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Full Length Research Paper

Agricultural characteristics and evaluation locations of sorghum plots with different eucalyptus arrangements

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The aim of the experiment was evaluate agronomic characteristics of grain sorghum plots, collected at different sites within four spatial arrangements of eucalyptus for the forest crop livestock integration system (iLPF). The work was implemented in the second crop in the agricultural year 2011-2012, after the soybean harvest in Uberlândia, Minas Gerais. For this, they were planted in areas adjacent seedlings of eucalyptus clone I 144 simple rows: 10 × 2 m; double rows: (2 × 3) + 15 m, (2 × 3) + 20 m in triple rows (3 × 2 × 3) + 20 m. At six months after planting clone, seeded through conventional tillage to cultivate sorghum 1G220 in the eucalyptus rows. It used the experimental randomized block design in split plot with five replications. The plots consisted of four spatial arrangements of eucalyptus and the subplots 3 sorghum assessment of sites within the plots (center, right side and left side of eucalyptus). The site assessment of the plots affect**assessment of the plots site affects the main agronomic characteristics of sorghum. In future experiments it is necessary that sorghum portions are formed by central and lateral lines within the wider spacing eucalyptus. The spatial arrangement (2 ×x 3) + 20 m and 10 ×x 2 m are more promising for the consortium . The definition of best arrangement will depend on the market for the sale of wood.**

Key words: Agroforestry system, sustainability, consortium.

INTRODUCTION

The integration of Crop-Livestock-Forest (iLPF) is an alternative to recover degraded areas, which are found in all regions of Brazil, including the cerrado. Thus, it is necessary to know how to implant this system to improve production of involved agricultural species. Intercropping has emerged as an important system of land recovery since it increases the efficiency of land use, diversifies production and optimizes use of natural resources such as soil, water, temperature and radiation (Oliveira et al., 2013). Therefore, the choice of species that make up the integration system is directly related to the viability of this system (Bravin and Oliveira, 2014).

Cereals most frequently used for the implementation of this system are corn, sorghum, millet, beans, soybeans,

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rice and sunflower. In Brazil, there are successful experiences with various shaded crops. However, further studies of some crops and areas of interest are necessary. Additionally, the generated technologies should be tested in production areas and not only in experimental areas (Oliveira et al., 2011).

According to Alvarenga et al. (2010), crops of corn and sorghum, both for the production of grain and silage, stand out within the iLPF due to the potential they offer regardless the size of the property. Cereal crops, forage and eucalyptus are consortium in Brazil, but this system is rarely used in other countries (Borghi et al., 2013).

Currently, there is a high demand for more sustainable production models, such as the agroforestry systems (Cubbage et al., 2012). These systems are important to enhance soil fertility (Jackson and Ash, 1998; Pinho et al., 2012), increase the farm economical return (Dube` et al., 2002; Souza et al., 2007; Prasad et al., 2010; Pacheco et al., 2013), and sequester the carbon that can be stored in the tree biomass and in the soil (Montagnini and Nair, 2004; Haile et al., 2010; Howlett et al., 2011; Tonucci et al., 2011), among others. Therefore, the agroforestry systems and techniques such as no-tillage were included in the Brazilian government financing program called low carbon agriculture to enable the recovery of degradedBrazil has large stretches of degraded land, mainly occupied by pasture (Paula et al., 2013).

Sorghum is the fifth most produced cereal in the world. The nutritional value is similar to corn, and has the advantage of the absence of gluten. It is recommended for iLPF systems because of its competitiveness when associated with other crops, as forage and tree species.

The sorghum (*Sorghum bicolor* (L.) Moench) has long been used because it is easily intercropped, offers good productivity and adapts to drought conditions (Oliveira, 2011). However, it is necessary to know the best spatial arrangements of trees to obtain the best production output.

In integrated systems, the adaptation of forage species and agronomic crops in consortium with trees must be well known, especially because of the microclimate conditions and competition among plant components for the available natural resources. Some studies show increased canopy growth under shade (Soares et al., 2009).

There are numerous studies that show the influence of solar radiation on grasses grown in agroforestry systems (Macedo et al., 2006; Oliveira et al., 2009; Almeida et al., 2014), however few studies have been made regarding the sorghum grain crop (Albuquerque et al., 2013). Sorghum could be an alternative in consortium systems, especially with tree species (Dan et al., 2010).

The objective of this study was to observe the effect of different spatial arrangements of eucalyptus on the main agronomic characteristics of grain sorghum in iLPF system in Uberlândia, MG.

MATERIALS AND METHODS

Location and description of the experimental area

The experiments were established in November 2011 with the planting of eucalyptus and soybean, and in March 2012, after harvest of soybean, with the planting of sorghum in the experimental area in the city of Uberlândia, MG, located at the coordinates of 18º50'S and 48º14'W at an altitude of 785 m.

The climate according to Köppen classification is an Aw type, characterized as rainy tropical, megathermal, typical for savannah, with dry winter, with average rainfall of 1550 mm and annual average temperatures of 23.1°C.

Variations for ten-day periods, during the conduct of experiments involving the sorghum crop, are shown in Figure 1. The soil was classified as Dystrophic Red Latosol of medium texture. The chemical attributes, before planting of sorghum are shown in Table 1, EMBRAPA (2009).

Plant component

The clone of Eucalyptus used in this experiment was I 144 hybrid, a cross between *Eucalyptus grandis* x *Eucalyptus urophilla*. It is widely commercialized in the region; its wood is widely used for energy production, for pulp and paper industry, and for the production of poles and stakes (Terra Forte Florestal, 2014).

Regarding sorghum, the 1G220 hybrid was used, from Dow AgroSciences, which has early cycle, low height and half-open panicle.

Evaluated spatial arrangements

The effect of spatial arrangements of the eucalyptus agrosilvopastoral system was studied with single line: 10 × 2 m; double lines: (2 × 3) + 15 m, (2 × 3) + 20 m and with triple lines (3 × 2 × 3) + 20 m. The evaluations of sorghum characteristics took place between the lines of trees, where spacing is as greater as described in the followingin Figure 2.

Table 2 shows the arithmetic mean of eucalyptus clone height and number of trees per hectare in different arrangements after harvest sorghum.

Deployment and conducting of the experiment

Liming took place in August 2011 with subsequent plowing in order to incorporate and increase the base saturation to 70%. For this, 4.6 t ha^{-1} of dolomitic limestone was used. In November harrowing was carried out in order to prepare the soil for planting eucalyptus in different spatial arrangements. The planting of eucalyptus was made according to the terraces in the area. Thus, the lines were placed in north-south direction. Magnesium thermophosphate (120 kg ha^{-1} P_2O_5) was applied into the furrows during planting of eucalyptus seedlings. Top-dressing fertilization of Eucalyptus took place 45 days after planting via lateral furrows with 240 g of a 08-28-16 fertilizer, along with 3 g of Borax per plant. The eucalyptus fertilization followed the recommendations of the 5th approach (Ribeiro et al., 1999). Mechanized sowing of the 1G220 sorghum took place in March 2012. For this a vacuum planter was used, set with 0.50 m spaces. The density of 140 thousand plants ha^{-1} was used for sorghum. To evaluate sorghum, there were randomized blocks in split plots with five replications. The experimental plot consisted of four spatial arrangements of eucalyptus and the subplots of three locals of assessment (center, right and left side of eucalyptus) of the agronomic characteristics of sorghum. To delimit the useful area of each spatial arrangement, two central

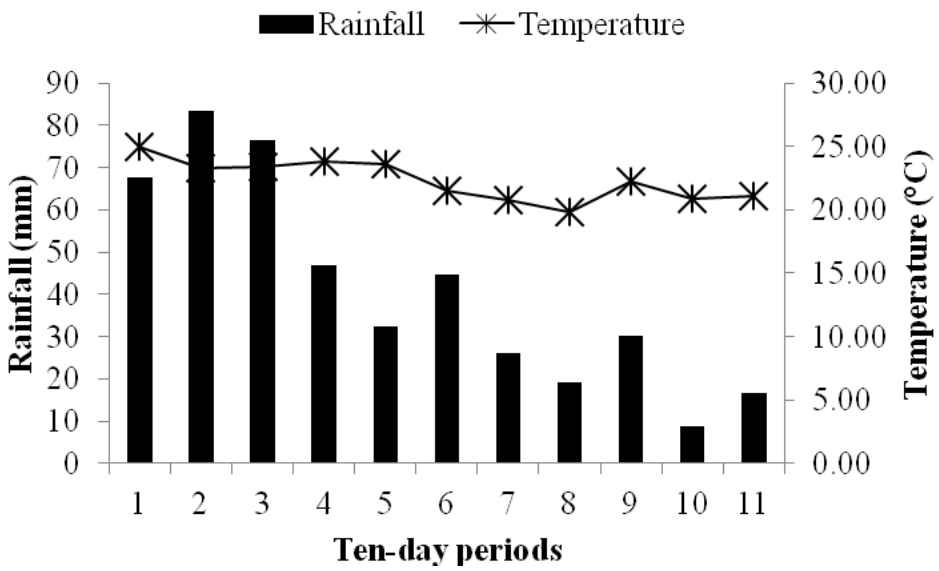

Figure 1. Temperature and rainfall per ten-day periods, in Uberlândia, MG, from 01/03/2012 to 01/06/2012. Data provided by UFU weather station in Uberlândia, MG.

Table 1. Soil chemical properties, in the layer of 0.00-0.20, Uberlândia, 2012.

