture of 120 kg of Oxisol (Rhodic Hapludox) and cattle manure, in a proportion of 3:1 v/v, respectively. A completely randomized design was used, 3x4, with three repetitions, analyzed in split plots. The treatments were combinations of three levels of replacement water (75, 50 and 25% of available water) and four N rates (0, 60, 120 and 180 kg ha^{-1}, equivalent to pots), which were also the same doses for the treatment residual N rate. In both cycles, at harvesting the stem yield and the total recoverable sugar (TRS) were determined for calculation of gross income of sugar and alcohol. The plant cane was not affected significantly by any of the factors evaluated. Interactions between the residual N doses and water replacements influenced the TRS and the gross income of sugar and alcohol in ratoon cane, proving that the N applied on a cycle can be leveraged by another; the stem productivity was affected only by water replacements.

Key words: Residual nitrogen, sugar yield, alcohol yield, irrigation, stem productivity.

INTRODUCTION

The sugarcane is a crop that stands out in Brazil for its great socioeconomic importance. Sugarcane constitutes the raw material for various byproducts and is responsible for a large share of biofuels used in the

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world. Currently, Brazil is the world largest sugarcane producer. In the 2014/2015 growing season, Brazil cultivated approximately 9.00 million hectares of sugarcane crop, harvesting 634.76 million metric tons.

The sugarcane yielded a total production of 35.56 million tons of sugar and 29.21 billion liters of alcohol. Goiás State has the second largest planting and production area for sugarcane in the country; they participate with 9.49% of the total area and 10.44% of production, being surpassed only by the São Paulo State (CONAB, 2015).

Currently, the main objective of the sugarcane industry is to increase productivity and reduce costs associated with the improvement of the quality of the raw materials and final products. To attain it, the agricultural and industrial areas must work together to obtain a higher quality and industrial yield.To achieve high income and productivity of sugarcane, it is necessary to combine the genetic and environmental factors, so that both can and are manipulated anthropically. Genetics favor the cultivation associated with managements in various regions of Brazil and the world. Among the environmental factors, nitrogen fertilization and irrigation are some of the practices that have been increasing.

Consequently, researches have been developed in the search of information regarding the optimization of more rational use of water and nitrogen fertilizers, to maximize production, under technical, economic and environmental aspects. According to Oliveira et al. (2015), agriculture demands large quantities of water to supply the plant's needs; and among the irrigated crops, sugarcane stands out for its responsive characteristics to irrigation.

There are lots of literature information on the response and benefits of N for sugarcane; however there still is no consensus on the crop response to the application of N fertilizers. Various authors have reported the response to the nutrient in general, are more frequent in cycles of ratoon sugarcane than in plant cane (Wiedenfeld, 1995; Franco et al., 2011; Teodoro et al., 2013).

The objective of the present study is to evaluate the productivity of stem and the agro industrial yield of sugarcane in the cycle of plant cane and first ratoon, grown in the SE region of the Goias State under different levels of water replacement *via* surface drip and different N rates as urea; and to evaluate the residual effect from N rate for first sugarcane ratoon.

MATERIALS AND METHODS

Soil and weather conditions

The study was carried out in the Southwest region of Goiás state, Brazil, municipality of Rio Verde, from October 2013 to August 2014 (sugarcane plant cycle) and September 2013 to May 2014 (first sugarcane ratoon). Plastic pots with dimensions of 0.6 m (upper) diameter × 0.45 m (lower) diameter x 0.45 m (height), filled with a mixture of soil with cattle manure above the layer of gravel were used. The experiment was carried out in the open, in an experimental area belonging to the Instituto Federal Goiano, Campus Rio Verde, Goiás State, Brazil, situated at latitude 17°48'28" S and longitude 50°53'57" W, with average altitude of 720 meters. The climate of the region is classified (Koppen), as Aw (tropical), with rainfall in the months from October to May, and with drought from June to September. The annual average temperature ranged from 20 to 35°C and the rainfall varied from 1500 to 1800 mm annually. Figure 1 show the total precipitation accumulated in the cycles of the sugarcane, in function of the days after transplanting (DAT) for cane plant and days after cut (DAC) for first sugarcane ratoon.

The IAC 95-5000 variety, with a variety of a very high agricultural production, rusticity, precocity, indicated for a favorable environments for erect growing, excellent ratoon sprouting, good tillering and between rows covering, resistance to the main diseases and not presenting tipping and flowering was used.

Soil collected from 0 to 0.20 m depth of a Rhodic Hapludox according to United States (2006), a Latossolo Vermelho distroférrico, loamy, Cerrado phase by the Brazilian soil classification EMBRAPA (2013) was used along with cattle manure, in a proportion of 3:1 v/v, respectively. The chemical characteristics of the substrate (soil + cattle manure) used in the pots are presented in the Table 1.

Experimental design and characterization of treatments

In plant cane, the experimental design used was randomized blocks, with three replicates, analyzed in 3x4 split plots. The treatments were combinations of three rates of water replacement (75, 50 and 25% of available water) and four N rates (equivalent to 0, 60, 120 and 180 kg ha^{-1} as urea, represented by D1, D2, D3 and D4, respectively); where the plots are the rates of water replacement (WR) and the subplots, the nitrogen rates (ND). The difference for ratoon sugarcane was the only subplots, which correspond to four residual N rates (applied in the previous cycle), being 0, 60, 120 and 180 kg ha^{-1}, equivalent to the pots.

Fertilization of plant cane

All the N fertilization was done manually in accordance with their due treatments. There was planting fertilization for the treatments D2, D3 and D4.The (D0) treatment is the N control, that is, without N. The topdressing N fertilization was top dressed at 45, 60 and 90 DAT in accordance with the respective treatments.

Fertilization of the ratoon sugarcane

The N fertilization of 120 kg N ha^{-1} (urea) was applied as in the cane plant, so as to match all treatments, to make it possible to assess only the N applied in plant cane cycle, evaluating in the first ratoon cane as residual N. This in turn was divided into two applications, the first at 30 DAC and the last at 70 DAC.

Water replacement

The irrigation was done by the drainage lysimeter method, using four pots for each replicate, totaling twelve pots (lysimeters).

The soils of pots were maintained at field capacity for 18:00 hours and the drained water was collected 12 h later. After the collection of the drained water volume of each pot, the data were applied to equation 1, to reach the field capacity of each pot and the average value was considered and extrapolating for the rest of the pots. The pots of reference (lysimeter) also received the plant and the treatments corresponding to each line of drip. All irrigations were performed in the morning.

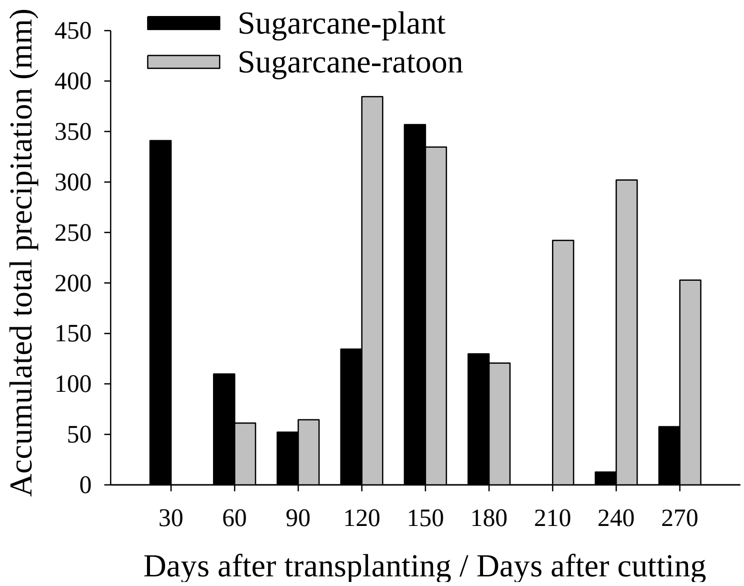

Figure 1. Precipitation accumulated in two cycles of sugarcane, plant cane and ratoon cane. Source: Meteorological Station of UNIRV - University of Rio Verde, Brazil.

Table 1. Chemical characterization initial of the soil + cattle manure, used in pots.

pH	K	Ca	Mg	Al	SB	CEC	H+Al	O. M.
$CaCl_2$				mmolc dm^{-3}				g dm^{-3}
6.9	14.5	139	33	<1	186.9	198.8	12	51
M	**V**	**P**	**S**	**Cu**	**Fe**	**Mn**	**Zn**	**B**
	%				mg dm^{-3}			
0	94	305	70	4.8	38	8.6	9.8	0.33

SB, sum of bases; CEC, cation exchange capacity; V, bases saturation; O. M., organic matter; m, Al saturation.

$$FC = WA - WD \quad (1)$$

Where: FC = field capacity (L); WA = volume of water applied (L); WD = volume of water drained (L).

The irrigation interval used was two days. This to ensure that the quantity of water to be applied was always proportional to the quantity sufficient to elevate the moisture to their respective treatments of 25, 50 and 75% field capacity of the pots. Field capacity was determined from each reference pots, established as 22 L.

At the end of the experiment the effective precipitation and the net water blade applied to the soil (via irrigation system) were calculated, for the period which the crop culture was not in the field. Whereas, an available water capacity (AWC) of 25 mm in a water catchment area of the precipitation of 0.2827 m^2. For the total cycle of plant cane and ratoon cane the values of precipitation effective calculated for each pot were 337.60 mm and 484.70 mm, respectively.

The net blade of water for the WR of 75, 50 and 25% in plant cane the values were 824.35 mm, 662.1 mm and 499.85 mm respectively. For the first ratoon cane the values were 1033.70 mm, 850.7 mm and 667.7 respectively. Irrigation was done using drippers of a turbulent flow with the flow of 1.65 L h^{-1} to 1.0 bar, spaced 0.50 m, being installed in a lateral line of dripper for each treatment, giving a total of six lines of dripping, 10 m in length.

Analyzed variables

Productivity was determined through manual cutting with the aid of a digital dynamometer, precision of 20 g. The mass in kg of all the plants collected per pots, transforming it to productivity in t ha^{-1}, using the area occupied by the pots (1.595 m^2) was measured.

To determine the total recoverable sugar (TRS) of sugarcane, five plants were collected per pot, with a total of 60 whole culms per repetition, eliminating, only the pointer and the dry leaves. Then, stems were taken to the Center for Sugarcane APTA/IAC Laboratory, located in Piracicaba, SP for the technological parameter analysis used to estimate the gross income of sugar and alcohol, according to the instruction manual of the Council of Producers of Sugarcane, Sugar and Alcohol of the São Paulo State (CONSECANA, 2006).

For the calculation of the gross income of sugar and alcohol, the equation 2 and 3, respectively, were used:

$$GIS = \frac{Productivity \times TRS}{1000} \quad (2)$$

$$GIA = \frac{GIS \times 0.51}{0.79} \quad (3)$$

Where: Productivity (t ha^{-1}); GIS: gross income of sugar (t ha^{-1}); GIA: gross income of alcohol (m^3 ha^{-1}); TRS: total recoverable sugars (kg t sugarcane^{-1}).

Statistical analyzes

The data were submitted to analyze the variance and when the F test was significant, the mean Tukey test (0.05 probabilities) was proficient for the water replacement factor, due to the fact that there were only three rates. For the N rate factor, linear and quadratic polynomial regression test were applied. The statistical software SISVAR-ESAL® (Ferreira, 2011) and the SigmaPlot®11 (Systat Software Inc.) were used.

RESULTS AND DISCUSSION

According to Table 2, the interaction between the N rates factor (ND), and water replacements (WR) were not influenced significantly for the variables analyzed in the plant cane.

Due to the ideal conditions for growth and the use of the PSS method, the sugarcane plants remained in the field for about nine months, thus anticipating its cycle and reaching the ideal maturation index for the harvest up to 270 DAT. The fact that water replacements did not affect these variables could be explained by the following attributes: resistance to drought presented by the variety- Good water availability (from precipitation) which coincided with the time of maximum development of sugarcane (Figure 1): and high content of organic matter present in the soil (exceeding 5%) - according to Machmuller et al. (2015) one of the benefits of organic matter is to increase water retention capacity of a soil. Despite this result, the average values found for the TRS were satisfactory, ranging from 141.91 to 152.63 kg t cane^{-1}. The maximum value was higher than that obtained by Moura et al. (2014), 151 kg ton cane^{-1}, in WR of 75% and with NDR 100 kg ha^{-1}.

The results found for the TRS corroborate with Oliveira et al. (2011) and Moura et al. (2014), where irrigation did not influence the TRS in the plant cane circle. As reported in several studies, some sugarcane do not respond to N fertilization, both on crop yield and technological quality, because some factors such as the roots strength, organic matter mineralization and the biological N fixation are assigned to this behavior (Urquiaga, 1992; Silva et al., 2013; Rosa, 2012). In the face of these facts, the production and total recoverable sugars, which are part of the crop industrial quality reflected in the insignificance of the gross income of sugar and alcohol when subjected to the evaluated factors, since the calculation to reach these variables takes into account only the TRS and yield.

Table 3 is different from plant cane; the evaluated factors influenced significantly the analyzed variables of sugarcane in its cycle of first ratoon. It is observed that the interaction between residual N rates and water replacement in the TRS, GIS and GIA, influenced the productivity significantly only by water replacement. These results confirm that the ratoon cane responds to N fertilization and that the N applied in the previous cycle can be leveraged by sugarcane (residual effect), thus influencing the gross income of sugar and alcohol of crop.

As the factors influenced the TRS and the SP, it was expected that the gross income of sugar and alcohol were also influenced, in view of the direct relation of these variables. Oliveira et al. (2016), studying productivity variables of sugarcane also noted an increase in sugarcane yield with increasing WR, reaching the value of 230 t ha^{-1} in the WR of 75%, higher than what was obtained in this study, 183.68 t ha^{-1}. The comparison between these studies, however, is not feasible because of difference in used varieties and also considering that our experiment was carried out in pots. But the increase in productivity that promotes WR is clearly seen in both studies.

It was observed in Figure 2 the unfolding of the interaction between WR x RND. For water replacements within each level of residual N rates, there was a significant difference between the RND 60 and 180 kg ha^{-1}, so that for these rates, the WR50% was statistically equal to WR of 25 and 75%, with difference only between the WR of 25 and 75% (Figure 2A). For the residual N rates within each level of water replacement, there was no difference between the WR of 25 and 75%, but only the WR25% adjusted to one of the models studied. The equation adjusted for this WR was to the quadratic, so that there will be a decrease in the TRS until the RND of 67.5 kg ha^{-1}, noting that when using a low WR (25%), in ratoon sugarcane, the N fertilization up to 140 kg ha^{-1} in the preceding cycle is not favorable for the TRS of ratoon cane but the RND above this N rate provide increment quite expressive, so that for the RND of 0, 140 and 180 kg ha^{-1}, the TRS estimated in WR of 25% was 122.6, 124.0 and 138.8 kg t cane^{-1}, respectively (Figure 2B).

For the GIS and GIA, the unfolding of RND within each level of WR did not submit any significance to the equations studied. For the unfolding of the WR within each RND, there was significant difference only in the rates of 60 and 120 kg N ha^{-1} (Figure 3A and 3B). The N rate that differed in the WR and presented higher values for incomes was the 75% which was not statistically different from 50%. The lowest values were found in WR, 25%, which was statistically not different from the 50% (Figure 3A and 3B). Silva et al. (2014), studying growth and yield parameters of sugarcane variety RB92579, under irrigation scheme in Brazilian Semi-arid, found a gross income of sugar and alcohol for the first ratoon of 20.89 t ha^{-1} and 15.01 m^3 ha^{-1}, respectively. This is very

Table 2. Summary of the analysis of variance for total recoverable sugar (TRS), stems productivity (SP), gross income of sugar (GIS) and alcohol (GIA), sugarcane (plant cane) subjected to different water replacement (WR) and nitrogen doses (ND).

Variation source	DF	Mean square			
		TRS	SP	GIS	GIA
WR	2	361.72^{ns}	2142.68^{ns}	28.58^{ns}	11.90^{ns}
Block	2	493.26^{ns}	570.83^{ns}	60.66^{ns}	25.29^{ns}
Residue (a)	4	193.46	664.05	12.36	5.17
ND	3	198.13^{ns}	1205.70^{ns}	46.67^{ns}	19.45^{ns}
WR x ND	6	286.09^{ns}	1296.03^{ns}	26.05^{ns}	10.83^{ns}
Residue (b)	18	160.99	777.41	39.72	16.55
CV a (%)	-	9.35	16.59	15.34	15.37
CV b (%)	-	8.53	17.95	27.49	27.49
General means		**kg t cane^{-1}**	**t ha^{-1}**		**m^3 ha^{-1}**
		148.69	155.33	22.92	14.8
LSD		23	37.47	5.11	0.64

^{ns}not significant to 0.05 probability by F test; DF, Degrees of freedom; LSD, least significant difference.

Table 3. Summary of the analysis of variance for total recoverable sugars (TRS), stems productivity (SP), gross income of sugar (GIS) and alcohol (GIA), sugarcane (plant cane) subjected to different water replacement (WR) and residual N rates (RNR).

Variation source	DF	Mean square			
		TRS	SP	GIS	GIA
WR	2	52.59^{ns}	5080.14*	96.71*	40.31*
Block	2	49.58^{ns}	1249.94^{ns}	23.97^{ns}	10.00^{ns}
Residue (a)	4	42.71	599.46	11.16	4.66
RND	3	58.28^{ns}	886.22^{ns}	11.93^{ns}	4.96^{ns}
WR x RND	6	290.29**	1886.50^{ns}	46.51*	19.39*
Residue (b)	18	45.30	730.22	17.13	7.12
CV a (%)	-	5.14	15.23	16.27	16.28
CV b (%)	-	5.29	16.80	20.15	20.13
Means (WR%)		**kg t cane^{-1}**	**t ha^{-1}**		**m^3 ha^{-1}**
25		124.74^a	143.80^b	18.05^b	11.65^b
50		128.68^a	154.95^{ab}	19.96^{ab}	12.88^{ab}
75		127.93^a	183.68^a	23.63^a	15.25^a
LSD		9.50	35.60	4.86	3.14

**Significant among themselves the 0.01 probability by F test; *Significant 0.05 probability by F test; ^{ns}not significant to 0.05 probability by F test; means followed by the same letter in the columns do not differ statistically at 0.05 probability by Tukey's test; DF, degrees of freedom); LSD, least significant difference.

close to the general mean found in this study both for plant cane and the ratoon sugarcane (Tables 2 and 3).

In accordance with the results of this study, the benefit of the N fertilization associated with the irrigation provides for the cultivating of sugarcane. These are responsible for the significant increase in the final product of commercial interest that is the sugar and alcohol. Taking into account a soil with at least 5% of organic matter, and assuming physical, structural, chemical and biological benefits, availability of nutrients mainly N, through mineralization (Lehmann and Kleber, 2015), it is recommended that for obtaining good agro industrial productivity with small quantities of soil N it is not necessary for N fertilization on plant cane. For the first cycle of ratoon cane, the recommendation of N fertilization is quite variable, because it will depend on the fertilization performed in planting, since that soil residual N influences the sugarcane yield from one cycle to the other. But what is clear is the need for applications of N fertilization in ratoon sugarcane, even with a soil rated with a high content of organic matter.