Layer	pH H_2O	P	K	Al	Ca	Mg	H+Al	SB	t	T	V	m	MO
M							$cmol_c\ dm^{-3}$				%		$dag\ kg^{-1}$
0.00-0.20	6.25	11.73	0.14	0.06	0.73	0.78	1.75	1.65	1.71	3.40	47.82	5.64	2.11

P, K = (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}) available P (Mehlich-1 extractor); Ca, Mg, Al, (KCl 1 mol L^{-1}); H+Al = (Buffer Solution – SMP a pH 7.5); SB = Bases Sum; t = effective CTC; T = CTC at pH 7.0; V = Base saturation; m = Aluminum saturation (EMBRAPA, 2009).

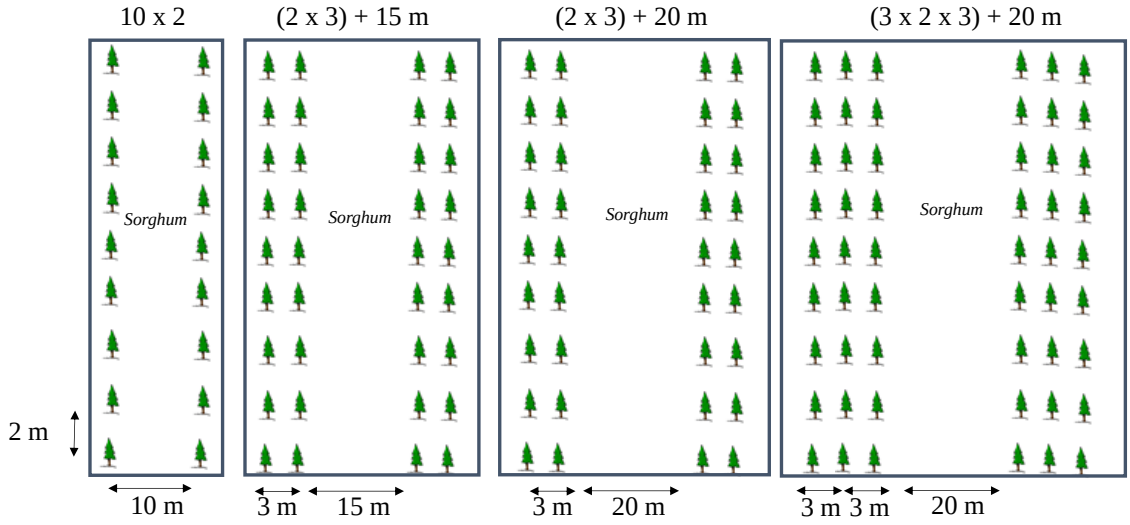

Figure 2. Spatial arrangements of the eucalyptus agrosilvopastoral system.

lines of sorghum were considered, planted between the greatest distance from the eucalyptus rows and two lateral lines on the right

Table 2. Number of trees per hectare in the various arrangements and arithmetic mean of eucalyptus height after sorghum harvest (EH).

Arrangement	Number of trees	EH (m)
2 × 3 + (15 m)	556	3.10
2 × 3 + (20 m)	435	2.97
2 × 10 m	500	3.29
3 × 2 × 3 + (20 m)	577	3.06

and two lateral lines on the left of the tree component. There was a border line with sorghum in the direction of eucalyptus on both sides.

The panicles were covered with paper bags at flowering to protect against birds. Pesticide applications to control pests, diseases and weeds took place during the experiment, according to recommendations and practices commonly adopted in the region.

Evaluated characteristics

Plant height (m)

The plant height was measured from the insertion of the upper panicle to the ground, expressed in meters, with four plants from the useful area per plot after physiological maturity of grain.

Grain weight (kg)

It was obtained by weighing the grain of the plants from two rows of 4 m in different installments.

Grain yield (t ha^{-1})

Data regarding grain weight from the plots after threshing were corrected for moisture of 13% and converted to t ha^{-1} using Equation 1.

$$P13\% = PC(1-U)/0.87 \quad (1)$$

In: P13%: grain yield (t ha^{-1}) corrected to standard moisture of 13%; PC: grain yield without correction; U: grain moisture observed at harvest.

Effective grain yield (kg ha^{-1}) for spatial arrangement

Using grain yield (kg ha^{-1}), the effective yield was calculated, which is based on sorghum productivity in integration with the forest system, discounting the area occupied by trees (m²). It was determined by Equation 2.

$$\text{Effective Produtivity} = \text{Produtivity} \times (10000 - \text{area occupied by forest}/10000) \quad (2)$$

Statistical analysis

Individual analysis for spatial arrangements was held and subsequently the data were submitted to the normality and homogeneity tests for further realization of joint analysis of variance and Scott-Knott test at 0.05 significance.

RESULTS AND DISCUSSION

The temperature and the rainfall were considered normal during the experiment (Figure 1). The interaction of spatial arrangements of eucalyptus and sites of sorghum evaluation was significant (P<0.05) for plant height, grain weight, average productivity and effective productivity of sorghum grains, as well as the local of plots and spatial arrangements of eucalyptus (Table 3).

For the height of sorghum plants in consortium in spatial arrangement of simple line of eucalyptus (10 x 2 m) there was no difference between the locals of evaluation (Table 4). Regarding the other three spatial arrangements, there was difference in sorghum height depending on the place of evaluation. Thus, for the system of double lines (2 × 3) + 15 m and triple lines (3 × 2 × 3) + 20 m the height of sorghum plants was greater in the center, with values of 0.85 m 0.88 m (Table 4). Lower height in the (2 × 3) + 15 m spatial arrangement was observed on the left side, with 0.71 m; however, for the triple-line system, a lower height was on the right side, of 0.59 m.

Regarding the (2 × 3) + 20 m spatial arrangement, the center had the highest plants, with 0.87 m. The left side, with 0.81 m, and the right side, with 0.76 m, obtained lower values (Table 4). Moreover, when sorghum was grown in simple spatial arrangement (10 × 2 m) no difference was observed in plant height, regardless of the assessment site. However, for the other three spatial arrangements, the height of sorghum was affected by the location, where the center obtained higher values under these conditions.

These results were similar to those reported by Oliveira (2011), in a study of intercropping sorghum with eucalyptus in iLPF system, where the height of the plants in the lines close to the trees was lower comparing with the center lines of the plot. The reduction in sorghum height occurred as approaching the tree, resulting in smaller sorghum plants in the vicinity of eucalyptus.

The same was observed by Macedo et al. (2006), who found higher values of the average height of the consortium of corn with eucalyptus situated 4.5 to 5.4 m away from the eucalyptus lines than those located 1.8-2.7 m from eucalyptus possibly because of the greater light

Table 3. Analysis of variance for plant height (PH), grain weight (MG), average productivity (PR) and effective productivity (PREF) of sorghum grown with different Eucalyptus spacings. Ajustar as siglas para ingles.

Sources of variation	Gl	PH (m)	MG (kg)	PRme (t ha^{-1})	PRef (t ha^{-1})
			QM		
Bloc	4	0.013	5.552	6855556.720	3957716.192
Arrangement	3	0.046*	12.758*	15751155.097*	13656695.179*
Loc. evaluation	2	0.085*	9.806*	12106683.606*	6561457.458*
Local arrangement*	6	0.022*	4.238*	5232345.963*	3184279.199*
Error	44	0.001	0.608	751143.339	450717.757
Average		0.799	4.300	4.478	3.655
CV (%)		5.23	18.14	18.14	18.37

*: Significant at 5% error probability by F test.

Table 4. Average height (m) of sorghum plants in eucalypt spacing in function of the local of assessment of the iLPF system.

Spacing (m)				
Local of evaluation	**10 × 2**	**(2 × 3) + 15**	**(2 × 3) + 20**	**(3 × 2 × 3) + 20**
Right side	0.88^{Aa}	0.77^{bB}	0.76^{bB}	0.59^{cC}
Center	0.88^{aA}	0.85^{aA}	0.87^{aA}	0.88^{aA}
Left side	0.83^{aA}	0.71^{bC}	0.81^{aB}	0.72^{bB}

Average followed by different letters, lowercase and uppercase on the line in the column, different by the Scott-Knott test at 0.05 significance.

incident in the middle between rows of eucalyptus clones, oriented east-west. Such evidence can be explained by the fact that the sorghum evaluated in the center, that is, far from the eucalyptus lines, received the most of solar radiation input and had less competition for water and nutrients from the tree component of the system. Thus, there is greater photosynthetic rate and greater redistribution of assimilates to the plant, since sorghum is a C4 plant with high yields in sunlight.

When comparing the different space systems within each assessment site, one can observe that the heights of sorghum plants were affected by the used spacing. To the right, the plants in the spacing with single line (10 × 2 m) had greater height, however the triple line system led to lower plants (Table 4).

In relation to the center, there was no difference between the space systems of eucalyptus. On the left side, both arrangements 10 × 2 m, with 0.83 m and (2 × 3) + 20 m with 0.81 m, provided the highest plants, and did not differ between one another; the other two arrangements produced the lower values.

With regard to spacing within each trial site, the 2 × 10 m space system produced greater height on the right side (Table 4). On the left side, the highest values were found in the simple line spacing with 10 × 2 m and double (2 × 3) + 20 m. However, in the center the height was statistically equal in all, regardless of the spatial arrangement. Based on the obtained data, it can be inferred that, in the sample on the right side, the spacing with fewer trees (10 × 2 m) obtained the highest plants, due to weaker shading of the sorghum plants.

Similarly, for the left side, however, the (2 × 3) + 20 m spatial arrangement produced higher plants, perhaps because it has greater spacing between rows, allowing more light to the enter under the trees. The center of the samples was far from eucalyptus lines, and had higher brightness. Importantly, the planting of trees occurred in the north-south direction. This fact favored shading regularly in the morning, favoring the right side and in the afternoon the left side, depending on the position of the sun.

Another analyzed variable was the weight of grain, which affects an agronomic component of paramount importance showing the performance of a crop in a certain place and time. So, when sorghum was grown in the 10 × 2 m spatial arrangement and the weight of grain was evaluated on the right side and center, the values were statistically equal. However, on the left side, lower weight was noted (Table 5).

For the (2 × 3) + 15 m spatial arrangement no significant differences between the sites of evaluation were observed. Unlike what happened in the (3 × 2 x 3) + 20 m spatial arrangement where only the center had higher weight and right side lower (Table 5). For the (2 × 3) + 20 m spatial arrangement, the center and the left side showed the greatest weight and right side the lowest weight (Table 5). When sorghum was produced in the (2 × 3) + 15 m spatial arrangement, it presented the same

Table 5. Sorghum grain weight per plot (kg) in different eucalyptus spacings depending on the location of evaluation in the iLPF system.

Spacing (m)				
Local of evaluation	**10 × 2**	**(2 × 3) + 15**	**(2 × 3) + 20**	**(3 × 2 × 3) + 20**
Right side	5.31^{aA}	3.23^{bA}	4.38^{aB}	1.98^{cC}
Center	4.96^{bA}	4.24^{bA}	6.06^{aA}	5.06^{bA}
Left side	3.47^{bB}	3.66^{bA}	5.98^{aA}	3.27^{bB}

Average followed by different letters, lowercase and uppercase on the line in the column, different by the Scott-Knott test at 0.05 significance.

grain weight regardless of location. However, other spatial arrangements obtained variation depending on the site of evaluation.

By analyzing the evaluation site, it was observed that the sorghum on the right side had higher grain weight in 10 × 2 m, (2 × 3) + 20 m spatial arrangements and the triple-line spatial arrangement provided lower value (Table 5). In the center and on the left side the behavior was similar. However, the (2 x 3) + 20 m double-line system showed increased grain yield comparing with the other three systems.

It was evident that the sorghum in the center and left side of the subplots had the highest grain yield in the (2 × 3) + 20 m spatial arrangement. On the right side, both the 10 × 2 m system and the (2 × 3) + 20 m system had higher values (Table 5). Looking at the average yield of sorghum grain, the 10 × 2 m spatial arrangement had higher values on the right side and in the center. The double row (2 × 3) + 15 m system showed no difference in grain yield among the evaluated sites. The spatial arrangement with triple row had higher value only in the center. And the (2 × 3) + 20 m spatial arrangement obtained higher values both in the center and on the left (Table 6).

Thus it can be seen that the highest productivity proved to be dependent on the spatial arrangement and the place of evaluation. The (2 × 3) + 15 m system was an exception where productivity was the same in three locations.

However, for the 10 × 2 m and (2 × 3) + 20 m systems, the center obtained the highest values, but on the right side of the 10 x 2 m system, productivity was equal to the center. In the (2 × 3) + 20 m system, the productivity on the left side was equal to the center. For the triple system, only the center showed higher value. Importantly, in all spatial arrangements, the center presented high productivity.

These results contrast those found by Almeida et al. (2014), in an experiment to evaluate the productivity of soybeans between eucalyptus rows in different positions (west, the center and east of the trees). These authors found that the number of plants per hectare, the number of pods per plant and the number of seeds per pod did not differ between the sampling positions. The yield of grain per hectare was higher in the western position, followed by the center strip, and the east provided the lowest productivity, with the orientation of trees in north-south direction.

These results highlight the importance of planning the plots in future work involving sorghum intercropped with eucalyptus. There is the need to include the center and the sides to constitute a sorghum experimental plot in iLPF systems. According to Oliveira et al. (2013), studies related to morphological and physiological characteristics of shaded forage are relevant to better understand the response of these species and fill gaps in literature on the subject. By analyzing the places of evaluation, it was observed that the right side had a higher grain yield in 10 × 2 m, (2 × 3) + 20 m spatial arrangements, and the triple system proved to give the lowest value. Already at the center and on the left side productivity was higher in the (2 × 3) + 20 m system than in the other three arrangements of eucalyptus.

Similar results were found by Albuquerque et al. (2014), where the production of sorghum in the (2 × 3) + 20 m spatial arrangement was higher comparing to denser systems, where the (2 × 3) + 9 m system showed a decrease of 37.7% in yield in relation to production without any shade. In all evaluated sites, the (2 × 3) + 20 m system showed higher productivity, however the 10 × 2 m system had no significant difference on the right side (Table 5).

The light plays an important role in regulating numerous chloroplastid enzymes; when missing or in excess it may trigger disorders associated with photosynthetic processes (Albuquerque et al. 2014).

According to these authors, low amount of light limits photosynthesis with negative effects on tillering, growth rate and consequently the production of biomass. The average productivity of sorghum in 2012, for the state of Minas Gerais, was 3.51 t ha^{-1} (CONAB, 2012). In the experimental area, the obtained average was 4.77 t ha^{-1}, 35% higher comparing with the national average. This may be related to precipitation in place during the experiment, mainly during the first month which is the most critical, providing good results.

When assessing the effective productivity, that is productivity without the area occupied by the tree, it was

Table 6. Average productivity of sorghum grain (t ha^{-1}) in different spacings of eucalyptus depending on the evaluation location for the iLPF system.

Spacing (m)				
Local of evaluation	**10 × 2**	**(2 × 3) + 15**	**(2 × 3) + 20**	**(3 × 2 × 3) + 20**
Right side	5.90^{aA}	3.58^{bA}	4.86^{aB}	2.19^{cC}
Center	5.51^{bA}	4.71^{bA}	6.73^{aA}	5.62^{bA}
Left side	3.85^{bB}	4.06^{bA}	6.64^{aA}	3.63^{bB}

Average followed by different letters, lowercase and uppercase on the line in the column, different by the Scott-Knott test at 0.05 significance.

Table 7. Effective grain yield (t ha^{-1}), in different spacings of eucalyptus depending on the local of evaluation for the iLPF system.

Spacing (m)				
Local of evaluation	**10 × 2**	**(2 × 3) + 15**	**(2 × 3) + 20**	**(3 × 2 × 3) + 20**
Right side	5.01^{aA}	2.51^{cA}	3.69^{bB}	1.60^{dC}
Center	4.68^{aA}	3.29^{bA}	5.11^{aA}	4.10^{bA}
Left side	3.27^{bB}	2.84^{bA}	5.05^{aA}	2.65^{bB}

Average followed by different letters, lowercase and uppercase on the line in the column, different by the Scott-Knott test at 0.05 significance.

noted that in the 2 × 10 m system, both the right and the center provided higher values than the left (Table 7). In the (2 × 3) + 15 m arrangement, there was no difference between the evaluated sites.

The spacing with triple rows of eucalyptus provided better effective grain yield when sorghum was produced in the center, with 4.10 t ha^{-1}, unlike what happened on the right side that got the lowest value, 1.60 t ha^{-1}. But the (2 × 3) + 20 m arrangement had higher productivity in the center and on the left side (Table 7).

By observing the spatial arrangements within each assessment site, sorghum on the right side provided the best effective productivity (5.01 t ha^{-1}) in 10 × 2 m spatial arrangement, and the (3 × 2 × 3) + 20 m obtained lower productivity (1.60 t ha^{-1}). In the center, this characteristic was superior in both systems, in the 10 x 2 m and in the (2 × 3) + 20 m system, while the other two provided less effective productivity. Finally, on the left side, the spacing with the highest value was the (2 × 3) + 20 m comparing to the other three (Table 7).

Introduction of the trees in the integration of production systems promotes profound changes that need long-term care in its planning and execution. According to Alvarenga et al. (2010), special attention should be given to the selection of species, intended use and spatial arrangement. These authors pointed out that the forestry component can achieve improved profitability was driven for timber production by sawmills, veneer lamination, with a low density of trees / hectare. Thus, due to this simple arrangement the work 10 and double row ×x 2 m (2 ×x 3) + 20 m would be most suitable because they have a lower density of trees / ha and greater productivity grain sorghum, however, experiments long-term to assess the forestry component becomes necessary to characterize the wood.

If the producer requires faster economic return of the forest component, the same can opt for the arrangement with more trees. In this case the market would be coal or energy. It is important to note that after the sorghum harvest, the area can be used for a few months paragraph tofor grazing animals, animals; it is common in the emergence of Brachiaria in the area.

Coelho et al. (2014) evaluate the dry matter production of forage in different arrangements concluded, the use of eucalyptus double lines implies reducing radiation incident in the understory, but does not imply reducing pasture productivity compared to the simple arrangement.

Conclusions

The site assessment of the plots affectassessments of the plots affect the main agronomic characteristics of sorghum. In future experiments, it is necessary that sorghum portions are formed by central and lateral lines within the wider spacing eucalyptus.

The spatial arrangement (2 ×x 3) + 20 m and 10 ×x 2 m are more promising for the consortium . The definition of best arrangement will depend on the market for the sale of wood.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Albuquerque CJB, Camargo R, Souza MF (2013). Extração de macronutrientes no sorgo granífero em diferentes arranjos de plantas. Revista Brasileira de Milho e Sorgo, Lavras. 12(1):10-20.

Albuquerque CJB, Alvarenga RC, Viana MCM, Clemente MA, Wendling B, Araújo SAC (2014). Cultura do sorgo em sistema integração lavoura pecuária floresta. Informe agropecuário, Belo Horizonte 35(278):63-73.

Almeida LF, Calonego JC, Catuchi TA, Tiritan CS, Araújo FF, Silva PCG (2014). Produtividade de soja em diferentes posições entre renques de eucalipto em cultivo consorciado. Colloquium Agrariae 10(1):33-44.