Regarding the use of irrigation, it is quite variable not

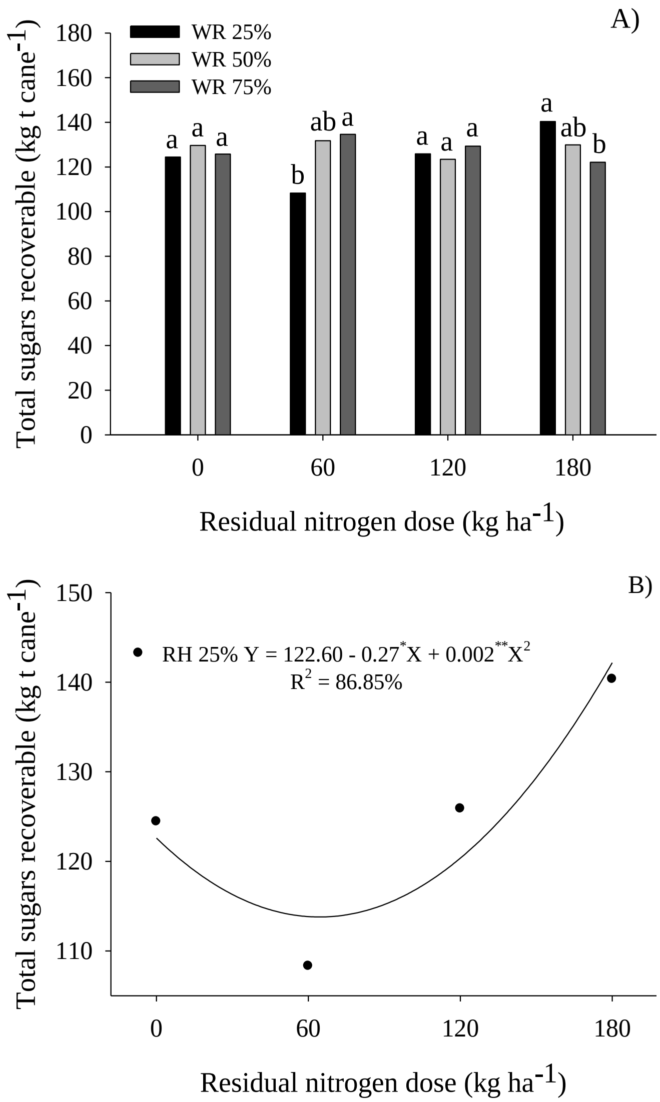

Figure 2. Total recoverable sugars of the sugarcane (TRS), as a function of water replacement within each residual N rate (A) and total recoverable sugars of the sugarcane, as a function of residual N rate within each water replacement (B).

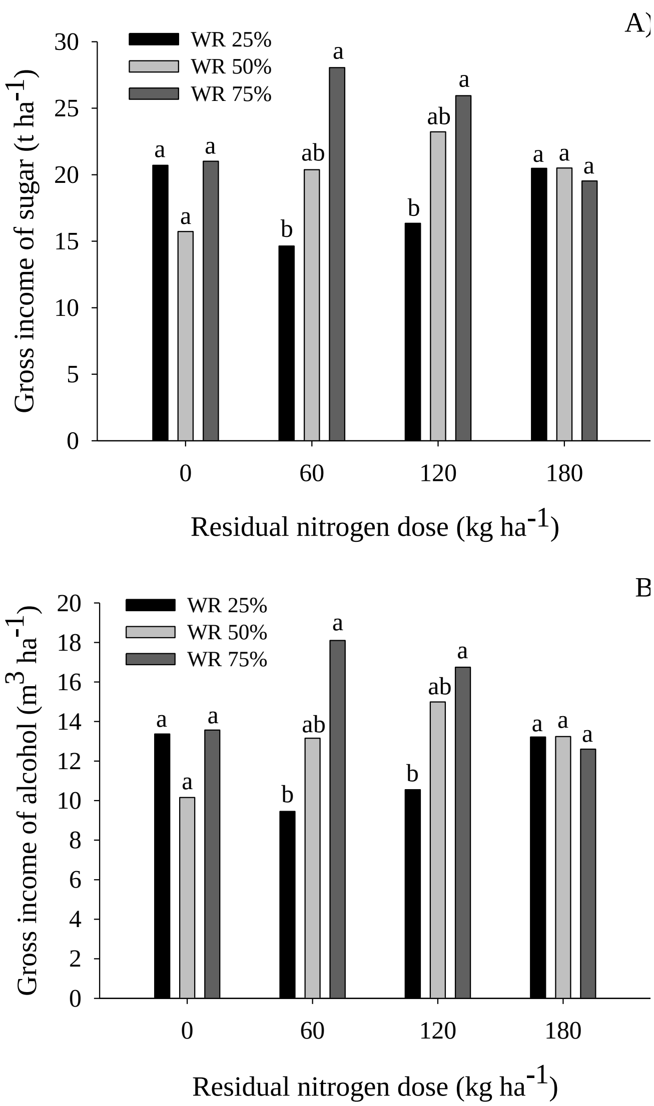

Figure 3. Gross income of sugar (A) and of alcohol (B), as a function of the water replacement, within each residual N rate.

depend on the sugarcane cycle, but on the distribution of rainfall, because, as observed, when there is a favorable distribution of rainfall particularly at the time of establishment and growth of sugarcane (fact occurring in plant cane) the minimum WR of 25% water, there is a certainty of achieving high yields, but when the distribution of rainfall is not so favorable (fact occurring in ratoon sugarcane), WR average of 50% is the most recommended.

Conclusion

The N applied on plant cane is utilized by the crop in the first ratoon cane (residual effect), but does not influence the stems productivity. Water replacements interact with residual N, so as to influence the total recoverable sugar and consequently the gross income of sugar and alcohol in ratoon cane.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Companion plants associated with kale increase the abundance and species richness of the natural-enemies of *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae)

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The effects of intercropping of Brassicaceae with coriander (*Coriandrum sativum*), dill (*Anethum graveolens*), African marigold (*Tagetes erecta*) and calendula (*Calendula officinalis*) on the abundance, species richness and diversity of predators and parasitoids of *Lipaphis erysimi* have been assessed. The numbers of aphids, parasitized aphids and natural enemies were determined during two consecutive phases. The first period comprised the vegetative phase of companion plants up to the onset of flowering and the development of kale up to the start of harvesting, while the second period encompassed the late flowering of companion plants up to senescence and the complete harvesting phase of kale. The establishment of *L. erysimi* and its natural enemies during the first period was enhanced by the climatic conditions and the additional nutritional resources offered by companion plants. Over the complete 13 week period, the abundance of natural enemies in kale intercropped with African marigold, calendula, coriander and dill increased by factors of 3.1, 2.1, 2.0 and 1.6, respectively, compared with the kale monoculture, while species richness increased by 1.8-fold in kale/African marigold intercrop and by a factor of 2.7 in the other treatments. The predominant predators were Syrphidae larvae and *Hippodamia convergens* whereas the predominant parasitoid was *Diaeretiella rapae*. The diversity of natural enemies was similar in all crops owing to the high proportion of syrphids in relation to the other groups of insects. The improved resources offered by companion plants can be exploited in the conservative biological control of insect pests.

Key words: Conservation biological control, natural enemies, insect seasonality, Hemiptera, Aphididae, Asteraceae, Apiaceae, *Lipaphis erysimi*, abundance, richness, Syrphidae, ladybird, kale

INTRODUCTION

Increasing plant diversity within an agriculture-dominated landscape can bestow a setting approximating to that of the natural environment. Diversification may also serve to increase the abundance of beneficial insects by providing increased floral resources, alternative prey and hosts, and additional sites for hibernation, mating and oviposition for natural enemies of crop pests (Alignier et al., 2014).

Implementation of an organic production system coupled with diversification represents an alternative strategy to the use of insecticides for regulating insect communities. Indeed, the expansion and intensification of monoculture farming has been considered one of the main factors responsible for loss of arthropod diversity around the globe (Altieri, 2009; Welch and Harwood, 2014). While the selection of appropriate companion plants for successful management of agricultural landscapes is important, relatively few studies have focused on the influence of habitat on the relationship between insect pests and their natural enemies (Chaplin-Kramer and Kremen, 2012).

Kale (*Brassica oleracea* L. var. *acephala* D. C.) is one of the most popular vegetables in Brazil, and is of considerable economic importance, especially to small-scale farmers, so we use this plant as a model, however *Lipaphis erysimi* (Kaltenbach, 1843) (Hemiptera: Aphididae) being one of the most important pests of brassicas on the world. Kale and other species of brassicas crops throughout the world are constantly plagued by *L. erysimi*, a specialist brassica aphid that not only attacks the terminal portions of stems and inflorescences, causing curling and yellowing of the plant, but also acts as a vector for phytopathogenic viruses (Blande et al., 2008).

A number of reports are available concerning the impact of diversification on the population dynamics of insect communities associated with brassica crops (Hooks and Johnson, 2003). It is known that the intercropping of food crops with flowering species of the families Asteraceae and Apiaceae can enhance the efficiency of pest predators by increasing their longevity, fecundity, colonization and permanence in the cropping system (Walton and Isaacs, 2011).

Members of the Asteraceae have been shown to maintain the biodiversity of predators and parasitoids when employed as a companion crop in onion fields (Silveira et al., 2009). Regarding the Apiaceae, aromatic species attract numerous insects that forage for pollen and nectar, while the floral architecture provides shelter for prey and/or preferential or alternative hosts. The bright yellow color of the flowers and the nutritional value of the pollen are highly attractive to ladybirds and wasps, while the floral architecture is compatible with the head morphology and foraging behavior of the coccinellids (Patt et al., 1997; Walton and Isaacs, 2011).

Considering the beneficial effects that companion flowering species may have in the suppression of crop pests, we propose that intercropping kale with species of Apiaceae or Asteraceae in an organic culture system would increase the attraction and permanence of natural enemies of the aphid *L. erysimi.* In order to test this hypothesis, we evaluated the effect of intercropping kale with African marigold, calendula, coriander or dill on the abundance, species richness and diversity of aphid predators and parasitoids.

MATERIALS AND METHODS

Experimental design

The experiment was carried out between March and June 2012 at the organic crops research station of the Universidade Federal de Lavras (UFLA) (21°14'43" S; 44°59'59" W; 930 m altitude). The fully randomized block design consisted of five treatments, with five replications each, and involved 25 plots (4 x 1.7 m) comprising kale monoculture (control) and kale intercropped with African marigold, calendula, coriander or dill. Seeds of kale and the companion plants were germinated in a greenhouse in separate polystyrene trays (200 cells per tray) containing commercial Plantmax® substrate (Eucatex Agro, Paulínia, SP, Brazil), and transplanted to the experimental plots 30 days after germination. The soil in the plots was analyzed prior to experimentation and found to contain 11.05 mg dm^{-3} of P, 84 mg dm^{-3} of K, 3.30 $cmol_c$ dm^{-3} of Ca^{2+} and 1.00 $cmol_c$ dm^{-3} of Mg^{2+}.

In each of the plots, kale was arranged in five rows with three plants each (15 plants/plot) spaced 1.0 m between rows and 0.8 m between plants. Companion plants were arranged between the rows of kale, with each of the four rows containing four plants (16 plants/plot) spaced 0.4 m from one another. The applicable plot was considered to be the three central rows containing nine kale plants. Plots within a block were separated by 1.5 m weed-free aisles, and blocks were separated by pathways (1.0 m wide). The soil was covered with plastic mulch to suppress weeds that could interfere with the results and to conserve humidity, and the plants were irrigated daily by sprinkler irrigation aspersion. Meteorological data (monthly mean temperature and relative humidity and monthly accumulated rainfall) were collected at the weather station at the UFLA determined throughout the experimental period.

Sampling procedures

Determination of the numbers of aphids, parasitized aphids and natural enemies present in the kale crops commenced 20 days after transplanting seedlings to plots. Sampling was performed weekly in the morning over a period of 13 weeks until senescence of the companion plants. In order to facilitate evaluation of the fluctuating populations of insects, the sampling period was divided in two stages. The first period (P1) extended from 26th March to the 7th May and covered the vegetative stage of the companion plants up to full flowering, and the development of the kale up to the start of harvesting. During this period, six observations were performed, one at the end of March, four in April and one at the beginning of May. The second period (P2) extended from 17th May to 22nd June, and covered the late flowering of companion plants up to their

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senescence and the complete harvesting phase of the kale. During this period, seven observations were carried out, four in May and three in June.

Since collection and counting procedures at each sampling were carried out on the same plants, the predators and parasitoids had to be removed before counting the aphids and collecting the parasitized aphids (mummies) in order to avoid dispersion of the natural enemies. Predators and parasitoids of *L. erysimi* were collected from three randomly selected plants in each plot (75 plants/week) using a manual aspirator and brush on each whole plant for 5 min. The insects were subsequently transported to the laboratory for counting and identification. Adult insects were placed in acrylic flasks containing 70% ethanol, while immature insects were transferred to Petri dishes containing *L. erysimi*-infested kale leaves and incubated at 24±1°C and 70±10% relative humidity under a 12 h photoperiod until they matured into adults. Taxonomic classification of insect predators was carried out with the aid of specific dichotomous identification keys.

The numbers of aphids present on leaves from plants that had been previously sampled for the presence of natural enemies were determined with the aid of a manual counter. In order to ensure consistency of sampling, leaves were classified as:

Apical – young leaf not fully expanded
Median – adult and fully expanded leaf; and
Basal - senescent leaf with visible yellowing.

One leaf of each type was selected randomly from each plant prior to visualization of aphids on the abaxial side. After counting, mummies were collected from the same leaves.

Statistical analysis

Analyses were performed using R software (R Development Core Team 2014) with the level of statistical significance set at 5%. The effects of sampling period (P1 and P2) and treatments (explanatory variables) on the numbers of predators, parasitoids, aphids and mummies (response variables) were evaluated by analysis of variance (ANOVA) and regression analysis using generalized linear models with Poisson distribution errors and chi-square test (p<0.05) (Buckley et al., 2003; Crawley, 2005).

Subsequently, non-significant qualitative terms for kale monoculture (control) and kale intercropped with African marigold, calendula, coriander or dill factors were compared by contrasts, in order to establish similarities between treatments in the full model. The ecological parameters (*n*, *S* and *H'*) were calculated using PAST software version 2.04 (Hammer et al., 2001), tested for homogeneity of variance, and analyzed by ANOVA and Kruskal-Wallis tests.

Ecological parameters

The true number of species or species richness (*S*) in relation to the observed number of species (S_0) was established using the Jackknife estimator. The Shannon-Wiener diversity index (*H´*; range 0 - 5) was used to characterize diversity since it combines species richness and abundance.

RESULTS

Influence of sampling time and treatments on the population of aphids and natural enemies

The study periods P1 and P2 showed significant effects on the population of *L. erysimi* over time (χ^2 = 54215.0; *P* < 0.00001), with the highest incidence of aphids being observed during P1 (Figure 1). Moreover, the incidence of *L. erysimi* was significantly lower (χ^2= 73.52705; *P* < 0.00001) in the treatment pair kale monoculture x kale/dill compared with the pair kale/coriander x kale/calendula (Figure 1). The kale/African marigold intercrop resulted in a lower incidence of aphids in comparison with others treatments (153.74; p < 0.00001), and the highest incidence was observed during P1 coinciding with the period in which the population of predators was lower (Figure 2).

In contrast, the study periods did not exhibit significant influence on the population of parasitoids (χ^2= 7.5815; *P* = 0.10817) or the occurrence of mummies (χ^2 = 2.7910; *P* = 0.09479) over time. Comparison of treatment pairs revealed that the incidence of predators was significantly higher (χ^2 = 24.09329; p < 0.00001) in the treatment pair kale monoculture x kale/dill compared with the pair kale/coriander x kale/calendula (Figure 2). However, the kale/African marigold intercrop resulted in a higher incidence of predators in comparison with the other two treatment pairs (χ^2 = 94.71723; *P* < 0.00001).

Considering the two sampling periods together, the incidence of parasitoids was not significantly influenced by the treatments (χ^2= 7.5815; *P* = 0.1082), although the numbers of parasitized aphids were affected significantly (χ^2 = 23.8320; *P* < 0.00001). The number of parasitized aphids was highest in the kale/African marigold intercrop followed by the kale/calendula intercrop (Figure 3).

Influence of climatic conditions on the aphid population

The mean temperature during P1 (March and April) was around 21°C, and the mean rainfall was approximately 39 mm with 75% relative humidity. During P2, average temperatures decreased to 17°C (May) and 18°C (June), and precipitation increased to 42 mm (May) and 95 mm (June) with 84% relative humidity. As shown in Figure 4, the percentage of aphids diminished markedly from P1 to P2 as the temperature decreased, and the humidity increased.

Evaluation of the ecological parameters

The abundances of various groups of natural enemies of aphids observed in kale crops during the period of March to June, 2012 are presented in Table 1. Larvae of aphidophagous Syrphidae (Diptera) were predominant in all treatments, particularly in the kale monoculture where the richness of species was markedly lower than in all other treatments and hoverflies accounted for 62.1% of all specimens collected. *Hippodamia convergens*

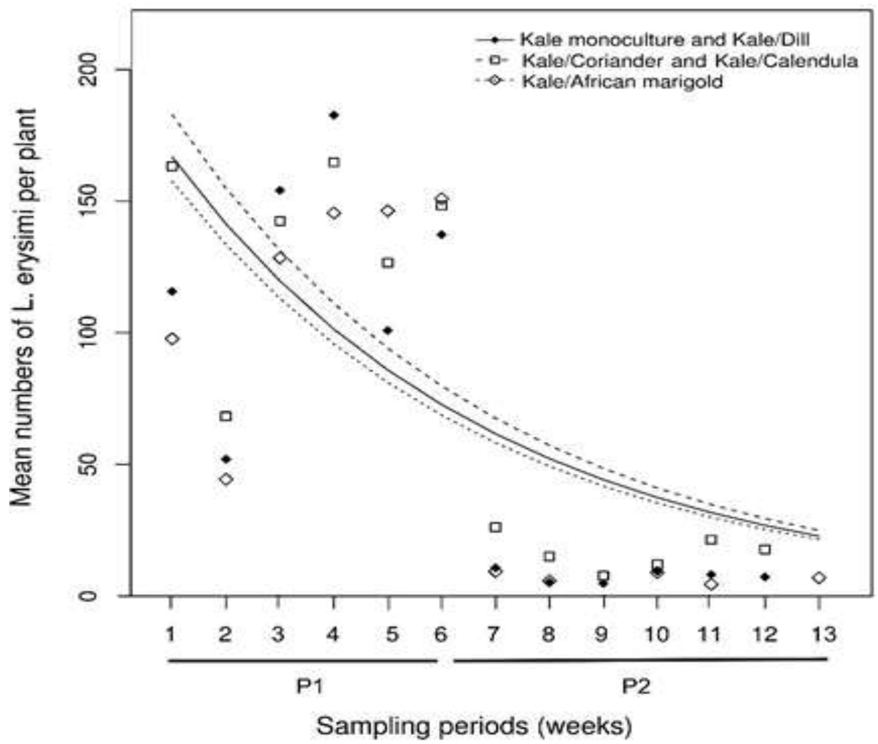

Figure 1. Mean numbers of *L. erysimi* aphids observed in kale crops during the sampling periods from 26 March to 7 May (P1) and from 17 May to 22 June (P2) 2012.