Alvarenga RC, Porfirio-da-Silva V, Neto MMG, Viana MCM, Vilela L (2010). Sistema Integração lavoura- Pecuária-Floresta: condicionamento do solo e intensificação da produção de lavouras. Informe agropecuário, Belo Horizonte 31(257):59-67.

Borghi E, Crusciol CAC, Nascente AS, Sousa VV, Martins PO, Mateus GP, COSTA C (2013). Sorghum grain yield, forage biomass production and revenue as affected by intercropping time. Eur. J. Agron. 51:130-139.

Bravin MP, Oliveira TK (2014). Adubação nitrogenada em milho e capim-xaraés sob plantio direto e preparo convencional em sistema agrossilvipastoril. Pesq. Agropec. Bras. Brasília 49(10):762-770.

Coelho JS, Araújo SAC, Viana MCM, Villela SDJ, Freire FM, Braz TGS (2014). Morphophysiology and nutritive value of signalgrass in silvipastoral system with different tree arrangements. Semina: Ciências Agrárias, Londrina. 35(3):1487-1500.

CONAB (2013). Companhia Nacional de Abastecimento. Acompanhamento da safra Brasileira. Brasília, DF: CONAB. Disponível em: http://www.conab.gov.br/conteudos.php?a=1253&.

Cubbage F, Balmelli G, Bussoni A, Noellemeyer E, Pachas NA, Fassola F, Colcombet L, Rossner B, Frey G, Dube F, Silva ML, Stevenson H, Hamilton J, Hubbard W (2012) Comparing silvopastoral systems and prospects in eight regions of the world. Agrofor. Syst. 86:303-314.

Dan HA, Carrijo MS, Carneiro DF, Costa KAP, Silva AG (2010). Desempenho de plantas sorgo granífero sobre condições de sombreamento. Acta Sci. Agron. Maringá 32:4.

Dube F, Couto L, Silva ML, Leite HG, Garcia R, Araujo GAA (2002) A simulation model for evaluating technical and economic aspects of an industrial Eucalyptus-based agroforestry system in Minas Gerais, Brazil. Agrofor. Syst. 55:73-80.

EMBRAPA. (2009). Empresa Brasileira de Pesquisa Agropecuária. Manual de análises químicas de solos, plantas e fertilizantes. 2 ed. rev. e ampl.. Brasílias, DF: Embrapa Informações Tecnológica 627 pp.

Haile SG, Nair VD, Nair PKR (2010). Contribution of trees to carbon storage in soils of silvopastoral systems in Florida, USA. Glob. Change Biol. 16:427-438.

Howlett DS, Mosquera-Losada MR, Nair VD, Rigueiro-Rodrıguez A (2011) Soil carbon storage in silvopastoral systems and a treeless pasture in Northwestern Spain. J. Environ. Qual. 40(3):825-832.

Jackson J, Ash AJ (1998) Tree-grass relationships in open eucalypt woodlands of northeastern Australia: influence of trees on pasture productivity, forage quality and species distribution. Agrofor. Syst. 40:159-176.

Macedo RLG, Bezerra RG, Venturini N, Vale RS, Oliveira TK (2006). Desempenho silvicultural de clones de eucalipto e características agronômicas de milho cultivado em sistema silviagrícola. Revista Árvore, Viçosa 30(5):701-709.

Montagnini F, Nair PKR (2004) carbon sequestration: an underexploited environmental benefit of agroforestry systems. Agrofor. Syst. 61:281-295.

Oliveira FLR (2011). Recuperação de pasto associada à produção de silagem de sorgo e madeira, em sistema de integração lavoura-pecuária-floresta. Montes Claros: UFMG. Dissertação Mestrado. 83 pp.

Oliveira FLR Machado VD, Veloso ACG (2013). Seleção de espécies para sistemas agrossilvipastoris. Revista Eletrônica de Pesquisa Animal, Formosa. 01(02):99-118.

Oliveira TK,de Macedo RLG, Venturin N, Higashikawa EM (2009). Desempenho silvicultural e produtivo de eucalipto sob diferentes arranjos espaciais em sistema agrossilvipastoril. Pesquisa Florestal Brasileira. 60:1-9.

Pacheco AR, Chaves RQ, Nicoli CML (2013) Integration of crops, livestock, and forestry: a system of production for the Brazilian Cerrados. In: Hershey CH, Neate P (eds) Ecoefficiency: from vision to reality. CIAT, Cali, Issues on Tropical Agriculture. pp. 51-61.

Paula RR, Reis GG, Reis MGF, Neto SNO, Leite HG, Melido RCN, Lopes HNS, Souza FC (2013). Eucalypt growth in monoculture and silvopastoral systems with varied tree initial densities and spatial arrangements. Agrofor. Syst. 87:1295-1307.

Prasad JVNS, Korwar GR, Rao KV, Mandal UK, Rao CAR, Rao GR, Ramakrishna YS, Venkateswarlu B, Rao SN, Kulkarni HD, Rao MR (2010) Tree row spacing affected agronomic and economic performance of Eucalyptus-based agroforestry in Andhra Pradesh, Southern India. Agrofor. Syst. 78:253-267.

Pinho RC, Miller RP, Alfaia SS (2012) Agroforestry and the improvement of soil fertility: a view from Amazonia. Appl. Environ. Soil Sci. pp. 1-11.

Soares AB, Sartor LR, Adami PF, Varella AC, Fonseca L, Mezzalira JC (2009). Influência da luminosidade no comportamento de onze espécies forrageiras perenes de verão. Revista Brasileira Zootecnia, Viçosa 38(3):443-451.

Souza AN, Oliveira AD, Scolforo JRS, Rezende JLP, Mello JM (2007) Viabilidade econoˆmica de um sistema agroflorestal. Cerne 13:96-106.

Terra Forte Florestal (2014). Disponível em www.terraforteflorestal.com.br/

Tonucci RG, Nair PKR, Nair VD, Garcia R, Bernardino FS (2011). Soil carbon storage in silvopasture and related landuse systems in the Brazilian Cerrado. J. Environ. Qual. 40:833-841.

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Phosphorus availability from natural and soluble phosphate sources for irrigated corn production

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This research's aims were to evaluate the mixture ratios of P fertilizers in irrigated corn yield in two seasons as well as evaluate the capacity of Resin and Mehlich 1 extractors to predict the P availability under field conditions in two soils: Hapludults and Oxisol. In the first crop, the experimental design was randomized block with four replications where two P sources, Triple Superphosphate (TSP) and Daoui Natural Phosphate (DNP), were tested in four rations (100%:0%; 67%:33%; 33%:67%; 0%:100%). The second planting was carried out in a similar manner, with some modifications to verify the residual effect of the fertilizers. DNP provided lower productivity and leaf P levels when compared to TSP in the first year but the DNP contributed with a higher residual effect through the test conditions. DNP fertilization provided higher residual effect in Hapludults than in Oxisol. Only Resin extractor, in Oxisol without TSP application in the second cultivation, was efficient in estimating the availability of P to the corn plants in the experiment conditions. In the experiment conditions, DNP provided productivity and leaf P levels similar to Triple Superphosphate in the first year and its residual effect is highly dependent on the soil type.

Key words: *Zea mays,* fertilizing, phosphorus, extractors.

INTRODUCTION

Northern Minas Gerais is part of Brazilian semi-arid region and presents areas recognized as having agricultural potential due to their topographical conditions and favorable soils for diverse several agricultural activities. However, poor rain distribution and droughts severely limit agricultural activities in a more intensive and competitive way, making it highly dependent on irrigation.

There is a great lack of information on corn (*Zea mays* L.) plants and P fertilizers in irrigated areas in the North of Minas Gerais, especially about natural phosphates which can be an interesting alternative to soluble phosphates (Moreira et al., 2002). Another concern is that Brazil has only 2% to 3% of the world reserves of P (Lopes, 1999), that is, raw materials used in phosphate fertilizer production are scarce, non-renewable and without substitution.

Natural phosphate rocks (PRs) (that is, natural phosphates of low reactivity or natural reactive phosphates) (Corrêa et al., 2005) for direct use on soil has gained attention worldwide as a natural raw material. However, more importance must be given regarding the

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Table 1. Chemical and physical properties of the 0 to 20 cm layer of the two soils studied.

Soil	pH[1]	P[2]	K[2]	Ca[3]	Mg[3]	Al[3]	H+Al[4]	Sand	Silt	Clay
		mg dm^{-3}	mmolc dm^{-3}					g kg^{-1}		
Hapludults	5.6	1	2.05	31.0	1.4	0.0	60.8	340	210	450
Oxisol	6.1	1	1.76	28.0	1.1	0.0	52.4	550	130	320

[1] pH H_2O; [2] Mehlich I extraction; [3] KCl extraction; [4] Calcium acetate extraction.

roles of phosphorus fertilizers on the accumulation of trace elements (As, Cd, and Pb) in production fields, which seems common and consequently may reach crops (Jiao et al., 2012). PRs are considered as a nutrient-rich P source for organic farming and, thus, interest in PRs as a "natural fertilizer" could open future markets (Smalberger et al., 2006). On the other hand, P fertilization in Brazilian Cerrado soils is mostly based on soluble sources (Ramos et al., 2009).

Soluble phosphate provides a higher response in the first year when compared to natural phosphates of small initial efficiency (Horowitz and Meurer, 2004). Associated to low efficiency, excessive or inappropriate use of fertilizers can affects the environment resulting in leaching by irrigation or precipitation, besides it causes pollute ground or underground water (Chen et al., 2009). However, P fixation occurs more intensely in high solubility sources than in natural phosphates, which release P more slowly and minimize the fixation process (Novais and Smyth, 1999). Consequently, the efficiency differences between the sources tend to decrease with time.

Many of the soil analysis laboratories in Brazil have adopted Mehlich 1 extraction and the anionic exchange resin to quantify phosphorous availability to plants. The objectives were to evaluate varying mixes of triple superphosphate and Daoui natural phosphate on the productivity of irrigated corn in northern of Minas Gerais, and to evaluate the potential of Mehlich 1 and anionic exchange resin to predict P availability under these conditions.

MATERIALS AND METHODS

Site characteristics

The experiment was carried out under field conditions in Montes Claros, State of Minas Gerais, Southeast Brazil located at 16°40'12,5" South and 43°50'40,1" West, 630 m above sea level, during the corn (2004/2005) season. The climate, according to the Koppen classification, is Aw, considered as tropical of savannah, with dry winter and rainy summer. Two low P level soils, a Hapludults and an Oxisol, were treated with dolomitic limestone (Table 1) and the cultivated corn hybrid was BR-201.

Experimental design and treatments

The P sources were the triple superphosphate - TSP (0 - 48 - 0; as standard or reference) and the Daoui natural phosphate – DNP (highly reactive phosphate rock of sedimentary origin from Morocco, with total P_2O_5 - 32%, soluble P_2O_5 in citric acid 2% (1:100) - 9%, Ca - 36%, physical nature: bran), used in different proportions. The irrigation control was done through tensiometer and the water supply was established when the matric potential reached -60 kPa.

The first cultivation was conducted in summer season. The experimental design was a completely randomized block with four P ratios from two forms of phosphate as aforementioned (100%:0%; 67%:33%; 33%:67%; 0%:100%, TSP and DNP, respectively) and four replications. Each experimental unit covered a total area of 75 m^2 (5 m x 15 m), with five rows, 15 m in length, spaced one meter. For yield evaluation, a central useful area of 9 m^2 was considered (three rows of three meters in length).

The second cultivation was carried out off-season, as described for the first one, with some modifications to verify the residual effect of the natural phosphate. The experimental design was a completely randomized block in 2 × 4 factorial scheme as follows: with and without application of triple superphosphate in the 2nd cultivation and 4 levels of DNP:TSP in the 1st cultivation, with four replications. Each plot was divided in two parts, where half received triple superphosphate fertilizer and the rest did not.

Field practices and management

P application rate was 100 kg P_2O_5 ha^{-1}, according to the recommendations for corn culture cultivated under low P-level soils in the Minas Gerais State (Alves et al., 1999). At soil tillage, in the first season, 2 Mg ha^{-1} of dolomitic limestone was applied to raise the pH in water of the soil close to 5.5, that is, an amount previously determined through a neutralization curve in laboratory. Inter row fertilizers were applied for amounts of N and K_2O, in potassium chloride and ammonium sulfate forms being 20 kg ha^{-1} of N and 70 kg ha^{-1} of K_2O for Hapludults and 20 kg ha^{-1} of N and 100 kg ha^{-1} of K_2O for the Oxisol. In both planting times and soils (Hapludults and Oxisol), a total of 120 kg ha^{-1} of N in urea form was applied on surface, split in two applications.

Measurements

In the beginning silking, leaf samples located below the corn ear were collected to determine the foliar P levels according to the methodology described by Malavolta et al. (1997). In the end of the growing season, grain yield was determined and grain moisture was adjusted to 12%. Before the second planting, soil samples were collected to determine "available" P. Two extractors were used: anionic exchange resins (Raij et al., 1986) and Mehlich 1 (Mehlich, 1978). The estimated P from these extractors was averaged and supported the recommendation of 80 kg P_2O_5 ha^{-1} in the form of triple superphosphate.

Statistical analysis

All variables were subjected to variance analysis. The addition or no addition of triple superphosphate in the 2nd season was compared by the F test and the DNP:TSP proportions in the 1st cultivation were compared by regression analysis.

Table 2. Yield and leaf P levels of Oxisol and Hapludults soils (first cultivation).

Treatment*	Yield	Leaf P Levels
	kg ha^{-1}	mg kg^{-1}
	Oxisol	
100% DNP:0% TSP	4,580	2.14
67% DNP:33% TSP	4,767	2.19
33% DNP:67% TSP	4,776	2.22
0% DNP:100% TSP	4,785	2.20
Average	4,727	2.19
	Hapludults	
100% DNP:0% TSP	4,608	2.08
67% DNP:33% TSP	4,712	2.10
33% DNP:67% TSP	4,978	2.25
0% DNP:100% TSP	5.008	2.31
Average	4.826	2.18

* DNP=Daoui Natural Phosphate and TSP=Triple Superphosphate. Treatments represent the percent of P_2O_5 applied from each source. Each data is mean of four replications.

RESULTS AND DISCUSSION

In the first experiment, there was no difference among the treatments for corn yield or foliar P concentrations for either soil (Table 2). However, in the first crop it must be considered that, although not statistically significant, the average yields of corn fertilized only with TSP were 5% and 8% higher, respectively, for the Oxisol and Hapludults compared to fertilization only with DNP. These results are in agreement with Richart et al. (2006) who did not verify superphosphate superior than natural reactive phosphate in an evaluation of the P and sulfur availability to soybean.

Some studies have demonstrated a low agronomic effectiveness of natural phosphates when applied alone (Prochnow et al., 2004; Franzini et al., 2009). However, the natural phosphates can be quite effective than higher solubility sources to supply P to plants, because they are sources of controlled P release and this minimizes the nutrient fixation processes (Rajan et al., 1996; Horowitz and Meurer, 2004; Resende et al., 2006). Therefore, natural phosphate could be used for the immediate supply of P to plants (Table 2), still providing a probable residual effect (Table 3). Thus, the residual effect becomes a very important component in agronomic and economic evaluation of phosphate fertilization practices (Resende et al., 2006).

In off-season cultivation, all the treatments that received triple superphosphate fertilization provided superior grain productivity than those without fertilizer application for both Oxisol and Hapludults soils (Table 3). For the two soils, the increment of soluble phosphate levels at the first cultivation did not influence yield and leaf phosphorus levels in the second cultivation with or without triple superphosphate application. These results corroborate with Fageria et al. (1991), which report that the residual effect of the triple superphosphate is usually low.

While the triple superphosphate application in the second cultivation strongly influenced corn yield, in the oxisol, there was no difference to grain production and P leaf concentrations in Hapludults in these conditions (Table 3). The highest production in Hapludults was 5.084 kg ha^{-1} under 59% of DNP and 41% of TSP applied in the first cultivation (Figure 1). Possibly this result is due to the differences in the soils' mineral constitutions. The natural phosphate dissolution is dependent to pH and Solution Ca and the existence of soil components, or plants, acting as P and mainly, Ca drains is considered as a major factor for the dissolution and effectiveness of the natural phosphates (Rajan et al., 1996; Novais and Smyth, 1999). If a soil has previously limed and contains only permanent charge, just a few quantity of the natural phosphate would be dissolved by the end of the second year after their application. However, whether a soil at these conditions contains variable-charge components, all natural phosphate applied would have dissolved in this soil by the end of the second year. These results corroborate the findings of Robinson et al. (1994), who also noted the dissolution of a reactive phosphate rock (Gafsa phosphate rock) in soils ever in the second year.

In the Table 4 presents phosphorus for the two soils. Overall, the extractors were not efficient in predicting phosphorus availability for corn plants in both soils and different proportions of phosphates studied. However, the resin extractor was numerically more efficient in predicting the availability of P regardless of the type of soil.

In general, there was no correlation between the P extraction by Mehlich 1 and anionic exchange resin and the corn yield under the experiment conditions (Table 5). These results differ from those obtained by Moreira

Table 3. Yield and leaf P levels of Oxisol and Hapludults soils (second cultivation).

Primary treatment (first year)	Secondary treatment (second year)	Yield	Leaf P Levels
		kg ha^{-1}	mg kg^{-1}
		Oxisol	
100% DNP:0% TSP	+ TSP	5,114*	2.12^{ns}
	- TSP	3,128*	2.09^{ns}
67% DNP:33% TSP	+ TSP	5,041*	2.15^{ns}
	- TSP	3,201*	2.16^{ns}
33% DNP:67% TSP	+ TSP	5,104*	2.20^{ns}
	- TSP	2,458*	2.22^{ns}
0% DNP:100% TSP	+ TSP	4,987*	2.19^{ns}
	- TSP	2,452*	2.22^{ns}
		Hapludults	
100% DNP:0% TSP	+ TSP	5,847*	2.22^{ns}
	- TSP	4,589*	2.45^{ns}
67% DNP:33% TSP	+ TSP	5,752*	2.14^{ns}
	- TSP	5,024*	2.35^{ns}
33% DNP:67% TSP	+ TSP	5,814*	2.45^{ns}
	- TSP	4,915*	2.31^{ns}
0% DNP:100% TSP	+ TSP	5,428*	2.14^{ns}
	- TSP	3,987*	2.31^{ns}

* - significant at 5% level; ^{ns} - not significant. DNP = Daoui natural phosphate and TSP = Triple Superphosphate. For the same proportion DNP:SP. Each data is mean of four replications.

Table 4. Available P by Mehlich 1 extraction and Resin capsule equilibrium from Oxisol and Hapludults.

Treatments	Mehlich 1	Resin
	mg dm^{-3}	
	Oxisol	
100% DNP:0% TSP	10	22
67% DNP:33% TSP	7	21
33% DNP:67% TSP	7	20
0% DNP:100% TSP	7	20
	Hapludults	
100% DNP:0% TSP	15	52
67% DNP:33% TSP	13	45
33% DNP:67% TSP	12	38
0% DNP:100% TSP	12	38

DNP=Daoui Natural Phosphate and TSP=Triple Superphosphate. Treatments represent the percent of P_2O_5 applied from each source. Each data is mean of four replications.