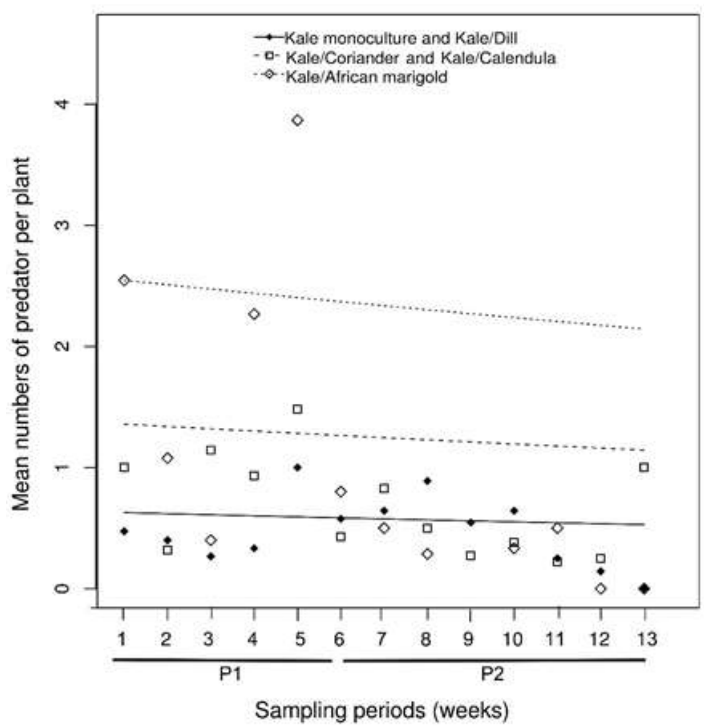

Figure 2. Mean numbers of predators observed in kale crops during the sampling periods from 26 March to 7 May (P1) and from 17 May to 22 June (P2) 2012.

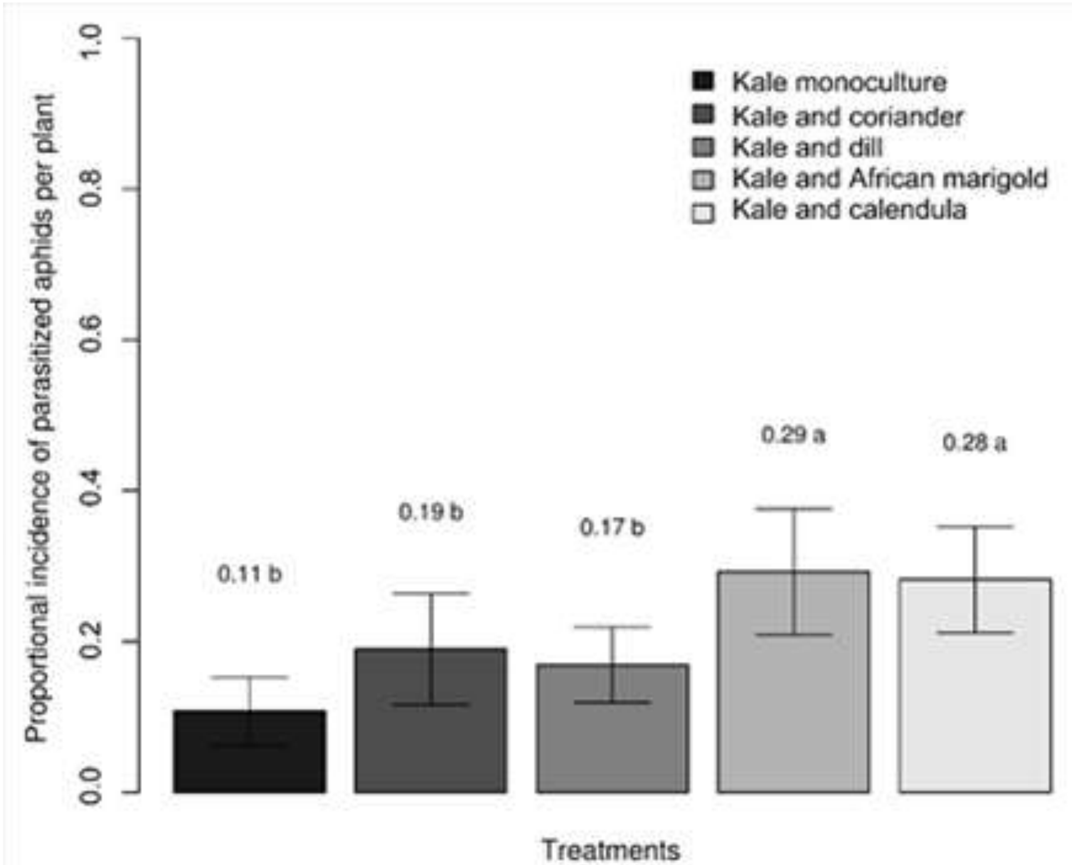

Figure 3. Incidence of parasitized aphids observed in kale monoculture or kale intercropped with companion plants during the whole sampling period from March to June 2012. Mean values (shown above the bars) followed by dissimilar lower case letters are significantly different (χ^2 test; $P < 0.05$).

(Guérin-Méneville, 1842) was also observed in all treatments, but the frequency was considerably higher in the kale/African marigold intercrop where the convergent lady beetle accounted for 21.1% of all natural enemies collected.

Parasitoid wasps accounted for around 10% of the natural enemies of aphids identified in the kale crops and included *Diaeretiella rapae* (McIntosh, 1855), which was present in all crop treatments and accounted for 89.6% of all parasitoids collected, *Aphidius colemani* (Viereck, 1912) (8.62%) and *Praon volucre* (Haliday, 1833) (1.72%) (Table 1). The highest abundance of *D. rapae* was observed in the kale/dill intercrop with 18 individuals collected.

Regarding the overall abundance (*n*) of natural enemies, significantly more beneficial insects were observed in the kale/African marigold, then Kale/ Calendula intercrop in comparison with the other treatments (Table 2). Species richness (*S*) in the kale monoculture was considerably lower in comparison with those of the intercrops, but no statistically significant between-treatment differences were observed in the Shannon-Wiener diversity index (*H'*).

DISCUSSION

Factors that are intrinsic to the host plant, especially those related to nutritional quality, influence the colonization and performance of phytophagous insects. For example, the reproductive rate of aphids is associated positively with the amounts of nitrogen in the plant (Zarghami et al., 2010) and these, along with the levels of proteins and carbohydrates, vary as the plant matures (Staley et al., 2011). In the present study, the colonization of kale plants by *L. erysimi* was markedly higher in P1 than in P2 (Figure 1), a finding that probably reflects the greater concentrations of soluble nitrogen translocated through the phloem in the young leaves of P1 compared with the old leaves of P2. According to Agarwal and Datta (1999), young leaves of mustard greens (*Brassica juncea*) are of superior nutritional quality in comparison with older leaves and, therefore, support the highest rates of fecundity and survival of *L.*

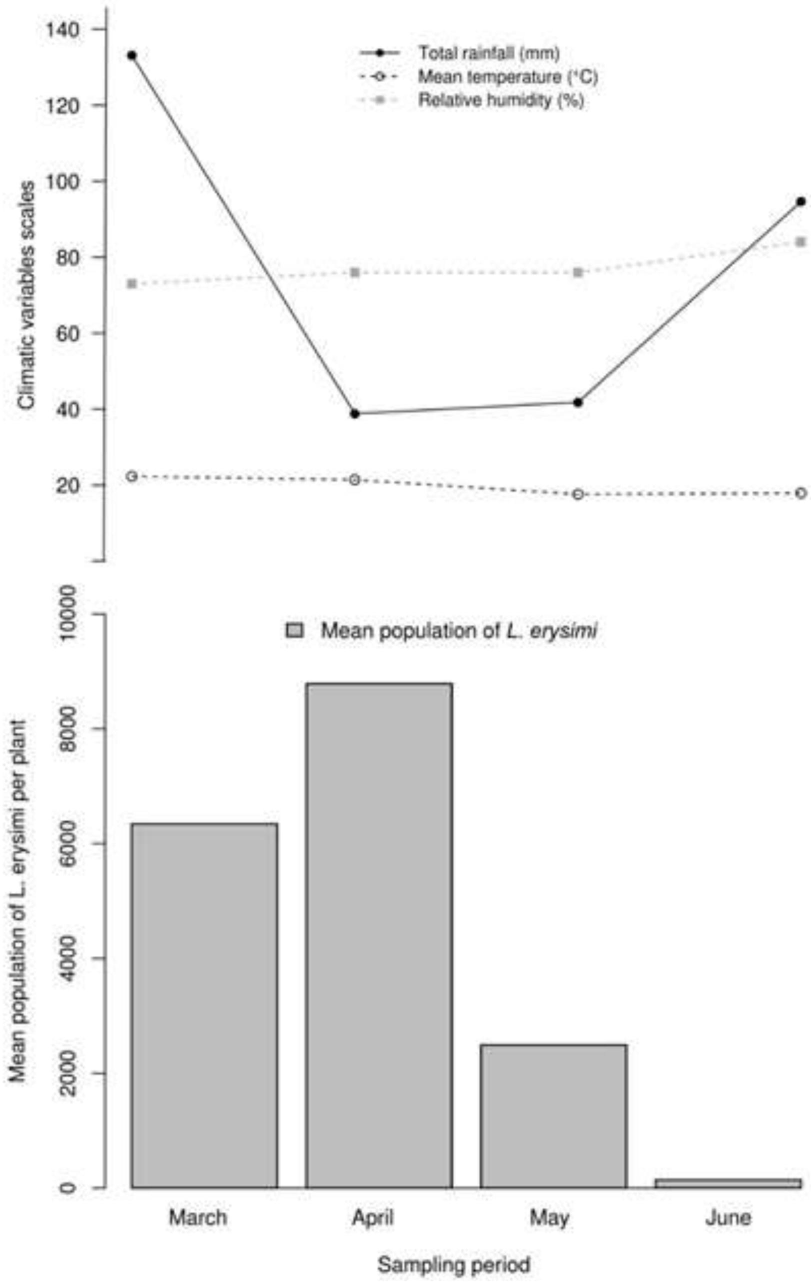

Figure 4. Variation in *L. erysimi* aphid population, rainfall, temperature and relative humidity during the whole sampling period of March to June 2012.

erysimi.

In the present study, the highest density of natural enemies of *L. erysimi*, particularly predators, was maximal in P1, the period during which the companion plants attained full bloom and the aphid population was at its highest level. During the flowering stage, members of the Apiaceae and Asteraceae are very attractive to the natural enemies of aphids, and the favorable dietary conditions serve to enhance the longevity, fecundity and preying capacity of beneficial insects (Walton and Isaacs, 2011) and, as a consequence, the aphid population tends to diminish.

Evaluation of the influence of treatments on the populations of aphids and their natural enemies during the crop cycle revealed that the kale/African marigold and Kale/Calendula intercrop attracted more predators than the other intercrop and also exhibited the lowest incidence of aphids (Figure 1). This is a promising result

Table 1. Abundance (*n*) of natural enemies of *L. erysimi* observed in kale monoculture and kale intercropped with companion plants during the whole sampling period of March to June 2012.

Taxon	Kale monoculture		Kale intercropped with companion plants							
			Coriander		Dill		African marigold		Calendula	
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Syrphidae larvae	43	62.1	62	48.1	47	48.5	104	59.2	48	42.0
Cantharidae	0	0	2	1.57	3	3.09	2	1.14	0	0
Carabidae	0	0	0	0	0	0	0	0	3	2.63
Coleomegilla maculata	0	0	4	3.14	1	1.03	4	2.28	0	0
Cycloneda sanguinea	0	0	3	2.36	0	0	0	0	1	0.87
Diomus sp.	0	0	0	0	0	0	0	0	1	0.87
Eriopis connexa	0	0	0	0	0	0	1	0.57	0	0
Harmonia axyridis	0	0	0	0	1	1.03	1	0.57	1	0.87
Hippodamia convergens	2	2.89	18	14.7	6	6.18	38	21.1	21	18.42
Hyperaspis sp.	0	0	0	0	0	0	0	0	1	0.87
Psyllobora rufosignata	0	0	0	0	1	1.03	0	0	0	0
Scymnus lowei	0	0	0	0	0	0	0	0	1	0.87
Scymnus rubicundus	0	0	0	0	1	1.03	0	0	1	0.87
Staphylinidae	0	0	1	0.78	0	0	3	1.71	0	0
Geocoris uliginosus	0	0	1	0.78	0	0	0	0	0	0
Macrolophus basicornes	0	0	6	4.72	0	0	0	0	2	1.75
Orius insidiosus	0	0	1	0.78	0	0	0	0	0	0
Orius thyestes	0	0	1	0.78	0	0	0	0	0	0
Paraproba sp.	0	0	0	0	0	0	2	1.14	1	0.87
Franklinothrips vespiformis	0	0	1	0.78	1	1.03	0	0	0	0
Stomatothrips angustipennis	0	0	0	0	1	1.03	0	0	0	0
Crisoperla externa	4	5.79	0	0	0	0	0	0	2	1.75
Hemerobiidae	0	0	2	1.57	0	0	0	0	0	0
Doru luteipes	0	0	2	1.57	1	1.03	0	0	0	0
Mantodea	0	0	1	0.78	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	1	1.03	1	0.57	1	0.87
Aphidius colemani	2	2.89	0	0	0	0	0	0	3	2.63
Diaeretiella rapae	6	8.69	7	5.51	18	18.55	7	4.57	14	12.28
Praon volucre	0	0	0	0	1	1.03	0	0	0	0
Araneae	12	17.39	15	11.81	14	14.43	11	6.28	13	11.4
Total	69	100	127	100	97	100	174	100	114	100

Table 2. Abundance (*n*), species richness (*S*) and Shannon-Wiener diversity index (*H'*) of natural enemies of *L. erysimi* observed in kale monoculture and kale intercropped with companion plants during the whole sampling period of March to June 2012.

Treatment	*n* [1]	*S*	*H'* [2]
Kale/African marigold	122.31±16.14[a]	11	1.18±0.08
Kale/calendula	83.46±9.85[ab]	16	1.77±0.21
Kale/coriander	80.31±9.77[bc]	16	1.60±0.15
Kale/dill	63.85±7.78[bc]	16	1.27±0.25
Kale monoculture	39.46±6.24[c]	6	1.81±0.15

[1] Mean values of *n* bearing the same superscript letter are not significantly different (Tukey test; $P >$ 0.02); [2] Mean values of *H'* are not significantly different (*Kruskal-Wallis test;* $P > 0.05$).

considering that the counting of natural enemies was performed on the kale plants themselves where predatory action against the aphids was more likely to occur. Silveira et al. (2009) demonstrated that African marigolds offer conditions that favor the maintenance of natural enemies of onion pests. Climatic conditions have been shown to influence the density of *L. erysimi* (Bapuji Rao et al., 2013).

Landin and Wennergren (1987) studied the intrinsic rate of increase of *L. erysimi* under different temperatures and concluded that the highest growth rate occurred around 25°C, while Bakhetia and Sidhu (1983) established that temperatures within the range 20 to 30°C favored the development and reproduction of the aphid. In the present study, the mean temperature of 21°C recorded during P1 probably contributed to the high incidence of aphids observed on kale crops during the first study period. Increased precipitation, which is considered to be a natural mortality factor of *L. erysimi* (Dogra et al., 2001), coupled with the reduced temperatures (17 to 18°C) registered during P2 were partly responsible for the increased aphid mortality observed during second study period. In contrast, relative humidity varied little between P1 and P2 (75 and 84%, respectively), and these levels were within the 75 to 85% range cited by Kulat et al. (1997) as favorable for the presence of *L. erysimi* in the field.

It is concluded, therefore, that relative humidity alone was not a factor in determining the variation in population size of the aphids during the kale cropping cycle.

Meteorological data gathered during the crop cycle are also important since climatic conditions may constitute a decisive factor in determining the timing of absence or peak infestations of aphids in the field (Chattopadhyay et al., 2005).

In the present study, predators predominated over parasitoids throughout the crop cycle. According Venzon et al. (2013) predators can benefit not only from the floral resources of the companion plants but also from the that allowed them to survive longer in the field, a factor that is particularly important when pest density is low. Most of the sampled predators were generalists or zoophytophagous (that is, spiders, syrphids, ladybird beetles and thrips) and were prevalent from the start of the culture period until the senescence of the companion plants. The main advantage of generalist predators is their ability to colonize an agro-ecosystem before the arrival of the primary pests and to remain in the field throughout the crop cycle by feeding on alternative prey (Aguiar-Menezes, 2003; Amaral et al., 2013).

Nevertheless, the results indicate that the density of predators accompanied changes in the phenology of the plants but that *S* was greater than S_0, most likely because the experiment was field-based and predators constantly appeared from the surrounding areas.

The three species of parasitoids observed were host-specific and all belonged to the Aphidiinae subfamily of parasitic wasps (Starý et al., 2007). This specificity explains the decrease in parasitoid density during P2, which occurred because of the decline in the *L. erysimi* population within this period (Figure 4). The results obtained herein indicate that the relative abundance and species richness of the natural enemies of *L. erysimi* vary according to the phenology (vegetative, flowering and senescence phases) of the companion plants, thus confirming previous reports relating to species of the families Asteraceae and Apiaceae (Silveira et al., 2009; Resende et al., 2012). In general, insect populations change over time according to the availability of food resources, microclimate and shelter offered by the host plants, and these elements clearly favored the continuous richness and addition of species during the present study.

In the present study, although the highest number of specimens of natural enemies was collected in the kale/African marigold intercrop ($n = 174$), species diversity was similar in all treatments as shown by the *H'* values (Table 2). This result was due to the high number of coccinellids, particularly *H. convergens*, which were present in all intercrops but mainly in the kale/African marigold treatment. A study conducted by Medeiros et al. (2010) demonstrated that the most common pollen grains found in populations of *H. convergens* within and around horticultural areas derived from members of the Asteraceae, thereby showing the importance of pollen as a food resource for these beneficial insects in a conservative biological control program. In contrast to the intercrop treatments, the kale monoculture presented a low *S* value with only 69 specimens collected, a finding that may be explained by the absence of extra food resources and the predominance of Syrphidae larvae (62.1%).

Although the other treatments also presented a preponderance of these larvae, there was greater homogeneity in the overall distribution of natural enemies compared with the kale monoculture. Syrphidae larvae were observed during the entire experimental period and their permanence in the kale monoculture and intercrops was favored by the constant presence of *L. erysimi*. The predator-prey dependency between aphidophagous Syrphidae and aphids observed in this study is also noteworthy. Tenhumberg and Poehling (1992) demonstrated that syrphids are sensitive to reduction in the aphid population, and examples of predator-prey dependency have been described involving larvae of *Aphidoletes* sp. and *L. erysimi* in cabbage crops (Silva et al., 2011). It is possible to infer that the permanence of syrphids in the present study depended on the presence of *L. erysimi*, since the density of these predators was associated with the density of aphids in the kale crops. Clearly**,** predator-prey interactions must be taken into account during the evaluation of the abundance of insect groups in field cultures, while the understanding of this relationship is very important for devising biological control strategies.

The attraction of syrphids towards Apiaceae plants has been widely investigated, and floral structure is considered to be one of the key factors. The morphology of flowers of the Apiaceae is compatible with the short mouthparts of adult Syrphidae hoverflies, thus facilitating access to nectar and pollen (Morales and Köhler, 2008). Flowers of the Asteraceae, including marigold species, are also attractive to syrphids, as verified by Robertson (1929), who reported that 25% of the 257 species of this family serve as hosts for hoverflies, and by Sajjad and Saeed (2010) who established that the Asteraceae is one of the families most visited by Syrphidae.

In the present study, *D. rapae* was the most abundant of the three species of parasitoids and was present in all treatments, especially in the kale/dill intercrop. The exposed nectaries of dill flowers emit odors that attract adult parasitoid species and the floral architecture facilitates their feeding behavior (Patt et al., 1997). The results of the present study indicate that the factors influencing the dynamics of multitrophic interactions present in an agro-ecosystem should be considered when assessing the population patterns of the pests and their natural enemies. However, the influence of climatic factors and the structure of the local landscape on the dynamics of arthropod populations require further elucidation in order to establish plant associations that would confer extra benefits to the main crop through the response of herbivores and their natural enemies.

Conclusion

The results presented herein revealed that establishment of *L. erysimi* and its natural enemies in kale crops were influenced by the climatic conditions and nutritional resources of the host plant. The abundance of natural enemies in kale intercropped with African marigold, calendula, coriander and dill increased by factors of 3.1, 2.1, 2.0 and 1.6, respectively, in comparison with the kale monoculture, while species richness increased 1.8-fold in kale/African marigold intercrop and by a factor of 2.7 in the other three intercrops. The data presented in this study support the original hypothesis that intercropping kale with Apiaceae or Asteraceae species in an organic culture system increases the attraction and permanence of the natural enemies of *L. erysimi* by virtue of the improved resources offered to these beneficial insects. Moreover, the information collected will be used to forecast the period when kale crops in the study area are more susceptible to the attack of pests, and to make suitable management decisions to reduce pest populations in a planned manner, that is, by configuring the kale crop and companion plants in such a way that natural enemies are attracted during the critical phase.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Wheat stem rust disease incidence and severity associated with farming practices in the Central Rift Valley of Kenya

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Stem rust (*Puccinia graminis* f.sp.*tritici*) is a major disease of wheat that occurs more in the main wheat growing regions of Kenya. The objective of this study was to assess the incidence and severity of wheat stem rust during the 2015 growing season. A survey was conducted in Mau-Narok, Njoro and Kabatini regions. During the survey work, 149 small scale wheat growers' fields were assessed. The results revealed that stem rust incidence of the three surveyed areas ranged from 11.3 to 77.8% and severity 20 to 60%. The survey confirmed that the incidence and severity were associated with the farming practices such as chemical control, varieties grown, use of certified or uncertified seed and cropping systems. The survey showed that high to moderate incidence and severity levels were found on fields with one or two sprays with a fungicide. The use of fungicide was the major practice by growers for stem rust control reporting Mau-Narok with 43.2%, Kabatini 38.9% and Njoro 17.8%. The varieties grown had a relationship to disease incidence and severity percent levels. The use of uncertified seed by farmers contributed to high disease incidence. About 50.6% growers preferred old varieties mainly Robin and NjoroBWII. About 97.8% of the farmers practiced crop rotation of wheat with legumes. A multi-tactic disease management approach mainly two fungicide sprays per growing season, use at recommended rates, planting of certified seed of resistant varieties and crop rotation of legumes with wheat are required as stem rust effective management strategies.

Key words: Wheat production, farm classifications, disease assessment, survey.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the worlds' most productive and important crops in the 21st century (Curtis and Halford, 2014). It is one of the key staple crops for global food security, providing more than 35% of the cereal calorie intake in the developing world, 74% in the developed world and 41% globally from direct consumption (Shiferaw et al., 2013). Due to increased consumption and demand for grain, for food (Curtis and

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Halford, 2014) wheat yields must be increased as this is seen as an important strategy to prevent food shortages (Curtis and Halford, 2014).

Wheat is the second most important cereal staple food after maize in Kenya (USAID, 2010). In Kenya it is grown mostly in the Rift Valley, some areas of upper Central province (Nyandarua, Nyeri) and parts of Meru (Timau) (USAID, 2010). Inspite of the importance of wheat, plant disease is still a major constraint to its production. Plant diseases have been reported to reduce crop yields worldwide, leading to significant crop losses (Khoury and Makkouk, 2010). Stem or black rust, caused by *Puccinia graminis*, has historically caused severe losses to wheat production worldwide (Njau et al., 2009).

In most wheat-growing regions of the world, existing environmental conditions will favour stem rust infection, which at times leads to epidemic buildup (Singh et al., 2011). The situation is worsened by the fact that susceptible wheat varieties are grown over large areas and that a large proportion of current breeding materials are susceptible to stem rust race *Ug99* and other newly identified races. It implies therefore that the stem rust pathogens have the potential to cause a wheat production disaster that would sourly affect food security (Singh et al., 2011).

Disease assessment is an essential task in the study of plant disease epidemics and vital to the knowledge of whether disease management practices are successful (Campbell and Neher, 1994). Disease severity evaluation is an important decision support for adoption of strategies and tactics for disease control. The most commonly used method to assess disease severity is visual (Bade and Carmona, 2011). Disease severity is determined by a function of the degree of infection, colonization, and damage of host tissues. Besides the amount of host development and growth is a function of disease severity (Gaunt, 1995).

Integrated disease management (IDM), which combines biological, cultural, physical and chemical control strategies in a holistic way of disease control as opposed to using a single component strategy is a better option apart from being sustainable (Khoury and Makkouk, 2010). It can be defined as a decision-based process involving coordinated use of multiple tactics for optimizing the control of the pathogen ecologically and economically (Khokhar and Gupta 2014). In practice and in most cropping systems today, emphasis is still being placed on a single technology (Khoury and Makkouk, 2010). Many problems have been associated with the use of fungicide such as the frequent emergence of fungicide resistance in pathogens and the harmful effects of fungicides to human health and the environment (Khoury and Makkouk, 2010).

Wheat production in Kenya mainly takes place on large- and medium-scale farms, using capital intensive technology. The technology on the medium- and large-scale farms is the same as that in Western Europe (Monroy et al., 2013). In contrast, small scale farms operations are smaller as compared to the large and medium (Monroy et al., 2013). The small scale wheat farmers complain of prohibitive production expenses and low production (caused by use of non-certified seeds and low use of inputs) and sub-regionof land as a major problem (MOA, 2013). Most large scale farmers are still holding stakes of wheat (Monroy et al., 2013). The cost of key inputs such as seed, pesticides is high for resource-poor farmers. Such high costs lead to low application and adulteration of inputs (GOK, 2010).

The main aim of this study was to assess the differences in disease incidence and severity in the major growing regions. The information on disease levels as connected with the farming practices' being carried out in the regions was not there. Therefore, the survey conducted was to determine the effectiveness of the management and control measure used in the regions.

METHODOLOGY

General information and percentage of the surveyed regions

A survey was conducted in three regions of Nakuru county, Njoro, Mau-Narok and Kabatini regions which represented 25.7, 35.1 and 39.2% respectively of the study area (Figure 1). The surveyed fields were early planted by the farmers during the 2015 season. In Mau-Narok, there were two major cropping seasons, early and late while, Njoro and Kabatini had one which were planted early. Only the fields with the early crop were surveyed in Mau-Narok. Most growers' small scale had planted early in Mau-Narok while most medium and large scale farmers planted late.

The locations surveyed in Mau-Narok region were Sururu, Mwisho Wa Lami, Likia and Mau-Narok. Mau-Narok had an average annual rainfall of 1300 mm, an altitude of 2900 m above sea level (masl), minimum and maximum temperatures range of 14°C and 26°C, respectively (Jaetzold et al., 2010) The second region was Njoro which had five locations mainly Piave, Lower Piave, Njoro and Kerima. Njoro region had an altitude of 2185 masl, average annual rainfall of 935 mm, and minimum and maximum temperatures of 9.7°C and 23.5°C, respectively. The third region was Kabatini having four locations mainly Karunga, Ngecha, Thayu and Ruguru which had many wheat growers. Kabatini had an altitude at 2135 masl with a minimum temperature of 10€C and maximum temperature of 26°C, and annual rainfall of 800 mm (Jaetzold et al., 2010).

Field survey

A questionnaire check list was used during the study. The part one of the questionnaire was about the general information, the name of the division, location and farm classification. Farm classifications were mainly three; small (<10 ha), medium (10-60 ha) or large scale (>60 ha) farms, adapted from MOA (2006). The second part of the questionnaire was about the farming practices in place, fungicide used, rate used and number of sprays. The other questions were about the wheat varieties commonly grown (FAO-SEC, 2012).

Sample size for disease assessment

A multi stage sampling technique was applied where fields were

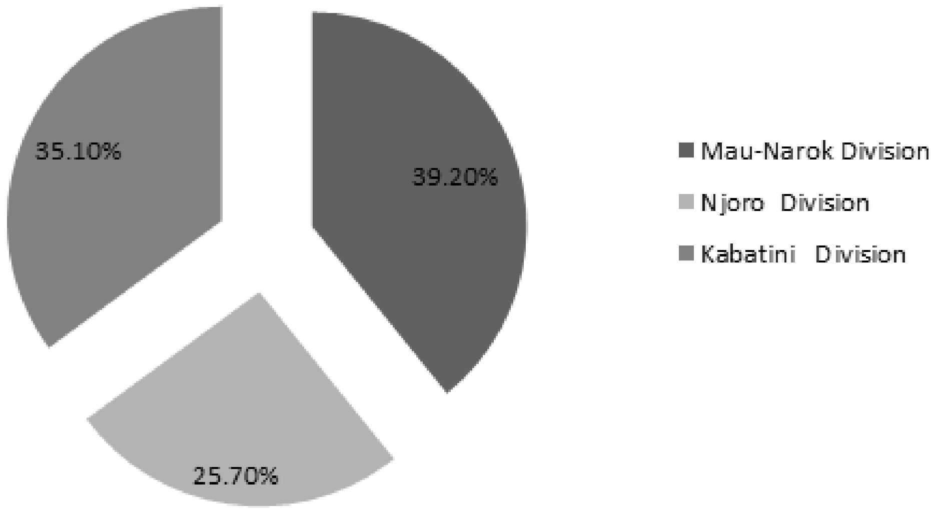

Figure 1. The frequency of fields surveyed in the three regions of three regions of Nakuru county, Kenya in 2015.

grouped as small, medium and large scale in the three regions of Mau-Narok, Njoro and Kabatini. The sample size of growers selected was done following the formula from Krejcie and Morgan (1970) as shown below;

$$S = \frac{X^2NP(1-P)}{d^2(N-1)+X^2P(1-P)} \quad (1)$$

S = required sample size, X^2 = the table value of chi-square for 1 degree of freedom at the desired confidence level (3.841), N = the population size, P = the population proportion (assumed to be 0.50 since this would provide the maximum sample size), d = the degree of accuracy expressed as a proportion (0.05). The total sample size was 58 in Mau-Narok, 38 in Njoro and 52 in Kabatini regions.

Assessment of disease intensity using incidence and severity percentages

A quadrate of 1 m by 1 m was used for both disease incidence and severity on the same field, and 1 m^2 used to obtain the two disease values. The incidence was the number of plant infected by disease and severity the percentage of foliage attacked by disease on the same plant. The stage of the wheat crop assessed was Zadoks GS 73 (early milk), GS 75 (medium milk), GS 77 (late milk), GS 83 (early dough) and GS 85 (soft dough) (Zadoks *et al*., 1974) which was wide-ranging from field to field across the study areas.

Disease incidence for three study areas

A quadrate was cast in the field randomly for the total number of farms visited. The proportion of stem rust infected plants to the total number of plants in the quadrate was calculated from the FAO-SEC (2012) formula as shown below:

$$DI = \frac{Number\ of\ diseased\ plants\ in\ the\ quadrate}{Total\ number\ of\ plants\ in\ the\ quadrate} * 100$$

Disease severity for three study areas

The same fields and plants used for disease incidence determination were scored for disease severity. Disease scoring was done following the modified Cobb scale as described by Peterson et al. (1948).

Data analysis

Data was input for analysis using the descriptive statistics, frequencies and cross tabulation. The frequencies were in percentages for all the entities. The entities included regions, locations, farm classification, fungicides used, the rates and number of sprays. The percentage for varieties grown, fertilizer use, seed source and a yes and no response for wheat grain yield as being high, medium and low was done. Each component was worked out in percentages among the three regions and arranged in tables accordingly.

RESULTS

Stem rust disease incidence

Data illustrated in Figure 2 revealed that Mau-Narok as having high levels of disease incidence, Kabatini having moderate to low and Njoro low. In Mau-Narok region, stem rust disease was observed in Sururu whose disease incidence were 12.8 to 23.5% and Mwisho Wa Lami 58.5%. Mau-Narok location had 7.9 to 60.3% and Likia location had 12.6 to 77.8%. In Njoro region, all the locations had no stem rust disease incidences. At Kabatini stem rust disease incidence occurred in Ngecha at 9.2 and 11.3% and Ruguru at 13.9% and 3.3%. The average disease incidence in Mau-Narok was 32.1 %, Kabatini 7.9% and Njoro 0%. The absence of disease in Njoro (Figure 2) was explained by the growers as an

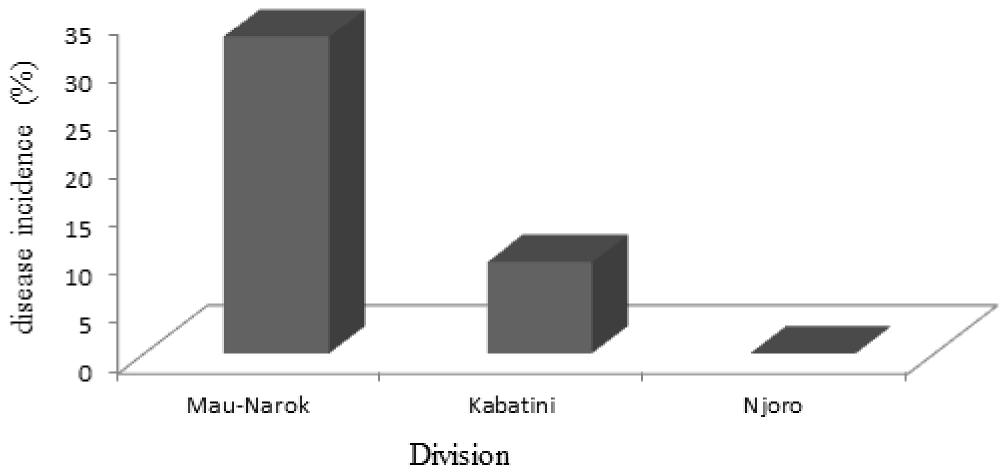

Figure 2. The average disease incidence (%) occurrence in the three regions of Nakuru county, Kenya in 2015.

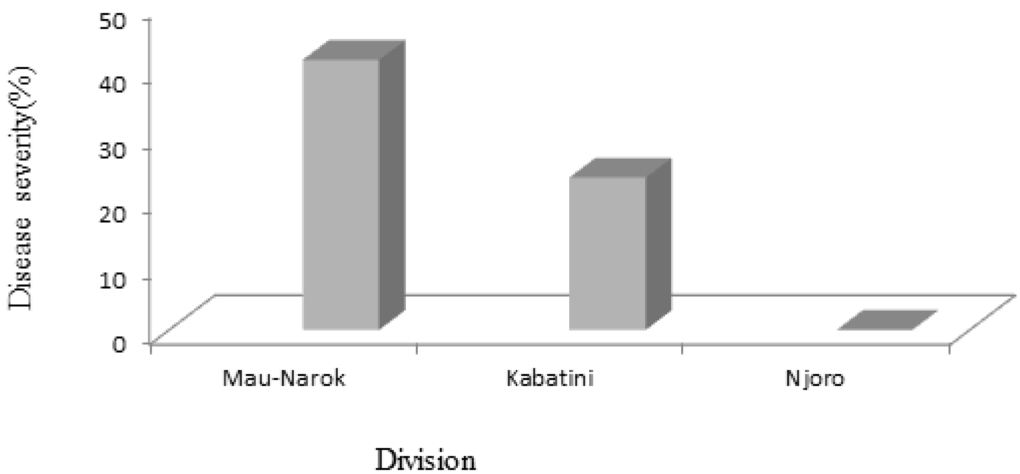

Figure 3. The average disease severity (%) occurrence in the three regions of Nakuru county, Kenya in 2015.

escape due to the changing rainfall patterns.

Stem rust disease severity in the three study areas

In Mau-Narok region, stem rust disease occurred in Sururu, where disease severity levels ranged from 30 to 40% and Mwisho Wa Lami had 20%, while Mau-Narok had upto 30 to 60%. In Likia, the disease severity was 40 to 60%. The disease severity in Mau-Narok was high by the figures obtained from one location to another. Kabatini reported stem rust disease severity in Ngecha was in the range of 20 to 30%. The figures in Kabatini showed that the disease severity levels were low. In Njoro region, all the locations surveyed had no stem rust disease severity. The average disease severity in the three regions was Mau-Narok 41.4%, Kabatini 23.3% and Njoro 0% (Figure 3).

Fungicide use, spraying rates and number of sprays in association with stem rust disease incidence

The number of sprays per growing season in Mau-Narok was observed at 34.5% of the growers spraying once, 34.5% of the farmers sprayed twice while 17.2% of the farmers sprayed thrice (Figures 4 and 5). About 42.1% of growers in Njoro did not spray fungicide on the wheat fields. Few growers (5.3%) used the earlier mentioned recommended rates, recommended rates were 52.6%

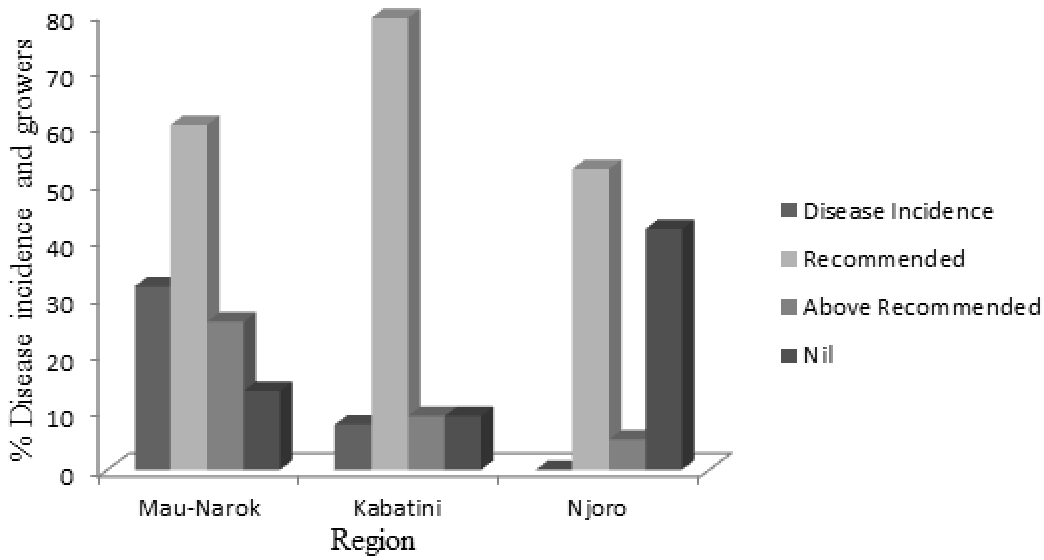

Figure 4. Fungicide spraying rates.

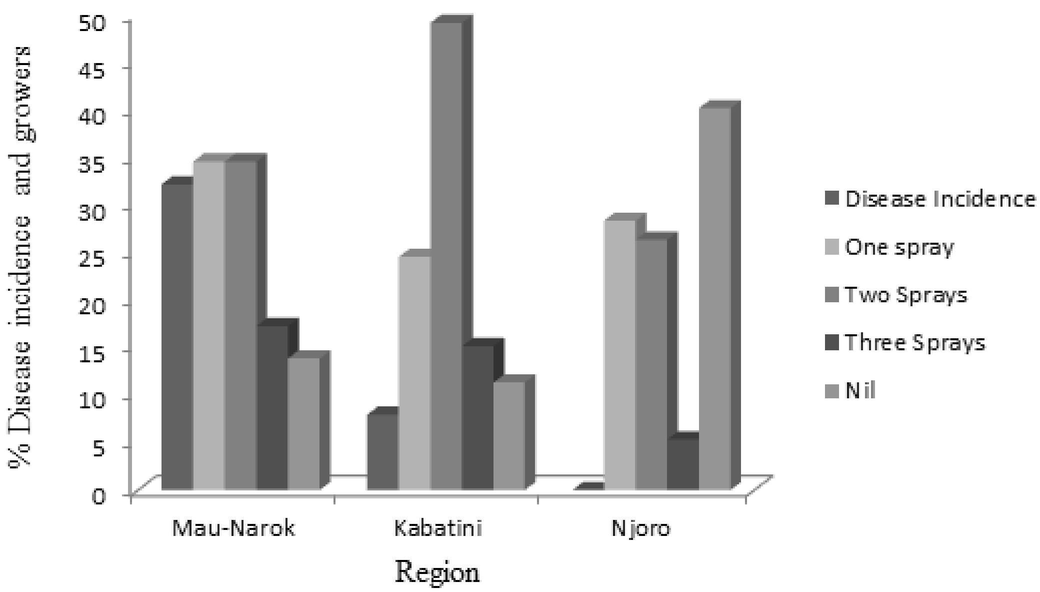

Figure 5. Disease incidence and number of fungicide sprays per growing season.

due to disease escape. In Njoro, few growers sprayed their fields once (28.9%). About 26.3% sprayed twice, and 2.6% sprayed thrice. The frequency of farmers who did not spray their field stood at 42.1% (Figure 4 and 5). In Kabatini, 9.4% used the earlier mentioned recommended rates, the recommended rates were at 79.2%, and those who did not spray at 11.3%. In Kabatini, 24.5% sprayed once, 47.1% twice, 15.1% sprayed thrice and 11.3% no spray (Figure 4 and 5).