(1997) and Faria et al. (2006), who found a high correlation between the two extractors for P available (r=0.96*) and P plant and P soil concentration (r values higher than 0.90), respectively.

Only Resin extractor, in Oxisol without TSP application in the second cultivation, was efficient in estimating the availability of P to the corn plants in the experiment conditions (Table 5). In assessing the liming-phosphated

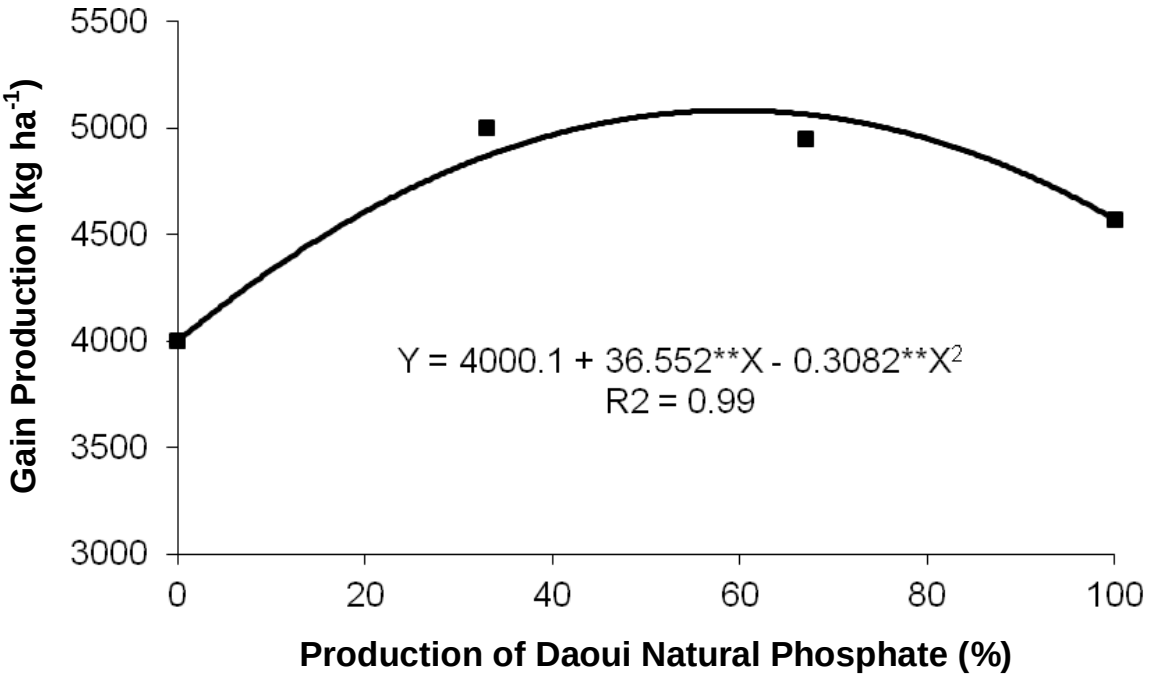

Figure 1. Residual P response of corn to proportions of Daoui Natural Phosphate levels grown on Hapludults without application of triple superphosphate, in the second year of cultivation.

Table 5. Correlation coefficients for the Mehlich 1 and Resin extraction in Oxisol and Hapludults, with grain production in the second cultivation.

Extrator	Oxisol		Hapludults	
	With TSP	Without TSP	With TSP	Without TSP
Mehlich 1	0.60^{ns}	0.52^{ns}	0.46^{ns}	0.05^{ns}
Resin	0.50^{ns}	0.87*	0.52^{ns}	0.24^{ns}

* - significant at 5% level; ^{ns} - not significant. DNP = Daoui Natural Phosphate; TSP = Triple Superphosphate.

fertilizing influence interaction on the critical P levels and on the growth of eucalyptus in a typical Dystroferric Red Latosol, the resin extractor was the most efficient in predicting the availability of P in the soil to the eucalyptus seedlings (Silva et al., 2007). The acid extractors are not suitable for soils that have recently received applications of natural phosphates because they tend to overestimate the P readiness of the plants, i.e., they are capable of extracting insoluble P from the natural phosphates (Novais and Smyth, 1999). In general, Mehlich 1 extracts preferably the fractions of phosphorus bound to calcium (Lacerda et al., 2013).

Conclusions

The productivity and leaf P levels in both soils were slightly higher when there was application of Triple Superphosphate compared to the application of Daoui Natural Phosphate in the first year of application, although was observed residual effect in the test conditions for Daoui Natural Phosphate.

Daoui Natural Phosphate fertilization supplied higher residual effect in Hapludults than in Oxisol in the test conditions being the residual effect highly dependent on the soil type.

In general, there is no correlation between corn yield and P extraction by Mehlich 1 and anionic exchange resin when natural phosphates are applied.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Alves VMC, Vasconcelos CA, Freire FM, Pitta GVE, França GE, Filho AR, Araújo JM, Vieira JR, Loureiro JE (1999). Milho. In: Ribeiro AC, Guimarães PTG, Alvarez VVH (Eds.). Recomendações para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação. CFSEMG, Viçosa, pp. 314-316.

Chen GQ, Jiang MM, Yang ZF, Chen B, Xi J, Zhou JB (2009). Exergetic assessment for ecological economic system: Chinese agriculture. Ecol. Model. 220:397-410.

Corrêa RM, Nascimento CWA, Souza SKS, Freire FJ, Silva GB (2005). Gafsa rock phosphate and triple superphosphate for dry matter production and P uptake by corn. Sci. Agric. 62(2):159-164.

Fageria NK, Baligar VC, Wright RJ (1991). Influence of Phosphate Rock Sources and Rates on Rice and Common Bean Production in an Oxisol. In: Wright RJ, Baligar VC, Murrmann RP (Eds.). Plant and Soil Interaction at Low pH. Phosphorus Kluwer Academic Publisher, pp. 539-546.

Faria CMB, Silva DJ, Pinto JM, Gomes TCA (2006). Efeito de fosfatos naturais em plantas de melão cultivadas em vasos. Rev. Bras. Ci. Solo 30(6):1083-1091.

Franzini VI, Muraoka T, Mendes FL (2009). Ratio and rate effects of 32P-triple superphosphate and phosphate rock mixtures on corn growth. Sci. Agric. 66(1):71-76.

Horowitz N, Meurer EJ (2004). Eficiência agronômica dos fosfatos naturais. In: Yamada T, Abdalla SRS. (Eds.). Fósforo na agricultura brasileira. Piracicaba: Potafos, pp. 665-687.

Jiao W, Chen W, Chang AC, Page AL (2012). Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. Environ. Pollut. 168:44-53.

Lacerda JJJ, Furtini Neto AE, Resende AV, Santos JZL, Carneiro LF, Oliveira CHC (2013). Crop growth response and dynamics of inorganic phosphorus fractions after application of reactive Arad phosphate rock in Oxisol with different land use histories. Afr. J. Agric. Res. 8(26):3454-3461.

Lopes AS (1999). Recomendação de calagem e adubação no sistema plantio direto. In: Ribeiro AC, Guimarães PTG, Alvarez VVH. (Eds.). Recomendações para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação. Viçosa: CFSEMG, pp. 93-98.

Malavolta E, Vitti GC, Oliveira SA (1997). Avaliação do estado nutricional das plantas: princípios e aplicações. Piracicaba: Potafos. P. 319.

Mehlich A (1978). New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. Comm. Soil Sci. Plant Anal. 9:477-492.

Moreira A, Malavolta E, Moraes LAC (2002). Eficiência de fontes e doses de fósforo na alfafa e centrosema cultivadas em Latossolo Amarelo. Pesq. Agropec. Bras. 37:1459-1466.

Moreira A, Malavolta E, Virgens Filho AC, Silveira RLVA, Abreu JBR (1997). Avaliação da disponibilidade do fósforo no solo por métodos isotópico, químicos e biológico. Sci. Agric. 54:78-84.

Novais RF, Smyth TJ (1999). Fósforo em solo e planta em condições tropicais. Viçosa, Universidade Federal de Viçosa. P. 399.

Prochnow LI, Chien SH, Carmona G, Henao J (2004). Greenhouse evaluation of phosphorus sources produced from a low-reactive Brazilian phosphate rock. Agron. J. 96:761-768.

Raij BV, Quaggio JA, Silva NM (1986). Extration of phosphorus, potassium, calcium and magnesium from soil by an ion-exchange resin procedure. Comm. Soil Sci. Plant Anal. 17(5):547-566.

Rajan SSS, Watkinson JH, Sinclair AG (1996). Phosphate rocks for direct application to soils. Adv. Agron. 57:77-159.

Ramos SJ, Faquin V, Rodrigues CR, Silva CA, Boldrin PF (2009). Biomass production and phosphorus use of forage grasses fertilized with two phosphorus sources. Rev. Bras. Ci. Solo 33:335-343.

Resende AV, Furtini Neto AE, Alves VMC, Muniz JA, Curi N, Lago FJ (2006). Resposta do milho a fontes e modos de aplicação de fósforo durante três cultivos sucessivos em solo da região do cerrado. Ciênc. agrotec. 30(3):458-466.

Richart A, Lana MC, Schulz LR, Bertoni JC, Braccini AL (2006). Disponibilidade de fósforo e enxofre para a cultura da soja na presença de fosfato natural reativo, superfosfato triplo e enxofre elementar. Rev. Bras. Ciênc. Solo 30(4):695-705.

Robinson JS, Syers JK, Bolan NS (1994). A simple conceptual model for predicting the dissolution of phosphate rock in soils. J. Sci. Food Agric. 64(4):397-403.