The connection between the number of sprays and disease incidence percentage is shown in Figure 6, where the two sprays per growing season had 0 to 13.8% and three sprays had 0%, one spray had 36.8 to 77.8% diseases incidence. A higher percentage of growers using fungicides were found in Mau-Narok, followed by Kabatini and Njoro. As compared to Njoro and Kabatini, Mau-Narok had the highest percentage of growers of the earier mentioned recommended rates which were 5.3, 9.4 and 25.9%, respectively. Njoro regionhad a great number of fields that were not sprayed at 42.1% as compared to Mau-Narok 13.8% and Kabatini 11.3%. Despite the use of the earlier mentioned recommended

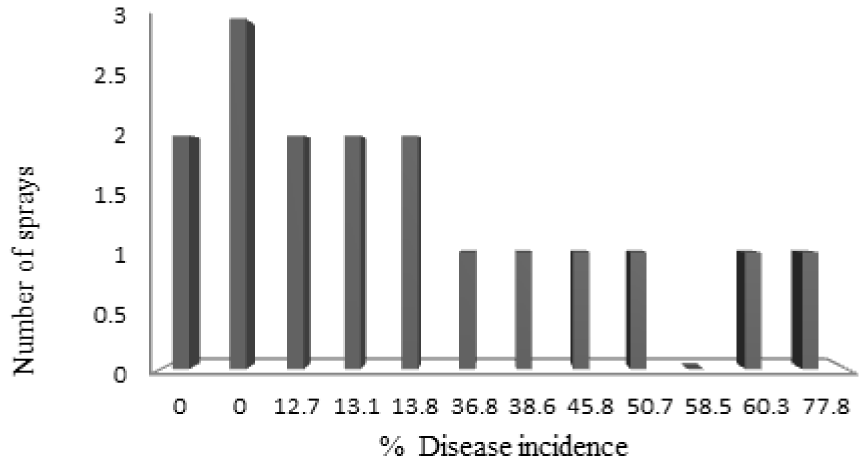

Figure 6. The association between number of sprays per growing season and disease incidence.

rates by growers in Mau-Narok disease incidence was moderate to high (Figure 2). In Njoro, during the growing season there was no disease, and growers sprayed only once or did not spray. In Kabatini, most of the growers sprayed twice at the recommended rates.

Varieties grown and seed source in association to stem rust disease incidence

Mwamba variety of certified seed was grown in Mau-Narok region where disease incidence of 23.5% was observed. In the same region, the fields with 12.8 and 13.3% disease incidence were planted with certified seed of the variety Robin. The field with 7.9, 12.6 and 18.8% disease incidence had certified seed of the variety NjoroBWII. The field with disease incidence at 19.8% was planted with certified seed of the variety Heroe (Figure 7 and 8). In Kabatini, the crop with disease incidence of 3.9, 11.3 and 9.2% had certified seed of variety Robin. The field with 3.3% disease incidence in the same region was planted with certified seed of variety NjoroBWII (Figures 7 and 8).

The field that reported the highest disease incidence of 77.8% was found in Mau-Narok region planted with uncertified seed of the variety Mwamba. The same region reported 38.6, 50.7, 58.5 and 60.3% disease incidence on the crops having uncertified seed of variety NjoroBWII. The field with 45.8% disease incidence was planted with uncertified seed of variety Robin (Figures 7 and 8). The percentage of growers using uncertified seed in Mau-narok was 70.7%, Njoro 23.7% and Kabatini 22.6% (Figures 7 and 8). The region with many gowers using certified seed was Kabatini 77.4%, Njoro 76.3% and Mau-Narok had the least percentage of 29.3%.

In the Mau-Narok region, the growers who planted the variety NjoroBWII was 53.4%, followed by Robin at 27.6%, Eagle10 at 1.7% and Korongo 1.7%. Mwamba was at 5.3% and Kwale 5.2%. The varieties Heroe, Ngami and Farasi were only found in Mau-Narok. In Njoro region, the growers have the following varieties: NjoroBWII (23.6%), Robin (34.2%), Mwamba (23.6%), Eagle 10 (2.7%) and Korongo (7.9%). Duma (2.6%) was the only variety grown in Njoro division and Kwale (5.4%). In Kabatini, the growers with Robin were 64.2%, NjoroBWII was 22.5%, Korongo was 5.7%, Kwale was 5.7%, Duma was 0% and Mwamba was 0% (Figure 9).

Cropping systems

In Mau-Narok, 100% of the growers used a rotation of wheat and peas or wheat and potatoes. In Njoro, 94.7% used a rotation of Maize and wheat, and 5.3% of growers were found using an intercrop of wheat and Boma rhodes grass. It was the only region where wheat was intercropped. In Kabatini, 100% used a rotation. The rotation involved wheat, tomatoes or wheat, beans, Kales instead of tomatoes (Figure 10).

DISCUSSION

The stem rust disease incidence in the three regions showed that there were many factors that were related to the % disease observed. For the entire study, areas incidence ranged from 3.3 to 77.8%. According to FAO-SEC (2012), incidence of over 40% is regarded as high. This implies that in Mau-Narok, stem rust disease incidence of 45.6 to 77.8% was high (Figure 2). In Kabatini area, the disease incidence was low at 3.3%. The factors that affected disease incidence levels were

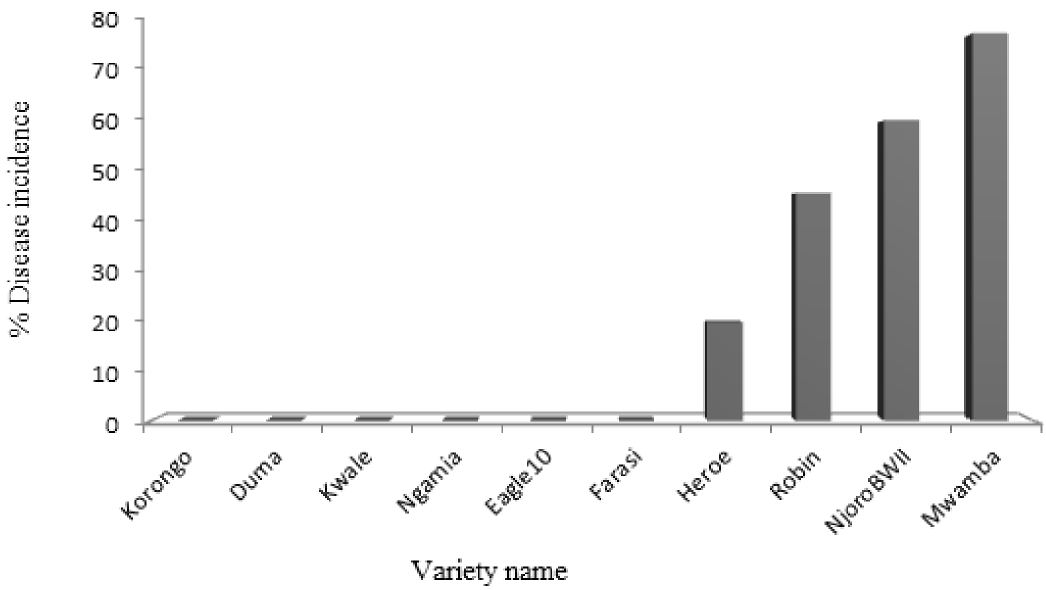

Figure 7. Variety of uncertified seed and disease incidence percentage.

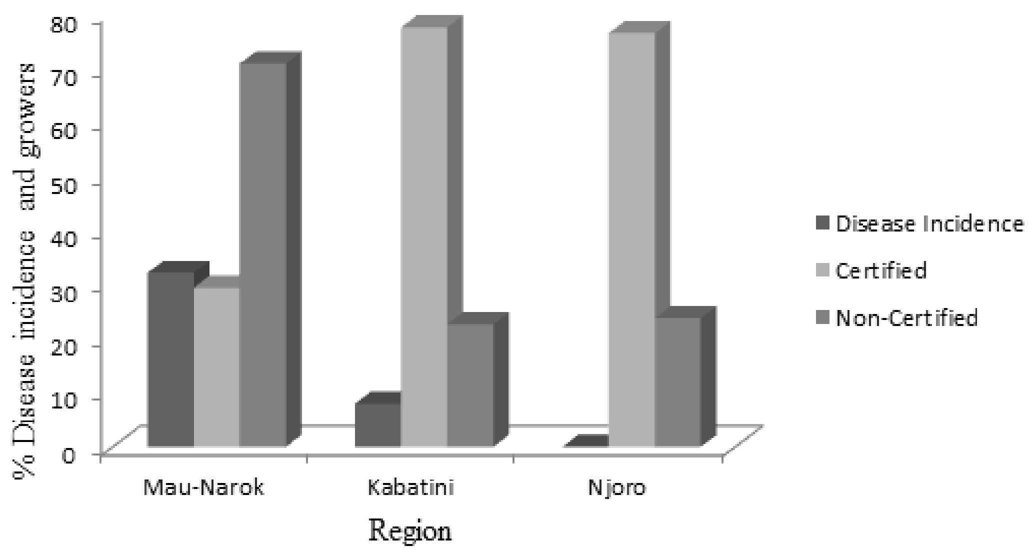

Figure 8. Disease incidence and seed source of certified or uncertified.

variety grown, fungicide use such as the rate of spray, number of sprays, seed source and crop management. In the case where the disease incidence is 77.8% in Mau-Narok, the crop was weedy, uncertified seed of variety Mwamba was used and one spray was done. The implication is that crop management and production process may affect the level of disease incidence. The fields with disease incidence ranging from 38.6 to 77.8% showed uncertified seeds were used and disease was not controlled using the right recommended spraying regimes.

The fields of Kabatini or Mau-Narok with disease incidence ranging from 0 to 12.8% had certified seed of the varieties Mwamba, Korongo, Robin and NjoroBWII being popular. The same fields were sprayed twice or thrice with a fungicide at the recommended rates (Figure 5 and 6). However, in Mau-Narok where 25.9% of growers sprayed at above the recommended rate was a sign of stem rust disease weighing heavily on growers' management attempts.

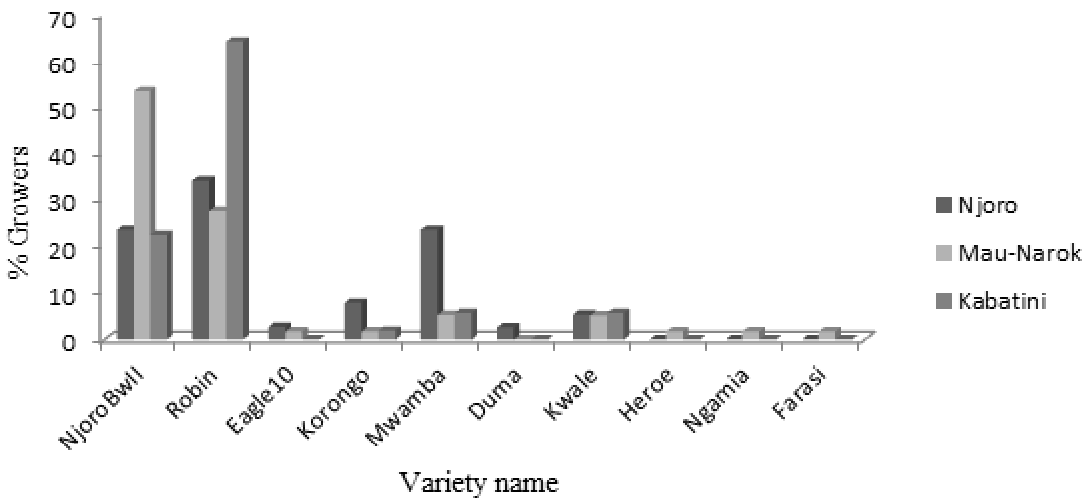

Figure 9. The percentage of varieties commonly grown in the three regions.

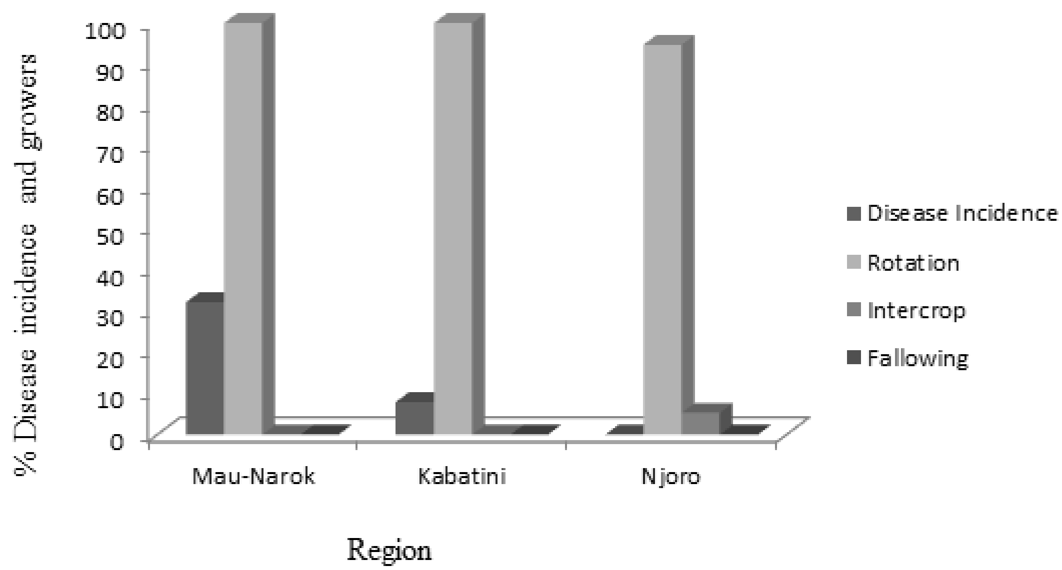

Figure 10. Cropping systems in the regions and disease incidence.

The stem rust disease severity in all the regions ranged from 20 to 60%. According to Taye et al. (2014), 0 to 20% indicates low disease severity, 21 to 40% is medium while greater than 41% is considered as high. Disease severity was high in Mau-Narok, low in Njoro and medium to low in Kabatini. The trend of disease severity was similar to disease incidence. However, fungicide use, spraying rates, number of sprays and varieties grown determined the severity levels. The fields sprayed twice or thrice with recommended or above the recommended rate reported medium to low severity of stem rust disease.

The number of fungicide sprays also affected disease incidence or severity. Prabhu et al. (2003) reported that two applications of tricyclazole or benomyl controlled panicle blast in rice, as indicated by lower values of disease progress curve and relative panicle blast severity, and increased grain yield. Ganesh et al. (2012) observed that three fungicide applications in rice Tricyclazole or Ediphenphos or Kitazine sprayed thrice at weekly interval managed leaf blast disease in rice. The percent use of fungicides in Mau-Narok was 43.2%, Kabatini 38.9% and Njoro 17.8%. The percentage of fungicide use reflected well with the disease pressure that was being experienced in the three regions with Mau-Narok being the most affected.

Fungicide application as described by Ghazanfar et al.

(2009) had an effect on the yield of Paddy rice Rabicide, particularly three applications resulted in increased yield. Gianesssi and Reigner (2005) stated that more effective fungicides have been introduced and used by growers to prevent losses caused by fungal pathogens, and Tadesse et al. (2010) also proved that fungicide treatments have effectiveness in reducing disease severity. As stated by Wegulo et al. (2012) fungicides used to control foliar fungal diseases of wheat belong to two major classes with a broad spectrum of activity against fungal pathogens. Fungicide application by the growers was not clear whether the spraying was done before or after disease onset. As explained by Balardin et al. (2010) fungicide application prior to any contact between pathogen and host is considered to be preventative. After inoculation and just before initial symptoms, the application is curative. All applications made after the onset of symptoms is eradicative. There is therefore a need of fungicide technologies to substantiate on the effective use and control of stem rust disease.

All the commonly grown varieties were released as resistant to wheat stem rust but resistance has been breaking down over the years due to mutation of the fungal pathogen. The two most commonly grown varieties across the three regions were Robin at 41.2% and NjoroBWII at 35.1%. The other varieties Mwamba (10.1%), Duma (5.4%) and Korongo (3.4%) were also found across the regions. High disease incidence and severity were found in the fields with Robin and NjoroBWII which appeared to have become susceptible to stem rust. Generally, the fields with Korongo, Duma and Kwale did not report any disease incidence largely due to the number of fungicide sprays used which was twice or thrice as recommended. The low disease incidence could be attributed to genetic resistance which according to Park, (2008) remains the most economical means of rust control. Resistant cultivars also contribute significantly to reducing off-season rust survival. Similarly, Singh et al. (2011) suggested that reducing the area currently occupied by susceptible wheat varieties should become the highest priority.

According to the growers, wheat varieties tend to be replaced for disease management purposes rather than market preference. The two most commonly preferred varieties by the growers in all the areas were Robin (59.1%) and NjoroBWII (40.9%) across the three regions. In contrast, varieties Mwamba, Kwale and Korongo were preferred by 10.1, 5.4 and 3.4% of the farmers respectively. This implied that most farmers preferred old varieties as compared to the newly released varieties. The farmer preference was based on yield and seed quality attributes rather than the disease reaction by the variety.

The most common cropping system in the three regions was wheat legume rotation. In Mau-Narok region farmers practiced wheat/peas and wheat potato rotation. The major crop rotation in Kabatini was a rotation of wheat and beans or wheat tomatoes. In Njoro, a rotation of wheat and maize was preferred. Overally, 100% of farmers in Mau-Narok practiced crop rotation while 94.7% and 5.3% of the farmers did the same in Kabatini and Njoro respectively. Crop rotation as reported by Khoury and Makkouk, (2010) is one of the most important means of managing disease in small grains. Cultural control methods such as crop rotations, fertilizer use and certified seed not only serve in promoting the healthy growth of the crop, but are also effective in directly reducing disease inoculums potential. Besides, crop rotation enhances the biological activities of antagonists in the soil.

Three of wheat fields in Njoro region lower Piave location had an intercrop of wheat and Rhodes grass which according to FAO (www.fao.org) is defined as planting alternating rows of maize and beans, or growing a cover crop in between the cereal rows. FAO (www.fao.org) also reported that the practice is not beneficial because an intercrop may compete with the main crop for light, water and nutrients. This may reduce the grain yields of both crops.

Fallowing was not observed in the three regions. This could be due to the fact that land scarcity is compounded by low soil fertility as was observed by Mwangi (1996). This has resulted in the shortening or elimination of the fallow period without concurrent efforts to increase soil nutrients through fertilizer application or other soil management practices mainly found in Sub Saharan Africa.

Seed quality is critical for crop establishment and plant vigour. Clean seed ensures field hygiene. About 59.1% of farmers interviewed used certified seed while 40.9% used non-certified seed. Mau-Narok (70.7) had the highest number of farmers using non-certified seed followed by Njoro (23.7%) and Kabatini at (22.6%) in that order (Fig. 10). The fields with certified seed had lower or no cases of stem rust disease as the case in Kabatini where 11.3% disease incidence was reported.

Conclusion

Stem rust disease incidence and severity % were reportedly high in Mau-Narok, followed by Kabatini which had low levels and Njoro having none. However, the disease incidence and severity was associated with the management practices. Two or more sprays at the recommended or above recommended rates showed either no or low disease incidence or severity. In addition, the variety grown and seed quality determined disease incidence and severity. The use of uncertified seeds of susceptible varieties increased disease levels. A multi-tactic approach involving optimal use of fungicides, resistant varieties, quality certified seed and crop rotations with legumes would be the best option for stem rust disease control. Similarly, as a result of diminishing land sizes under wheat (crops) farming practices in place have to be intensified and maximized based on modern agricultural technologies for food security. Work needs to be done on the verification and validation for an effective integrated disease management approach for stem rust disease.

Conflict of Interests

The authors have not declared any conflict of interests.

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Effects of hormone balance on Korean Hackberry seed germination

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Korean Hackberry (*Celtis koraiensis* Nakai), an ornamental, deciduous tree, lacks species resources, and low germination percentage also prevent the exploitation. Thus it becomes necessary to investage the mechanism of seed dormancy. This research explored the effect of plant hormone and seed coat (endocarp and testa) on seed germination. Endogenous hormone concentration, protein content, total soluble sugars, total starch and amino acids during germination were studied and the germination recorded with different seed coat treatments. Four seed lots were evaluated: (i) fresh; (ii) imbibed; (iii) germinated; (iv) non-germinated seeds. In seeds treated with gibberellic acid (+GA_3), the rate of germination was higher than in untreated (control) seeds. Exogenous GA_3 also promoted the generation of endogenous GA_3, increasing the total bioactive gibberellins (GA) in the embryo during germination relative to the control. Endogenous abscisic acid (ABA) concentration decreased during germination, however, the GA/ABA ratio was consistently higher in germinated than non-germinated seeds. The GA_3 treatment had a significant effect on the GA/ABA ratio. One-*trans*-zeatin riboside (ZR) and indole-3-acetic acid (IAA) content increased in germinated seeds. From dormancy to germination, the seeds of Korean Hackberry underwent changes in protein content, decreasing in germinated seeds, while concentrations of total soluble sugars and amino acids increased. In addition, germination was higher in seeds with the covering layers removed. The results suggest that germination of Korean Hackberry seeds is controlled by plant hormones and is also affected by the seed coat.

Key words: Abscisic acid, gibberellic acid, germination, indole-3-acetic acid, one-*trans*-zeatin riboside, storage substances.

INTRODUCTION

Korean Hackberry (Celtis koraiensis), a deciduous tree, is naturally widespread in northern China and in Korea. The beautiful foliage makes it very desirable as an ornamental. In addition, Korean Hackberry is potentially economically important for use in the production of lubricants and soap; this wild species has great development value and possible applications. It is also potentially important in research, because it is highly drought resistant. However, low and erratic seed germination limits the availability of seedlings and seriously affects the cultivation of this tree. Seed dormancy is vital for plant survival and ensures that seeds germinate only when environmental conditions are

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suitable. It is an adaptive trait in seed-plant species to resist bad environments, enabling plants to survive under stressful conditions in nature (Finkelstein et al., 2008), but can be a barrier for plant exploitation. In order to understand how to break seed dormancy, we need to explore seed germination mechanism. Germination is an extremely complex process that is regulated by signaling molecules, such as hormones, enzymes and antioxidants (El-Maarouf-Bouteau and Bailly, 2008). A considerable amount of research has demonstrated that the transition between dormancy and germination is mediated by plant hormones (Koornneef and Bentsink, 2002; Kucera et al., 2005), especially dependant on the balance of hormones such as abscisic acid (ABA) and gibberellins (GA) (Koornneef and Bentsink, 2002).

Germination is not only a consequence of complex hormone interactions, but also involves changes in cell structure and storage substances. Einali and Sadeghipour (2007) have demonstrated that protein in the seeds of *Juglans regia* is degraded by activating hydrolases. In the seeds of *Corylus avellana*, the total starch content has been shown to increase (Li and Ross, 1990) probably as a result of gluconeogenesis is by products of reserve lipid hydrolysis.

Although biochemical and physiological mechanisms during germination have been studied in seeds of many species, the knowledge is deficient in certain genera of trees, such as Korean Hackberry. Hence, in order to understand the mechanisms of Korean Hackberry seed dormancy and germination, we studied the dynamic change of plant hormones and storage substances at different stages during germination in seeds with or without GA_3 treatment.

MATERIALS AND METHODS

Plant material

Freshly matured fruits were collected from Taishan, at an elevation 751 m, in Shandong province, China (36.23924N° 117.10623°E) during late November 2014. Seeds were removed from the pericarp by hand, and only intact seeds were used for experimental analyses. The word ‘seed’ is used to refer to the propagule with the epicarp removed (botanical seed plus its covering layers: lemma, palea, and pericarp).

Seed covering layers

To investigate the germination pattern affected by the seed covering tissues, mature seeds were dissected as follows: (1) endocarp removed - N, E; (2) endocarp and testa removed-N, E, T; (3) Intact seeds-Control. Seeds which had the endocarp removed were surface-sterilised with 1% mercuric chloride for 5 min, and 75% alcohol for 10 min, the intact seeds were sterilised with 1% mercuric chloride for 10 min, and 75% alcohol for 10 min. All the seeds were inoculated into MS culture medium (8 $g.L^{-1}$sucrose, 30 $g.L^{-1}$ agar) and incubated at a constant temperature of 25°C. Seeds were either left in the dark or in an alternating regime of 16 h of white light and 8 h of darkness. Each treatment included three replicates of 30 seeds each.

Germination

The seeds were rinsed for 30 min with distilled water, surface-sterilised with 3% potassium permanganate ($KMnO_4$) for 10 min, and mixed with sand (also sterilised with 0.5% $KMnO_4$ at 60% moisture content in polyethylene mesh bags. The seeds were stored for 5 months at 4°C. All seeds were observed and the germination rate recorded every month. The appearance of the radicle was the criterion adopted for the onset of germination (Ribeiro et al., 2011). The percentage was calculated as the number of germinated seeds/viable seeds ×100, as described by Conversa et al., (2010).

Analyses of plant hormones and storage products in fresh, imbibed, germinated and non-germinated seeds

The aim was to determine the dynamics of endogenous hormones and storage substances during germination in seeds with and without GA_3 treatment. Four physiological phases were classified for biochemical analyses: fresh seeds, F (stored at room temperature without imbibition); imbibed seeds, I (stratification about 2 months); non-germinated seeds, NG (undamaged intact seeds after stratification about 5 months without appearance of the radicle); germinated seeds, G (the appearance of the radicle as criterion). To determine the influence of GA_3 on germination, the control seeds, without GA_3 treatment, were soaked in distilled water for 48 h at room temperature. The seeds treated with GA_3 were submerged in 400 mg/L GA_3 for 48 h. The seeds were first removed, by hand, from pericarp. Three replicates of 200 seeds were used for each treatment, with every sample frozen in liquid nitrogen and stored at -80°C prior to use.

(i) Seed hormones
The levels of ABA, GA, ZR and IAA were determined using gas chromatographye-mass spectrometry-selected ion monitoring (GC-MS-SIM) (Chen, 2015).

(ii) Storage substances
Total soluble sugars, starch, protein and amino acids were extracted from mature seeds, randomly selected and quantified using ultraviolet spectrophotometry. Total soluble sugars and starch were measured at a wavelength of 620 nm, protein at 595 nm, and the amino acid content at a wavelength of 340 nm. Three replicates were used for each sample. All data analyses were carried out using the procedure of SAS.

RESULTS

Gibberellin effects on Korean Hackberry seed germination

The effect of GA_3 on germination was clear, resulting in a two-fold increase in the percentage germination (Figure 1). The difference between treatments was discernible after 50 days incubation. Germination of GA_3-treated seeds was significantly higher than the control ($F=45.00$ $P<0.01$) after 50 days incubation. Through 5 months cold stratification, the GA_3-treated seeds germinated to 18.6%, significantly higher than the control ($F=39.20$ $P<0.01$). From days 50 to 80, the increase in the germination percentage after GA_3 application was evident. From days

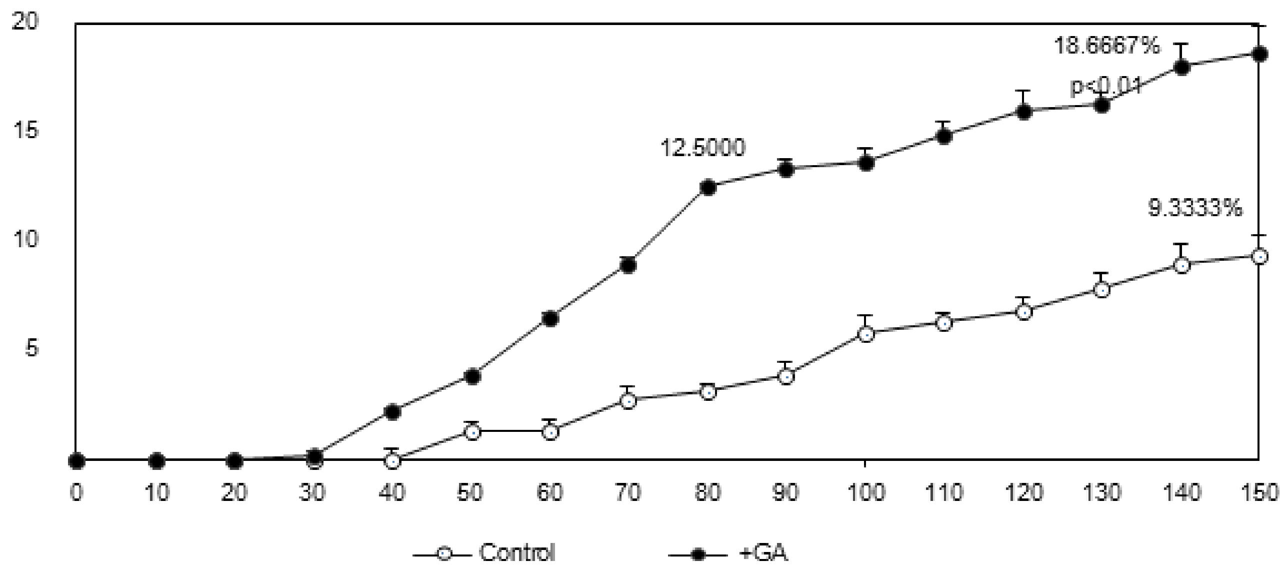

Figure 1. Cumulative germination and final germination percentage of untreated (control) and treated (gibberellic acid - GA3) seeds of Korean Hackberry seeds over 5 months. P-value from one way ANOVA denotes differences between treatments. Different letters means significant difference.

Table 1. Effects of different husk treatments on seeds germination of Korean Hackberry.

Explants	Sample number	Germination percentage			
		Culture time / days			
		5	15	30	60
Endocarp removed	30	2.2±1.90[a]	22.2±5.04[a]	54.43±12.61[a]	70±6.7[a]
Endocarp and testa removed	30	3.3±3.30[a]	18.8±1.96[a]	54.43±1.96[a]	75±5.09[a]
Intact seeds	30	0±00[a]	0±0.00[b]	0 ±0.00[b]	1.1±1.9[b]

Different letters means significant difference (P<0.01) NS no significant.

80 to 150, the effect of GA_3 was not significant (F=10.47; P>0.05). The change in variation of the control was similar to that of the GA_3 treatment.

Seed covering layers

In order to investigate whether a possible interaction exists between physiological embryo dormancy and seed coat (endocarp and testa) dormancy, the Korean Hackberry seeds were physically altered and germinated in MS culture medium. The results showed that there is indeed a difference in the seed germination depending on the treatment of the seed coat. There were no significant differences in any treatments after 5 days cultivation. Seeds without endocarp (N, E and N, E, T) had significantly higher germination rates than the control after 15 days incubation, but there was no difference between N, E and N, E, T seeds. Total germination increased by 70% points in N, E seeds and by 75% points in N, E, T seeds (Table 1).

Changes in endogenous hormone

Through 5 months cold stratification, there were major changes in the hormone levels in the seed embryo. Total ABA content decreased from the F to the G phase in both treatments. Except for the imbibition phases, there were significant differences between control and GA_3 treatments (Figure 2), although the total GA concentrations increased gradually in both. The addition of GA_3 significantly affected the endogenous GA concentration, the level increasing from 3.2ng. g^{-1} FW to 9.1ng. g^{-1} FW (P<0.01; Figure 2). These results indicate that the GA/ABA ratio in the G phase is significantly higher than in any other phase. There were also significant differences in the GA/ABA ratio between the control and the GA_3 treated seeds (P<0.01; Figure 2).

During germination, ZR and IAA were also detected in

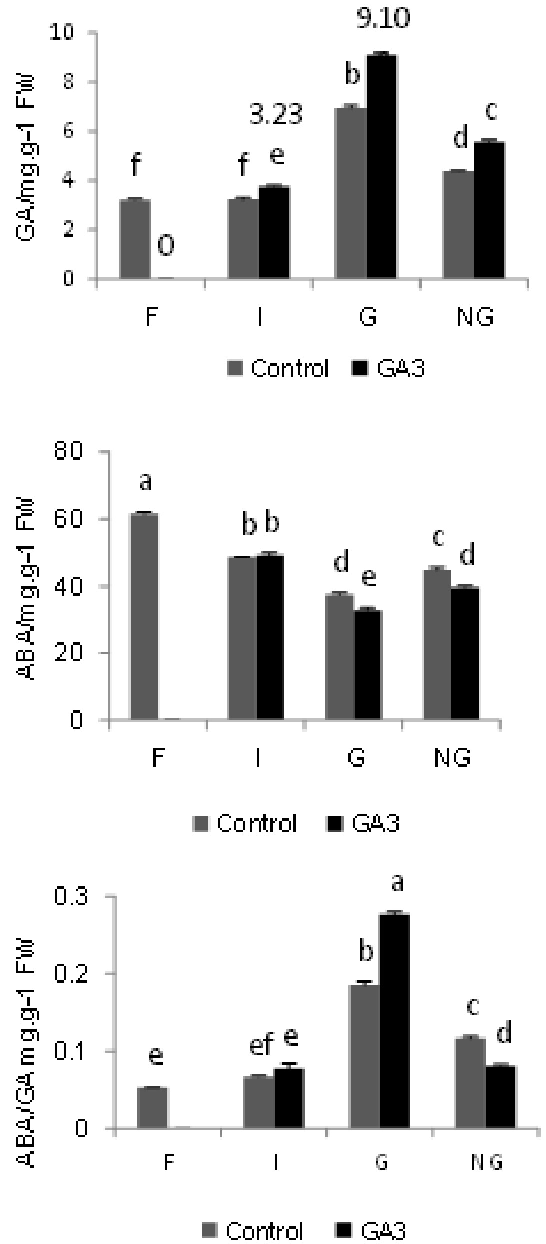

Figure 2. ABA and GA levels and GA/ABA ratio of Korean hackberry seeds. P-value from one way anova denotes differences between treatments and phases. Different letters means significant difference (P<0.01) NS no significant.

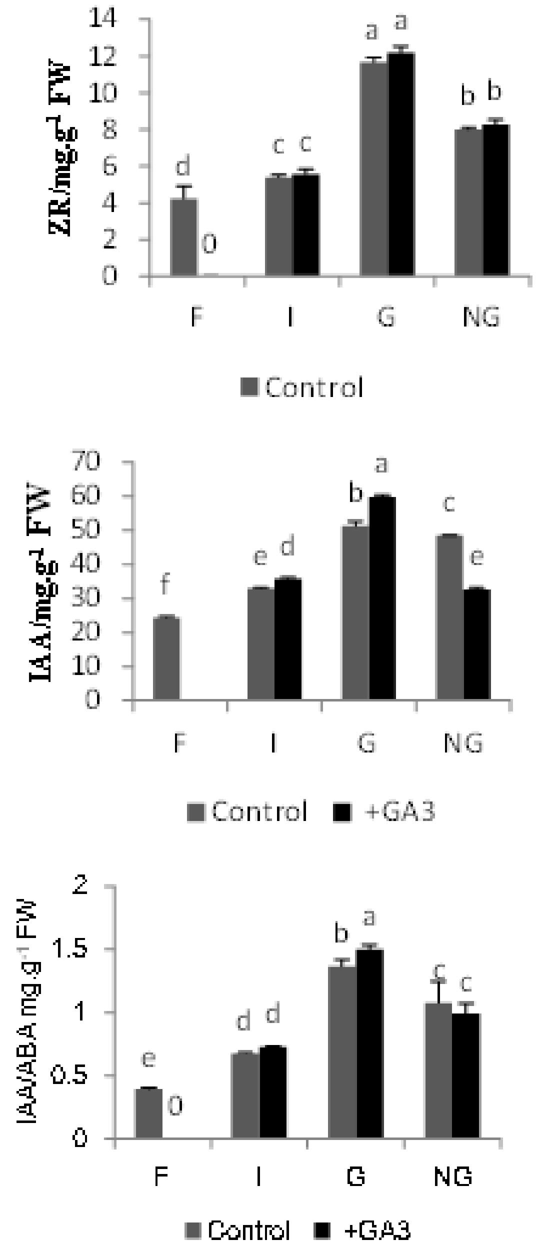

Figure 3. IAA and ZR levels and IAAA/ABA ratio of Korean hackberry seeds. P-value from one way anova denotes differences between treatments and phases. Different letters means significant difference (P<0.01) NS no significant.

the Korean hackberry seeds. The ZR levels increased from F to G in both the control and with GA_3 treatment, but there were no significant differences between them (Figure 3). The IAA concentration was higher in germinated seeds. The seeds treated with GA_3 attained maximum levels of 59.71 $ng.g^{-1}$ FW, and the IAA content differed significantly between the control and GA_3 treatment (P<0.01;Figure 3). Major changes in the IAA/ABA ratio in the Korean hackberry seeds also showed the degree of breaking dormancy, with significant differences from F to G in both treatments. However, the differences between the control and GA_3 treatment were only significant in the G phase.

Changes in storage substances

The concentration of total soluble sugar and starch in

Table 2. Total soluble sugar and total starch of Korean Hackberry seeds in fresh, imbibed, germinated and non-germinated phases.

Treatment	Total soluble sugar		Total starch	
	Control	**+GA_3**	**Control**	**+GA_3**
Fresh seeds	1.4033 ± 0.118^{c}	-	1.2846 ± 0.0355^{c}	-
Imbibed seeds	1.4067 ± 0.021^{c}	1.63 ± 0.089^{c}	1.3859 ± 0.027^{bc}	1.390 ± 0.193^{bc}
Germinated seeds	2.0033 ± 0.102^{b}	2.06 ± 0.05^{b}	1.6657 ± 0.006^{ab}	1.8506 ± 0.1032^{a}
Non-germinated seeds	2.1967 ± 0.015^{b}	2.50 ± 0.08^{a}	1.4070 ± 0.017^{bc}	1.5850 ± 0.0273^{ab}

Different letters means significant difference (P<0.01) NS no significant.

Table 3. Protein and amino acids of Korean hackberry seeds in fresh, imbibed, germinated and non-germinated phases.