Silva CA, Rangel OJP, Belizário MH (2007). Interação calagem-adubação fosfatada e sua influência nos níveis críticos de P e crescimento do eucalipto. Sci. For. 73:63-72.

Smalberger SA, Singh U, Chien SH, Henao J, Wilkens PW (2006). Development and Validation of a Phosphate Rock Decision Support System. Agron. J. 98:471-483.

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Farmer perceptions on the use of non-conventional animal protein sources for scavenging chickens in semi-arid environments

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For sustainable intensification of village production systems, it is important to understand the views of farmers who keep the chickens on the utilization of available protein sources. The objective of the study was to assess farmer perceptions on the use of non-conventional animal protein (NCAP) sources for scavenging chickens. Resource-poor households of Msinga local municipality in uMzinyathi district, KwaZulu-Natal province (n = 239) were interviewed using a semi-structured questionnaire. Logistic regression was used to analyse the data. Females were the prominent heads of households, followed by males, and then youths. Feed shortages were among the major challenges that limited chicken production. Provision of chicken housing and religion highly influenced (*P*<0.05) a household's likelihood of experiencing feed shortages. Farmers who did not provide overnight housing to their chickens were likely to not provide any supplementary feeding. Christian farmers were predisposed to chicken feed shortages compared to traditional-religious farmers. More than half of the farmers (56.6%) were aware that NCAP sources have a huge potential to be used as protein sources for chickens. Farmers commonly used termites as a protein supplement. Other common NCAP sources were earthworms and locusts. The potential of using NCAP sources were high on farmers with large village chicken flocks and female-headed households.

Key words: Scavenging chickens, resource-poor farmers, termites, earthworms, flock size, non-conventional animal protein (NCAP), scavengeable feed resource base (SFRB).

INTRODUCTION

Increasing productivity of village chickens has a huge potential to increase protein consumption among resource-poor households, particularly for children (Mwalusanya et al., 2001). Village chickens are usually raised with little or no investment in housing, feeding and health care (McAinsh et al., 2004). To increase meat and egg productivity, it is crucial to establish the scavenging behaviour of village chickens. Scavenging is an

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instinctive behaviour and skill that can be acquired from hens by their chicks. These skills are not well developed in most imported and synthetic chicken genotypes since they were selected under intensive indoor production systems. The scavenging feed resource base (SFRB) is highly variable and mainly composed of snails, flying insects, worms in the soil, grass seeds, berries and foliage (Sonaiya, 2004). Quality and quantity of the SFRB is inconsistent (Goromela et al., 2006) and depends on season, dominant crops grown, location and life cycle of insects, among other factors. Plants and grasses are the abundant feed resources that village chickens scavenge on. These green materials are rich in energy. Protein content of the SFRB is, therefore, likely to be below the requirements of the chickens (Goromela et al., 2006). There is, therefore, a growing interest in developing methods on the propagation, harvesting, processing methods, storage and optimum inclusion levels of preferred non-conventional animal protein (NCAP) sources for scavenging chickens. Non-conventional animal protein sources include earthworms, locusts, termites, fly maggots, caterpillars, cockroaches and snails.

The increased interest in understanding the contribution of NCAP sources for village chickens is also motivated by the desire to produce organic chicken meat and eggs (Mtileni et al., 2013). These products can fetch premium prices and enhance household income and rural livelihoods. The supply of such products in the markets is, however, erratic, low and unreliable. The contribution of NCAP sources to the diets of scavenging chickens should, therefore, be estimated. Before determining the nutritive value of these feed resources, it is essential to understand farmer perceptions on the potential of using NCAP sources so as to integrate their views in developing sustainable strategies to meet nutrient requirements for village chickens. The objective of the current study was, therefore to assess farmer perceptions on the use of NCAP sources for scavenging chickens.

MATERIALS AND METHODS

Study site

The study was conducted in Msinga local municipality in UMzinyathi district, KwaZulu-Natal province, South Africa. Msinga local municipality is located at 28°40'00"S and 30°34′00"E with an average altitude of 672 m above sea level. It is semi-arid, hilly and rocky with annual average rainfall of 400 to 900 mm (Zindove and Chimonyo, 2015). Most residents in Msinga rely on subsistence production of crops and livestock for consumption and sale. Village chickens are among important livestock that are imperative to the livelihood of households. The municipality is characterized by irrigable land and irrigation infrastructure that is situated near the Tugela river where there is wide alluvial plain. Alongside the Tugela river, informal agricultural endeavours are practiced in areas adjoining the irrigation scheme. Common agricultural produce from the irrigation scheme are tomatoes, butternuts, spinach, sweet potatoes, potatoes and onions. These products contribute considerably to the livelihoods and household economy. Agricultural activities in the rain-fed gardens include intercropping of maize and beans, cowpeas and pumpkin.

Sampling of households

Two villages were randomly selected from the municipality. Sampling of the households was based on chicken ownership and willingness to participate in the study. All farmers who owned chickens were randomly selected to participate in the study. Each farmer had an equal probability of being selected for the study. A pre-tested semi-structured questionnaire was administered to 239 households by eight trained enumerators. Enumerators were obtained from the local villages to ensure that farmers are comfortable to co-operate during the study.

Data collection

Discussions with key informants were held. The key informants were prominent livestock farmers in the municipality, officials from active non-governmental non-profit organisations, local traditional and political leadership, school headmasters and agricultural extension workers. A semi-structured questionnaire was also used to collect data. The questionnaire was granted ethical approval (HSS/0584/013M) by the University of KwaZulu-Natal. The questions were translated into the vernacular Zulu language to improve quality of data captured. The questionnaire captured data on household demographic and socio-economic status, uses and ownership patterns of chickens, challenges to chicken production, feeding practices and uses of NCAP sources. Data were also collected through direct observations of socio-economic status of farmers, housing structures and chicken genotypes used. Transect walks were also made in the communities to explore resource endowments in the area.

Statistical analyses

All the data were analyzed using SAS (2003). Household socio-economic status, uses of chickens, challenges to chicken production and the use of NCAP sources were analysed using PROC FREQ of SAS (2003). The PROC GLM procedure was used to analyse the effects of gender of head of the household on livestock herd size and chicken flock composition. Pair-wise comparisons of the least square means were performed using the PDIFF procedure. An ordinal logistic regression (PROC LOGISTIC) was used to predict the odds of a household to experience chicken feed shortages and farmer perceptions on the potential of using NCAP as a feed resource for chickens. The variables fitted in the logit model included age of the farmer, gender, marital status, religion, household size, production system, housing and flock size. The model used was:

$$\text{Ln}\,[P/1\text{-}P] = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots \beta_t X_t + \varepsilon$$

Where: P is the probability of household experiencing chicken feed shortages; [P/1−P] is the odds of the household to experiencing chicken feed shortages; β_0 is the intercept; $\beta_1 \ldots \beta_t$ are the regression coefficients of predictors; $X_1 \ldots X_t$ are the predictor variables; ε is the random residual error.

When computed for each predictor ($\beta_1 \ldots \beta_t$), the odds ratio for feed shortages was interpreted as the proportion of households that experienced chicken feed shortage versus those households that experienced no shortages. For farmer perception on the use of NCAP, odds ratio were interpreted as the probability of the farmer being aware of the potential of using NCAP as a feed resource versus those who were not aware of NCAP as potential feed

Table 1. Socio-economic status of heads of households of Msinga local municipality.

Status	Adult females (n = 148)	Adult males (n = 50)	Youth (n = 41)
Farmers who were christians (%)	47.9	52.9	64.2
Farmers who were single (%)	73.1	40.0	65
Major source of income (%)			
Old age grant	41.9	47.0	0
Child support grant	35.8	19.6	50.8
Casual work	15.4	15.7	44.1
Formal work	6.9	17.7	5.1
Chicken raised under extensive system (%)	97.3	90.2	94.9
Household size (mean ± S.E)	6.1 ± 0.27^{b}	7.7 ± 0.46^{a}	6.0 ± 0.73^{b}

Values with different superscript along the rows differ (P<0.05).

resources.

RESULTS

Household demographics and socio-economic status

The demographics and socio-economic status of farmers are given in Table 1. Adult males and females mostly relied on old age grants of South Africa rand of R14 400 per annum as their major source of income. Child supports grant of R 3 600 per annum and casual work were the main sources of income to youth. The common livestock species kept in Msinga local municipality are shown in Table 2. There was a large variation in flock size, with an average of 21.6 ± 12.82 ranging from 1 to 69 chickens per household. Surprisingly, chicks were fewer than hens. The cock: hen ratio was 1:3.5. Chicks were excluded because they were not sexually matured.

Chicken ownership patterns, gender participation and uses

Adult females (69.2%) were dominant household members who owned chickens followed by males (24.5%) and youth (6.3%). The management of chickens was mainly performed by females (69.3%), youth (21.4%) and lastly males (9.3%). Duties included feeding, housing, health management and sales. Chickens were largely used for meat, income and rituals in that order in female households (Table 3). Male-headed households mostly used chicken for meat, income and status. Youths used chickens mainly for meat, income and followed by manure.

Challenges to chicken production

Female-headed households were challenged by feed shortages, high disease prevalence and theft in that order (Table 4). The most prevalent diseases were reported as Newcastle disease, fowl pox, infectious bursal disease, ulcerative pododermatitis and diarrhoea. No definite diagnoses, were, however, conducted. High disease prevalence, predation and feed shortages were the major challenges faced by male-headed households. Farmers reported snakes, mongooses, dogs, hawks and wild cats as common predators. Youth-headed households were prone primarily to feed shortages, ecto-parasite infestation and predation in that order. Dominant ecto-parasites observed included scaly leg mites, chicken mites, Tampan fowl ticks and avian lice.