Treatment	Protein		amino acids	
	Control	**+GA_3**	**Control**	**+GA_3**
Fresh seeds	13.9796 ± 0.2787^{c}	-	0.453 ± 0.0806^{b}	-
Imbibed seeds	12.0107 ± 0.0317^{c}	13.1092 ± 0.121^{b}	0.219 ± 0.014^{c}	0.223 ± 0.007^{c}
Germinated seeds	8.2561 ± 0.1465^{e}	7.3948 ± 0.29^{f}	1.052 ± 0.214^{a}	1.018 ± 0.139^{a}
Non-germinated seeds	10.0191 ± 0.015^{d}	8.4637 ± 0.186^{e}	0.425 ± 0.339^{b}	0.417 ± 0.044^{b}

Different letters means significant difference (P<0.01) NS no significant.

fresh seeds was 1.40 mg. $(g\ DW)^{-1}$ and 1.28 mg.$(g\ DW)^{-1}$, respectively. The content of sugar increased on germination, and after 5 months cold stratified, there were significant differences in the content of sugar in non-germinated seeds (Table 2; P<0.01). The concentration in GA_3-treated seeds was higher than the control, but not significantly, except in the NG phase. One of the most remarkable things about this data was that the sugar concentration in non-germinated seeds was higher than in germinated seeds, especially on +GA_3 treatment. We found that the content of starch also increased from the F to G phases, with a similar trend and no significant difference between the control and GA_3 treatment (Table 2). For analyses of the protein, the concentration in fresh seeds was highest, with significant differences between the control and GA_3 treatment. The results for the total amino acid content were the reverse, increasing from the F to the G phase. However, the amino acid decreased during imbibition. The values in F, I and NG (control and +GA_3) were similar, being lower than in germinated seeds, and +GA_3 treatment did not significantly affect the concentration of amino acid (Table 3).

DISCUSSION

Widely used to increase germination in certain species, GA_3 treatment has been shown to significantly promote germination in macaw palm seed (Ribeiro et al., 2011), with +GA_3 treatment, seeds reaching maximum germination in four weeks, compared to control seeds at 18 weeks (Bicalho et al., 2015). However, there is no information on the effects of adding exogenous hormones on Korean Hackberry seeds germination. Here we found that Korean Hackberry seeds treated with GA_3 performed better than the control, with a significantly higher germination percentage. These findings support that exogenous GA_3 may be able to help break dormancy in Korean Hackberry seeds, even though GA_3 treatment did not fully promote germination, and the criterion suitable for sowing (>30%) was not reached (Figure 1). Whether a higher concentration of exogenous gibberellic acid may increase germination requires further study. Moreover, environmental cues also strongly influence seed germination. The transition of seeds from dormancy to germination is mainly mediated by the balance between two competing hormones, gibberellins and abscisic acid (Steinbach et al., 1997; Debeaujon and Koornneef, 2000; White et al., 2000; Denise et al., 2014). ABA can inhibit seed germination by affecting the cell cycle and synthesis of cell wall hydrolases (Miransari and Smithc, 2013). The germination rate of switchgrass seeds has been found to be reduced in solutions containing ABA (Duclos et al., 2014), and the embryos of germinated seeds of macaw palm have been found to have lower ABA concentration than in non-germinated seeds (Bicalho et al., 2015). In our research, major differences were found in the ABA concentration in all phases, in line with published research, with a lower ABA concentration detected in G seeds and a decrease of ABA during imbibition (Ali-Rachedi et al., 2004). During the imbibition phase, storage products are mobilized, and the cell wall is loosened by mannanases and mannosidases (Bewley et al., 2013). This impairs ABA concentration and leads to the decrease of ABA during imbibition. In addition, we found the ABA concentration was significantly lower in germinated than non-germinated seeds after 5 months cold stratification with and without the addition of GA_3. As stated by Barreto et al. (2014), the remaining ABA content in non-germinated seeds is related to dormancy maintenance and could protect seeds against biotic stress during storage. Our results confirmed that ABA could inhibit seed germination in Korean Hackberry seeds.

The plant hormone gibberellins are necessary for seed germination. Yamaguchi has showed that gibberellins stimulate the production of α-amylase, resulting in seed germination (Yamaguchi, 2008) and Ribeiro et al. (2014) suggested that GA increase has a central role in germination, with the absence of GA synthesis contributing to dormancy (Ribeiro et al., 2014). In our experiment, the concentration of GA significantly increased in germinated seeds, suggesting that GA does affect germination in Korean Hackberry seeds. In addition the highest concentration was observed in $+GA_3$ treatment, therefore indicating that addition of exogenous hormones could stimulate the production of endogenous hormones.

Recently, it has been showed that control of seed dormancy may depend on the ratio of GA/ABA rather than the individual amounts of these two plant growth regulators. Bicalho et al. (2015) found that germinated seeds had no significant GA increase, but ABA concentration was significantly lower in embryos of germinated seeds compared to non-germinated seeds, resulting in an increased GA/ABA ratio. Chen et al. (2015) also found that the decrease in the ABA/GA ratio during cold stratification for 12 weeks correlated with dormancy break. In this study, the higher GA concentration and lower ABA concentration of germinated seeds clearly resulted in an increased GA/ABA ratio in the control and treated seeds.

ZR and IAA also play a role in seed germination. ZR are active in all stages of germination and may affect the activities of meristematic cells in roots and shoots. Chiwocha et al. (2005) found that increases in cytokinins (CK) concentration were associated with completion of germination and post-germination events. Bicalho et al. (2015) also confirmed that CK and auxins are directly involved in growth events and strongly participate in post-germination development. And the function of CK in cell cycle progression is reflected by changes of the levels of individual CK forms, especially of the most active on, ZR (Dobrev et al., 2002). Here we found that the ZR concentration increased in germinated seeds and was significantly different to other phases. This data suggests that the accumulation of ZR could prove effective in speeding up germination. In addition, GA_3 application increased the content of GA and IAA in germinated seeds, but there was no change in ZR content.

Many plant physiologists state that auxin is not a necessary hormone for seed germination. However, auxin-related genesare expressed in the seed radicle tip during and after seed germination (Miransari and Smithc, 2013). We also found that IAA concentration increased in the G phase, confirming previous research. Liu et al. (2007) has also suggested that IAA could inhibit the ABA pathway through the microRNA60. In this research, the change in concentration of IAA was totally reversed: the IAA/ABA ratio increased in germinated seeds.

In addition to changes in hormones, there are also changes in the storage products. It has been showed that, during cold stratification, the number of protein bodies in fresh seeds is reduced and the proteins cleaved to oligopeptides and free amino acids by active proteolytic enzymes (Chen et al., 2015). In an experiment with *M. domestica* seeds, proteins were hydrolyzed by proteolysis, and there was an increase in the quantity of free amino acids with an increase in seed germinability (Dawidowicz-Grzegorzewska, 1989). Our data indicates that the protein decreased and total free amino acids increased markedly in germinated seeds. However, the free amino acids decreased during imbibition phase: the amino acids were most likely used to synthesize more protein to break dormancy. The ABA concentration also decreased in imbibed seeds, this change probably increases storage product breakdown. The role of protein in Korean Hackberry seeds is not well known, but as stated by Staszak and Pawlowski (2014), when seed dormancy is established, proteins in seeds are involved in carbon metabolism, energy production and antioxidant processes.

The soluble sugars provide energy directly to support early seedling growth. Normally, the total soluble sugar content increases in line with seed germination, and includes fructose, sucrose, glucose and maltose. Horbowicz and Obendorf (1994) found that the levels of fructose, sucrose, glucose and maltose decreased during germination, and may be involved in supply for cell division, but the levels of raffinose and trehalose did not change. In contrast, Chen et al. (2015) found that the total soluble sugars increased in germinated seeds of *A. morrisonense*, but the amount of sucrose significantly decreased after the radicle emerged. In our experiment, after 5 months cold stratification, the sugar content was higher in non-germinated seeds than in the germinated seeds. This is probably because soluble sugar was catabolized when the radicle appeared, acting as the direct source of energy for the germinating seeds. In the seeds of Korean Hackberry, those non-reducing sugars probably could not make up the balance of total soluble sugar concentration. The total amounts of starch increased, but there were no significant differences in fresh, imbibed, non-germinated and germinated seeds (Table 2), giving no obvious correlation between them.

The greatest response relative to the seed coat treatment occurred in the seeds without endocarp (N, E and N, E, E) (Table 1). These results showed that endocarp could be decreasing germination. The effect of seed coat on germination has been studied in other species. Neil and Horgan (1987) have found the bracts of switchgrass can hinder seed germination by interfering with ABA oxidation through oxygen deprivation, and this was confirmed by Duclos et al., (2014). Similar results have been found in barley seeds (Bradford et al., 2008), and the hull of seeds have been found to regulate oxygen availability to the embryo, and also influence the synthesis of and sensitivity to ABA and GA, altering the balance of hormones and their potential action to either promote or retard germination (Finch-Savage and Leubner-Metzger, 2006). In our work, the seeds without any covering layers also had a higher germination rate. The seed endocarp creates a physical barrier to radicle protrusion and acts as a block to inhibit oxygen or moisture (Kucera et al. 2005). Further research is needed to identify whether Korean Hackberry seeds have a seed-coat dormancy mechanism as in yellow cedar.

Transition between dormancy and germination is mediated by hormones, being stimulated by ABA and GA, with the possible involvement of IAA and ZR. To some extent, exogenous hormones can stimulate seed germination rate, and increase the ratio of GA/ABA, even though the mechanism is not clear in Korean Hackberry seeds. In addition, the storage products in Korean Hackberry seeds are affected and may be directly involved in dormancy release. Seeds without any covering layers also germinated better than controls, a result which also implies interaction with plant hormones. Our research increases understanding of the dynamic changes in endogenous hormones, storage products and the influence of seed coat on germination of Korean Hackberry seeds, and can contribute to investigating the dormancy mechanism and development of an effective method to break dormancy.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Motivational factors involved in development of dairy-based innovations

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Agricultural innovations are crucial for attainment of food security and poverty reduction; this fact is universally accepted. Farmers are not only recipients of introduced scientific technologies. It is a well known fact that agricultural innovations can emerge from multiple sources including farmers; in fact, farmers have been innovating long before the emergence of formal research and development. These innovations are commonly known as farmers' innovation or local innovation or grassroots innovation. There are various determinants responsible for farmers led innovation development process. Motivation happened to be an important parameter to make farmers innovators.

Key words: Motivation, innovation-development, dairy-based, process.

INTRODUCTION

Motivation is derived from Latin word 'movere' which means to move. Motivation may be defined as "the attribute that moves us to do or not do something". Motivation refers to the initiation, direction, intensity and persistence of human behavior (Atkinson, 1966). Internal and external factors that stimulate desire and energy in people to be continually interested and committed to a job, role or subject, or to make an effort to attain a goal is known as motivation (Business Dictionary). Motivation is of two types; intrinsic motivation, which refers to doing something because it is inherently interesting or enjoyable and extrinsic motivation, which refers to doing something because it leads to a separable outcome (Ryan and Deci, 2000).

Amabile (1983) stated that intrinsic motivation is encouraging to creativity, but extrinsic motivation is detrimental. Winston and Baker (1985) have done a review of more than 20 behaviorist studies, and finally concluded that there was compelling evidence that reward can be used to enhance divergent thought. Mumford (2003) assumed creativity to be strongly affected by interest in tasks for their own sake ("intrinsic motivation" or "intrinsic task interest").

'Innovations only develop in the laboratories', is a myth which prevails among a large section of our society. However the fact is that people also develop some innovations outside the limits of the laboratories (Biggs and Clay, 1981; Biggs, 1990; Reij and Waters-Bayer, 2001). Whenever farmers face a typical problem, they try to find solutions. And sometimes these solutions turn out

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to be innovations in making. Without motive, we never do anything; motive may be varying from individual to individual or situation to situation, even these may vary from time-to-time within the same individual. Motives have been linked with the human action and have been the subject of investigation by researchers (McClelland, 1961). There are a number of factors responsible for motivating farmers to develop grassroots innovations. Increasing climate variability affects livestock production systems in all parts of the world, and will inevitably impact the resource poor families whose livelihoods are wholly or partially dependent on livestock (Thornton, 2013) which may induce farmers to innovate in order to adopt. The scarcity of resources and availability of costly inputs in the market vis-à-vis dairying could also lead to the development of input-saving innovations. A grassroots innovator may innovate out of curiosity, serendipity, peer pressure or interest in increasing production or solving problems (Millar, 1994; Nielsen, 2001; Leitgeb, 2014). Therefore, this study was conducted to find out the motivational factors affecting the selected dairy-based innovators vis-à-vis innovation-development.

METHODOLOGY

In view of the nature as well as significance of the study, the "Case Study" method was adopted as the method of exploration. The cases were selected, purposively, on the basis of data-base compiled and documented by National Innovation Foundation (NIF), Ahmedabad. Other sources besides NIF were also used, viz. NGOs, Progressive Dairy Farmers Associations (PDFA) of states like Haryana and Punjab, personal interactions with Scientists and KVK staff working at the grass roots, etc. Nine such innovations were selected for this study, which were having relevance in the field of dairying. Data was collected with the help of motivational scale, which was developed for this particular study and the cases were analyzed for identifying the motivational factors involved in development of dairy based innovations.

RESULTS AND DISCUSSION

Without motivational drive, we never initiate anything. A number of factors are involved in the development of an innovation. Motivation is one of the prime factors behind development of an innovation. But motivational factors vary from innovator to innovator because of personal and contextual factors. For starting any creative work, an individual needs strong intrinsic motivation and self-determination drive; because, in creative thinking, there is always high probability of failure. In this study, motivational factors involved in innovation development have been identified and prioritized; and these have been given subsequently.

Under this study, seventeen motivational factors were identified. From the Table 1, it is quite evident that "problem" happened to be the prime motivation factor in development of innovation, followed by "challenging task" and "reducing the drudgery" respectively. Subsequently, "mental peace", "inability to afford available technologies" and "satisfaction" were found to occupy fourth, fifth and sixth rank, respectively. The moderately contributing motivational factors were "necessity", "hope of getting something novel", "attainment of self-fulfillment", "profit", "recognition", "ideas conceived after observing something new" which were occupying seventh, eighth, ninth, tenth, eleventh and twelfth rank, respectively. The least contributing motivational factors were "calculated risk taking behavior", "praise", "feel-good factor", "ensuring livelihood security" and "impressing others" which occupied thirteenth, fourteenth, fifteenth, sixteenth and seventeenth rank, respectively.

Motivational/reinforcement factors in innovation development process

Under this study, the nine cases of innovation development processes were analyzed with the help of longitudinal analysis of the respective processes. It was found that there were some motivational factors that played crucial role in the particular stages of innovation development process, which have been summarized as follows.

In Table 2 also it was quite evident that in all nine cases, the triggering-point of innovation development by the grass-root level innovators happened to be problems and/or challenging task. That indicated that the 'problem' and 'challenging task' were the prime motivating factors for innovation development at the grass-root level. In fact, all aforementioned factors are the cause of intrinsic motivation. From this study, it becomes clear that intrinsic motivation is directly responsible at the start of the innovation development process. Amabile (1983) claimed that people create something because of the intrinsic satisfaction they get from the very process of creation. These findings are in line with the findings of other related studies (Millar, 1993; Nielsen, 2001; Kummer, 2011). Eisenberger and Shanock (2003) delineated the importance of self-determination and intrinsic motivation for creativity. Mumford (2003) also stated creativity to be strongly affected by interest in tasks for their own sake ("intrinsic motivation" or "intrinsic task interest"). These three studies further strengthen the findings of this study. In Table 1 it was also found that "inability to afford available technologies", "satisfaction", "necessity", "hope of getting something novel", "attainment of self-fulfillment", "profit", "recognition", "ideas conceived after observing something new" which were occupying the ranks of fifth, sixth, seventh, eighth, ninth, tenth, eleventh and twelfth respectively. In Table 2 also, it was found that recognitions, award and profit were the important motivational factors responsible for further development and commercialization of technologies. Eisenberger and Shanock (2003) mentioned that careers of outstanding scientists and mathematicians suggest that anticipated rewards often increase creativity. Eisenberger et al. (1999) carried out research with college students

Table 1. Prioritization of Motivational factors involved in development of dairy-based innovations (n = 9).

S/N	Items	Weighted Mean value	Rank
1	Profit is the main motto behind development of an innovation	2.22	X
2	Inability to afford purchase of scientific technologies	2.89	V
3	Feel-good factor is the source of motivation behind development of an innovation	1.78	XIV
4	Satisfaction is the source of motivation behind development of an innovation	2.78	VI
5	Necessity is the source of motivation behind development of an innovation	2.67	VII
6	Reducing drudgery is the source of motivation behind development of an innovation	3.33	III
7	Ideas conceived after observing something new lead to development of an innovation	1.89	XII
8	Welfare of the society is the source of motivation behind development of an innovation	1.33	XV
9	Finding something novel is the source of motivation behind development of an innovation	2.56	VIII
10	Mental peace is the source of motivation behind development of an innovation	3.22	IV
11	Impressing others is the source of motivation behind development of an innovation	0.78	XVII
12	Ensuring livelihood security is the source of motivation behind development of an innovation	0.89	XVI
13	Solving the existing problem (s) is the source of motivation behind development of an innovation	3.89	I
14	Feeling of accomplishment after doing new and challenging task(s).	3.67	II
15	Calculated risk taking behavior of innovators	1.89	XIII
16	Attainment of self-fulfillment with my work	2.44	IX
17	Feeling of happiness and satisfaction when people recognize on account of the innovation developed	2.11	XI

Table 2. Motivational/reinforcement factors involved in ‘innovation development process’.

Case No.	Name of Innovation	Motivational/reinforcement factors in Innovation Development Process
I	Hand-Operated Milking Machine (developed by Mr. Raghav Gowda)	At the initially stage, it was intrinsic motivation that was the major drive on account of problems like milking problem, scarcity of labor, available milking machine being costly/expensive etc. Then, he got a solution, but that had a lot of shortcomings. In between he got publicity through local media. However, he was in dilemma, because his machine was not working properly. Then, he modified his machine 15 times. Here, the main motivating factor was the challenge which he got through publicity albeit, later on, the publicity itself acted as a motivational factor. Further he developed two more machines and started commercialization also. Here the main motivational reinforcement factors were NIF Award, a “certificate” given by University of Agricultural Sciences, Dharwad, during the Krishi Mela, recognitions/awards by private agencies, etc.
II	Advance Technology of Drinking Water for Dairy Animal (developed by Mr. Divakaran Nambiar)	Initially he faced the problems of drudgery, scarcity of labour and lack of continuous supply of drinking water for cows and unaffordable cost of water trough available in the market. These things developed ignition inside him for developing the innovation. Then, he got some recognition from Milma dairy cooperative and the state government, which motivated him, further.
III	Animal Lifting Machine (developed by Mr. J.R. Dhanraj)	Death of his cow which had developed a pain acted as the ‘triggering point’-cum-motivational drive for development of his innovation.
IV	Mixed Forestry [developed by Mr. Jagat Singh (*Jangali*)]	Once, he realized the pain & hardship faced by his mother and wife, he started thinking about the ways through which the drudgery of the women of village could be mitigated. Accordingly, he proceeded in that direction; and finally he was able to something for them (women) through his “Mixed Forestry”. Later on, he got recognitions/awards and huge publicity, which further motivated him for multiplication of his model in other places.

Table 2. Contd.

V	Azolla as a 'Bio-feed' (developed by Dr. P. Kamalasanan Pillai)	The question raised by the principal of the school about the use(s) of azolla other than as a 'Bio-fertilizer'. The question worked as challenge. He got funding from NDDB and DBT. He also got many recognitions and awards which motivated him for popularization of his innovation.
VI	Multi-purpose Processing Machine (developed by Mr. Dharam Bir)	When he started the processing of 'Aloe Vera' and 'Rose Waters' extraction, he needed machines for it but could not afford them, as the ones available in the market were very costly. Hence, he took this as a 'challenge' for him to develop something like those machines, but at cheaper rate. Then, he got a lot of recognition that acted as motivational drive for him vis-à-vis improvement and commercialization of the product/machine developed by him.
VII	Calf-Cage (developed by Mr. Jagdeep Singh)	When he faced the problems of calves mortality, improper feeding of calves and disease detection among the group of calves then he motivated to develop some special management of calves, resulting, he developed calf cage.
VIII	Forage Harvester (developed by Mr. Gurtej Singh Chaany)	He was motivated by the challenging task assigned to him by the dairy farmers for development of a machine which could perform cutting, chaffing and loading of forage at a time. For getting more benefit, he commercialized his machine.
IX	Milking Parlor (developed by Mr. Arvinder Singh)	There was a lot of difficulties in milking of so many cows. That motivated him for finding an alternative solution. He has developed own low-cost milking parlour, in order to help as many dairy farmers/entrepreneurs as possible.

indicating that a reward contingency requiring a high level of performance increased perceived self-determination and perceived competence, both of which enhanced intrinsic task interest. Moreover, in field studies, employees' expectation of financial rewards for high job performance was associated with perceived self-determination that, in turn, was related to heightened intrinsic interest in daily job activities. The positive relationship between reward expectancy and intrinsic task interest was greater among employees with a strong desire for control, indicating the importance of rewards as an indicator of self-determination. Winston and Baker (1985) have done a review of more than 20 behaviorist studies and concluded that there was compelling evidence that reward can be used to enhance divergent thought. These studies further enhanced the findings of this study.

Conclusion

The results of this study reveal that motivational factors varied from individual to individual. But, a few things were common: Intrinsic motivational factors are key in the beginning of innovation development process, followed by recognition, reward, and profit (extrinsic motivational factors), which play key role in further reinforcement of innovation development as well as development and commercialization of innovation.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Genetic divergence of colored cotton based on inter-simple sequence repeat (ISSR) markers

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The management of colored cotton is an agricultural activity widely adopted by farmers located at Brazilian semiarid region. The fiber colors currently available are still limited to green and shades of brown, however, there is possibility to broaden the variability for this trait by using accessions from *Gossypium* Brazilian bank in breeding programs. Therefore, it is necessary to know the genetic diversity of available accessions in the collection. Here, the genetic divergence in colored fiber accessions was estimated in order to identify promising candidates for further use in hybridization procedures of cotton improvement. DNA of twelve accessions were extracted from leaves and used in inter simple sequence repeat-polymerase chain reaction (ISSR-PCR) assays, using commercial oligonucleotides. The genetic divergence was estimated by clustering-unweighted pair group method with arithmetic mean (UPGMA) method. Five groups were clustered among them, three were contributive results for further use in hybridization procedures, including Brazilian cultivars and Peruvian accessions. Based on level of divergence, we suggest that lines generated from these materials could generate news shades of fiber colors in further use for selection procedures in cotton breeding.

Key words: *Gossypium*, molecular marker, variability, genetic improvement.

INTRODUCTION

Plant genetic resources represent a valorous portion of the biological diversity and contribute towards achieving security and sustainable development from preservation of cultivars, landraces, and wild relatives of important

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plant species. Germplasm banks are reservoirs with an important hole to preserve these resources for further use in both applied and basic researches. The primary importance of these banks is that they carry undefined variation that proves to be a valuable resource for breeders to develop new crop cultivars (Sachs, 2009). Maintenance of germplasm banks have generally occurred in regions and by nations associated with crop production and commerce.

Cotton (*Gossypium hirsutum* L.) represents the most important natural fiber in the world. The genetic resources of *Gossypium* are extensive, dispersed globally across five continents, and consist of approximately 45 diploid (2n=2x=26) and five allotetraploid (2n=4x=52) species (Harlan and Wet, 1971; Fryxell, 1992; Stewart, 1994; Brubaker et al., 1999). Two allotetraploid species, *G. hirsutum* L. (upland cotton) and *Gossypium barbadense* L. (Pima, Sea Island or Egyptian cotton), account for the majority of cotton world production, although the former is widely grown worldwide (>90% of the total area) due to fiber yield and broad adaptation to several environments (Campbell et al., 2010; Percy et al., 2014). The quality of fibers from *G. barbadense* L. is better than *G. hirsutum* L., however the transference of fiber traits into upland genotypes provides limited success due to hybrid breakdown and segregation toward either parents (Gore et al., 2012; Gore et al., 2014).

The major cotton collections are located in United States, Russia, Uzbekistan, China, India, Brazil, Australia, and France (Campbell et al., 2010; Percy et al., 2014). The National Cotton Germplasm Collection (NCGC) has nearly 10,000 accessions of *Gossypium* accessible at the website www.ars-grin.gov. Each one has a single plant introduction number (PI) at the time the accession enters the collection. The first major breeding effort to incorporate the development and maintenance of a cotton germplasm collection was implemented in Trinidad in 1926 by the Empire Cotton Growing Corporation (Frelichowski and Percy, 2015).

The Brazilian collection is maintained by the Brazilian Agricultural Research Corporation (Embrapa) at the National Center for Genetic Resources and Biotechnology and currently has more than 3,000 accessions, several of them used for cotton breeding program to Savanna (Cerrado) and semiarid regions.

White fibers are desirable for most Brazilian textile industries, because they provide a uniform substrate for dyeing and finishing. Onto new market trends, other niches have emerged, such as the naturally colored fibers, that required no or less dying in the textile processing, reducing the pollution to the environment due to minor residual chemical toxicant (Xiao et al., 2007; Yuan et al., 2012; Feng et al., 2013). This technology is a differentiated product and therefore with higher value-added, representing an alternative model of innovation, to promote social and sustainable transformations (Cavalcanti, 2012).

Colored fibers appear as brown or green during the fiber development process. Generally, the resistance, length and fiber percent are lower in colored than in white accessions due to the pleiotropic effects of fiber color genes (Carvalho et al., 2011; Lacape et al., 2005), but Brazilian breeders have made efforts in order to improve this trait via genetic improvement. According to Kohel (1985), several mutants conditioning fiber color and quality traits were described and mapped. Among the many fiber color variants described, almost all have had a dominant expression over the white fiber color of commercial cottons.

The Brazilian Company of Agricultural Research (Embrapa Cotton) coordinates a robust program to colored cotton, involving improvement to yield, fiber quality and environmental adaptation. Currently, six cultivars are commercially available and others top lines are in progress (Carvalho et al., 2011). Periodically, new different accessions are introduced and evaluated in selection procedures, aiming to identify promising materials to assist the colored fiber breeding. In this work the genetic divergence of new lines of cultivated and wild *Gossypium* accessions were estimated based on polymerase chain reaction-inter simple sequence repeat (PCR-ISSR) molecular markers.

MATERIALS AND METHODS

Genetic resources and ISSR-PCR assays

Seeds of twelve cotton accessions, including wild and commercial cultivars, were used in this work. The genealogy and origin of materials are found in Table 1. DNA from seeds were extracted (Dellaporta et al., 1983) and further used in PCR assays. Twelve ISSR oligonucleotides, from University of British Columbia, were used in reactions (Table 2).

The PCR assays were performed in a 0.2-ml reaction tube with total volume of 25 µl containing 20 ng of template DNA, 1 µl each of ISSR oligonucleotide (10 µM), 0.5 µl dNTP mix (10 mM), 1.4 µl $MgCl_2$ (25 mM), 1× PCR assay buffer, and 1 U Taq DNA polymerase (Fermentas). PCR amplifications were performed in Amplitherm Thermal Cyclers, with initial denaturation at 96°C/5 min followed by 30 cycles of denaturation at 96°C/45 s, annealing at 40°C/45 s, and extension at 72°C/1 min. A final extension step was added at 72°C/5 min. Amplicons were separated by agarose gel (1.5%) and photodocumented. All reactions were carried out in triplicate.

Genetic analysis of cotton accessions

Amplification products were scored as presence (1) or absence (0) of the band, for each accession. A binary data matrix was

Table 1. Genealogy and origin of cotton accessions used in ISSR-PCR assays.

Accession	Species	Genealogy	GB/Origin	Fiber color
PI 608.352	*G. barbadense*	*Wild/comensal*	Peru/GRIN	Orange brown
BRS Topázio	*G. hirsutum*	Cultivar	Paraíba, Brazil	Tan
BRS 336	*G. hirsutum*	Cultivar	Goiás, Brazil	White
BRS 200	*G. hirsutum*	Cultivar	Paraíba, Brazil	Brown
PI 435.250	*G. barbadens*	*Wild/comensal*	Peru/GRIN	Dark brown
PI 435.259	*G. barbadense*	*Wild/comensal*	Peru/GRIN	Purple brown
PI 528.086	*G. barbadense*	*Wild/comensal*	Peru/GRIN	Yellowish brown
BRS Verde	*G. hirsutum*	Cultivar	Paraíba, Brazil	Green
BRS Rubi	*G. hirsutum*	Cultivar	Paraíba, Brazil	Reddish brown
BRS 286	*G. hirsutum*	Cultivar	Goiás, Brazil	White
MO	*G. barbadense*	*Wild/commensal*	Peru/GRIN	Dark brown
V3	*G. hirsutum*	*Land race*	Paraíba, Brazil	White

GB: Germplasm bank; CENARGEN: Embrapa Genetic Resources and Biotechnology; GRIN: Germplasm Resources Information Network, EUA.

Table 2. Sequence of ISSR oligonucleotides used in genetic analysis of colored cotton fiber.

Oligonucleotide	Sequence (5'→ 3')	TNB	NBP	Polymorphism rate (%)
UBC 812	GAGAGAGAGAGAGAGAA	5	3	60
UBC 813	CTCTCTCTCTCTCTCTT	10	4	40
UBC 820	GTGTGTGTGTGTGTGTC	8	1	12
UBC 824	TCTCTCTCTCTCTCTCG	9	6	67
UBC 827	ACACACACACACACACG	13	7	54
UBC 834	AGAGAGAGAGAGAGAGYT	9	4	44
UBC 853	TCTCTCTCTCTCTCTCRT	7	5	71
UBC 866	CTCCTCCTCCTCCTCCTC	11	8	73
UBC 868	GAAGAAGAAGAAGAAGAA	9	4	44
UBC 872	GATAGATAGATAGATA	9	6	67
UBC 884	HBHAGAGAGAGAGAGAG	14	2	14
UBC 892	TAGATCTGATATCTGAATTCCC	9	5	56
Total	-	106	50	-

TNB: Total number of bands; NBP: number of polymorphic bands.

generated, from which it was calculated genetic similarity index between all individuals compared two by two, using the index agreement Jaccard (Sneath and Sokal, 1973).
The similarities (*Sji*) were calculated, according to the expression:

$$S_{ji} = \frac{a}{(a + b + c)}$$

where *a* means the presence of bands on both accessions; *b*, presence of band in first accession and absence in second and *c* is the presence in second and absence in the former.

Clustering was done using symmetric matrix of similarity coefficient. A dendrogram based on *Sij* values was constructed using clustering technique of unweighted pair group method with arithmetic mean (UPGMA). In order to eliminate the non-hierarchical effects, the cophenetic correlation coefficient was estimated (Sneath and Sokal, 1973). Analysis was performed using the software GENES, version 2013.5.1 (Cruz, 2013).

RESULTS AND DISCUSSION

The ISSR oligonucleotides used for genetic analysis were contributive to identify divergent groups in cotton accessions. An average of 9 bands/oligo was obtained, with polymorphism rate varying from 75 to 12% (Table 2).

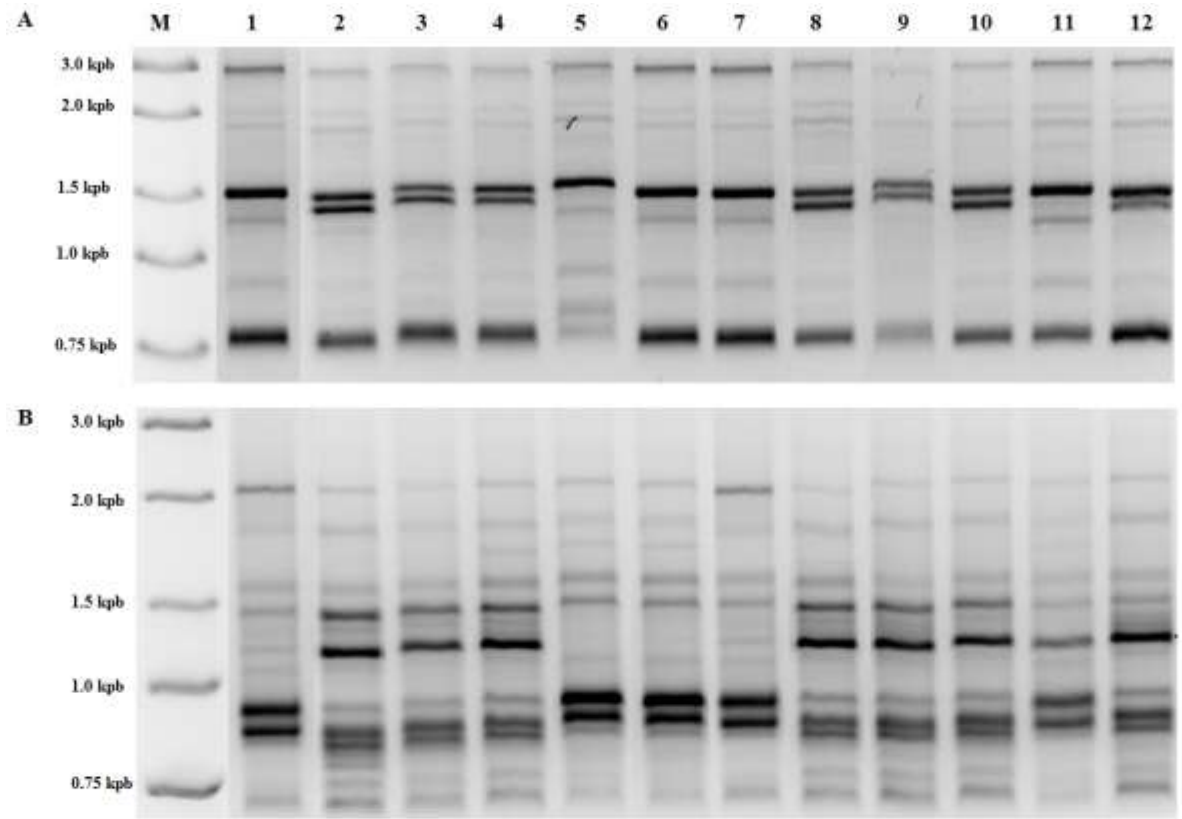

Figure 1. Band pattern obtained with oligonucleotides UBC 866 (A) and UBC 853 (B). M - marker 1 Kb (Ludwig Biotec); Accessions: 1. PI 608.352, 2. BRS Topázio, 3. BRS 336, 4. BRS 200, 5. PI 435.250, 6. PI 435.259, 7. PI 528.086, 8. BRS Verde, 9. BRS Rubi, 10. BRS 286, 11. MO, 12. V3.

UBC 866 and UBC 853, both rich in CT repetitions, were highly polymorphics, with rate of 73 and 71%, respectively. The pattern of bands obtained with these oligos is found in Figure 1.

Amplicons generated by ISSR-PCR assays were used to estimate the genetic divergence of cotton accessions by UPGMA method. A detail of fiber colors is found in Figure 2. Five groups were clustered (Figure 3), showing the following composition: Group A- compounded by five *G. hirsutum* L. accessions: BRS Topázio, BRS Verde and BRS Rubi, all colored fibers, and BRS 286 and V3, both white fiber. The peculiarity of this group is that all accessions are mid-cycle (140 to 160 days) and widely adapted to Brazilian Northeast region. V3 is a land race in pre-breeding proceeding and BRS 286 is full-sib of BRS Rubi. Both have the same parent, the drought tolerant CNPA 7H, developed by Embrapa to semiarid environments (Pedrosa et al., 2009; Carvalho et al., 2011).

About the other groups, the most relevant results were seen in B and D, both clustered wild *G. barbadense* accessions, from Peru, with fiber shades varying from cream to brown (Table 1 and Figure 3). Group C contained cvs. BRS 336 (white) and BRS 200 (brown), both *G. hirsutum,* with excellent fiber length. The last group contained only one *G. barbadense* accession, which remained distant from other Peruvians genotypes, because it is a land race with wild phenotype.

In overall, the use of accessions from groups A, B and D could be contributive to broaden the genetic basis of new lines of colored fibers. The white fiber accessions could contribute to improve the fiber qualities, providing genetic gains in selection procedures, while BRS Topázio and BRS Rubi, two Brazilian colored fibers of high yield and satisfactory fiber traits, could contribute to minimizing the deleterious effects often resulting from interspecific *Gossypium* crossings (Carvalho et al., 2011).

Based on results, there is a possibility to obtain new shades by using Peruvians accessions PI 608.352 (1), PI 435.259 (6) and PI 435.250 (5), with BRS 286 (10). For green shades, crossings between BRS Verde (8) and BRS 336 (3) is recommended. According to Morello et al.

Figure 2. Detail of fiber color of accessions: 1. PI 608.352, 2. BRS Topázio, 3. BRS 336, 4. BRS 200, 5. PI 435.250, 6. PI 435.259, 7. PI 528.086, 8. BRS Verde, 9. BRS Rubi, 10. BRS 286, 11. MO, 12. V3.

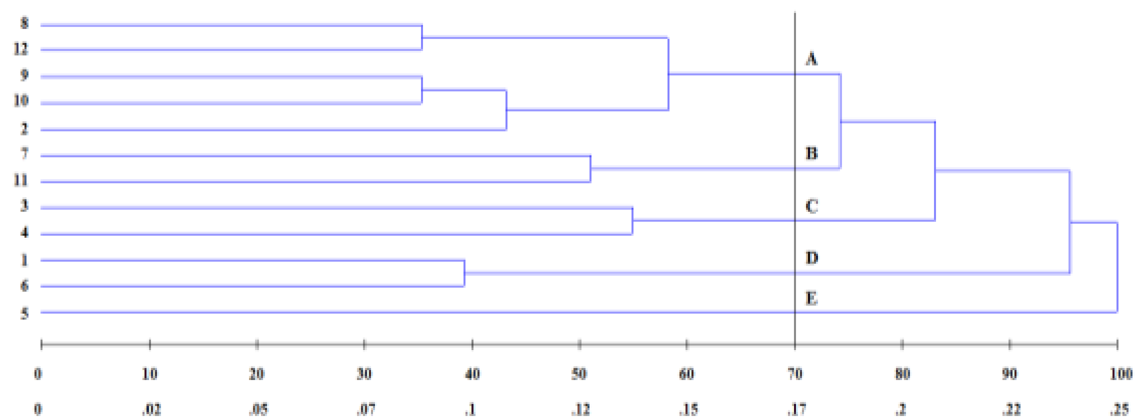

Figure 3. Dendrogram obtained by hierarchical clustering method UPGMA, from the dissimilarity matrix of 12 cotton genotypes. Cophenetic correlation coefficient: 0.80. Dotted line represents adopted selection screen based on the 70% similarity index. Access: 1. PI 608.352, 2. BRS Topázio, 3. BRS 336, 4. BRS 200, 5. PI 435.250, 6. PI 435.259, 7. PI 528.086, 8. BRS Verde, 9. BRS Rubi, 10. BRS 286, 11. MO, 12. V3.

(2012), this last cultivar has broad adaptability, high yield and excellent fiber quality.

Conclusion

Groups formed with cotton accessions offer opportunity to generate new colored lines, by using crossing works, with high yield and fiber quality for further use in selection procedures in breeding program.

Conflict of interests

The authors have not declared any conflict of interests.

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