Low availability and poor quality of housing

The majority of the households (77.5%) did not provide separate overnight housing for their chickens. Chickens that were not provided with housing mostly rested on tree branches. The housing materials commonly used were wood, mud and corrugated iron sheets, followed by combination of timber planks and nets and, to a lesser extent, bricks.

Predation

Snakes were the most important predator to growers and adults chickens followed by chicks and lastly eggs (Figure 1). Mongooses were also important to growers and adults chickens and lastly eggs. Dogs were a major problem to eggs and relatively less harmful to chicks and adult and growing chickens. Hawks were problematic to chicks, whilst growers and adult chickens and eggs were less affected. Wild cats were important predator to growers and adult chickens followed by chicks and to little extent, eggs.

Feeds and feeding practices

Thirty percent of the farmers practiced supplementary

Table 2. Least square means (± S.E) for livestock herd and chicken flock composition in Msinga local municipality.

Livestock herd size	Adult females (n= 148)	Adult males (n=50)	Youth (n=41)
Scavenging chickens	22.8 ± 1.03[a]	24.9 ± 1.75[a]	14.5 ± 1.95[b]
Cattle	2.6 ± 0.49[b]	5.9 ± 0.84[a]	3.5 ± 0.94[ab]
Sheep	0.1 ± 0.21[b]	2.0 ± 0.40[a]	0.1 ± 0.45[b]
Goats	10.3 ± 1.16[b]	18.6 ± 2.00[a]	7.4 ± 2.02[b]
Ducks	0.1 ± 0.06[b]	0.4 ± 0.10[a]	0
Pigs	0.1 ± 0.06[a]	0.2 ± 0.11[a]	0.3 ± 0.12[a]
Chicken flock composition			
Chicks	6.8 ± 0.68[c]	10.0 ± 1.16[a]	3.1 ± 1.29[b]
Hens	12.0 ± 0.65[a]	11.0 ± 1.12[ab]	8.1 ± 1.24[b]
Cocks	4.0 ± 0.22[a]	3.9 ± 0.37[a]	3.3 ± 0.41[a]

Values with different superscript along the row differ (P<0.05).

Table 3. The most important reasons (%) of uses of chickens in Msinga local municipality.

Uses	Adult females (n = 148)	Adult males (n = 50)	Youth (n = 41)
Meat	71.6	70.6	61.0
Eggs	2.0	2.0	2.4
Income	15.5	9.8	20.1
Rituals	7.5	2.0	6.3
Manure	3.4	7.7	10.2
Status	0.0	7.9	0.0

feeding to their chickens. The predominant feeds used to supplement chickens were unground rotten maize, kitchen waste, bought-in feeds, sorghum and rice. Non-preferential feeding was mostly practiced (88.8%). Birds were commonly supplemented once a day (76.3%) before they scavenge. At least 98.8% of the chicken keepers provided water to their birds.

Feed shortages

Chicken housing and household religion highly influenced *(P* <0.05) the household's likelihood to experience feed shortages (Table 5). Farmers with overnight housing for their chickens were less likely to experience feed shortages. Christian farmers were predisposed to chicken feed shortages compared to traditional-religious farmers.

Potential of using non-conventional animal protein sources to village chickens

Most farmers did not provide NCAP sources (94.6%) to their chickens. However, more than half of the farmers (56.6%) were aware that these NCAP sources have a potential of being used as chicken feed. One in four farmers (25.4%) cited lack of knowledge on the methods of collection and bulking them to feed a large flock of chickens. Few farmers (5.4%) supplemented chickens using termites. The members of the termite colony mostly used to feed chickens were soldiers and workers. These termites were predominantly found in tree stems, deteriorated wooden door frames and mounds. Farmers also trapped termites by opening a hole in the mound and incorporate clay pot with green materials then sealed with cover. Women were the main responsible household members to feed chickens with these animal protein sources.

The NCAP feedstuffs were relatively less available during the hot dry season, for example, 21.9% of farmers reported that earthworms are more available during the hot dry season whilst 78.1% observed less availability (Table 6). The NCAP sources were dominant in the rainy season. Earthworms, termites and locusts in that order were identified as NCAP sources with the highest potential for feeding village chickens. Farmers claimed that chickens preferred these NCAP sources because they are easy to obtain or hunt and are more available. Farmers also reported that NCAP are common animal protein feedstuffs consumed by scavenging chickens. However, they are low in proportion especially during dry season. They were mostly found in river banks, crop

Table 4. The most important challenges (%) to chicken production in Msinga local municipality.

Challenges	Adult females (n = 148)	Adult males (n = 50)	Youth (n = 41)
High diseases prevalence	20.0	30.7	7.6
Ecto-parasite infestation	7.3	9.5	22.5
Intestinal parasites infestation	2.5	2.7	5.3
Theft	15.3	4.8	7.3
Predation	9.8	21.6	9.8
Poor market	0.8	7.8	3.3
Poor availability housing	8.8	5.8	2.0
Feed shortages	35.5	17.3	42.2

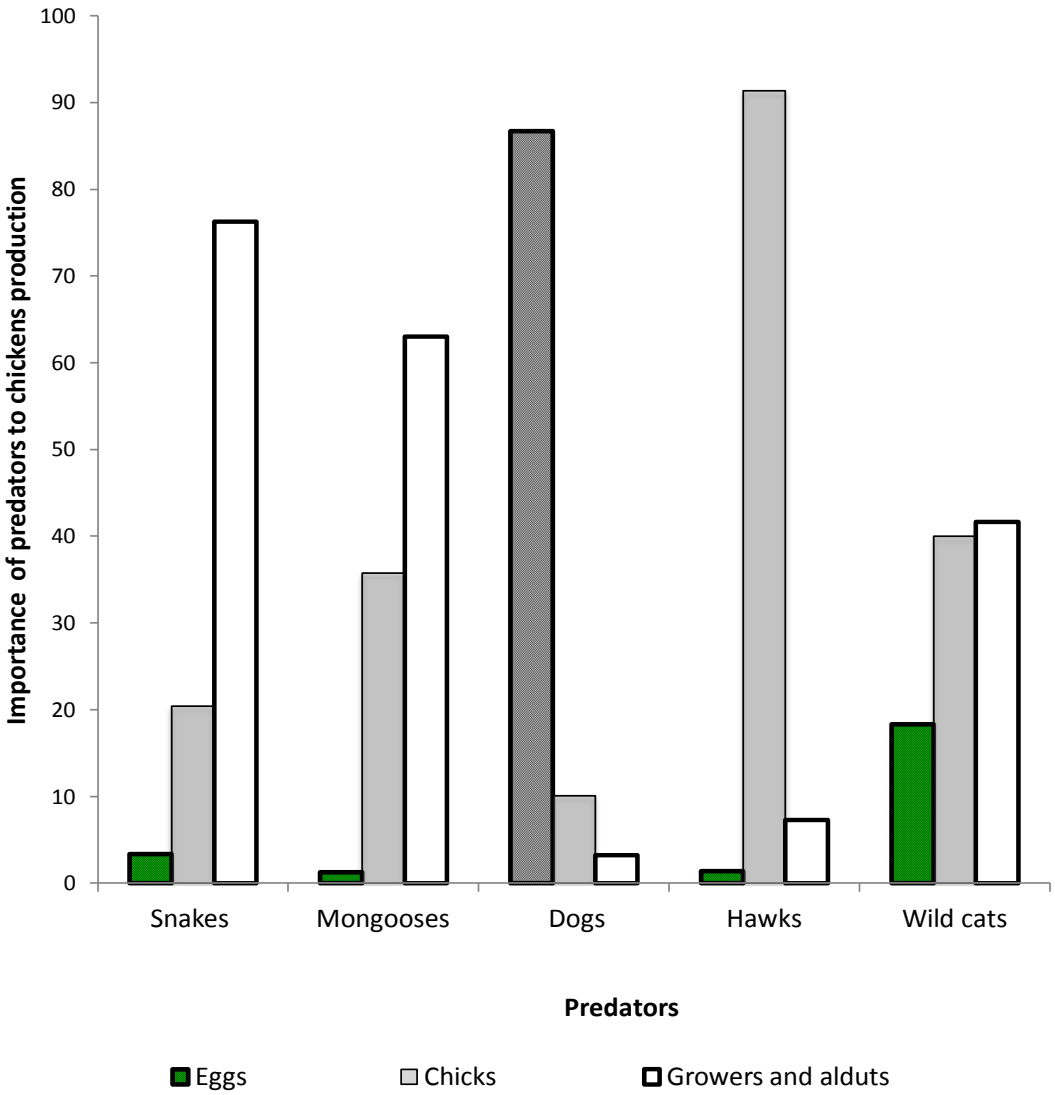

Figure 1. Percentage of the most important predators to chickens.

fields, kraals, wetlands and in deteriorated materials. The odds ratio estimates of farmers being aware of NCAP as potential feed resources to chickens were high on chicken flock size, gender of head of the household and household size (Table 7). Farmers with large chicken flock sizes were likely to be aware of NCAP as potential

Table 5. Odds ratios for chicken feed shortages.

Predictor	Odds ratio	Lower CI	Upper CI	Significance
Age (youth ≤35 versus adults >35 years)	2.4	0.62	4.78	ns
Gender (female versus male)	1.7	0.38	7.86	ns
Marital status (single versus married)	2.2	0.61	8.36	ns
Religion (tradition versus christian)	4.5	1.12	24.02	*
Household size (large >6 versus small ≤6 members)	1.4	0.36	5.39	ns
Production system (extensive versus semi-extensive)	3.9	0.58	26.14	ns
Chicken housing (no versus yes)	5.6	1.31	23.63	*
Chicken flock size (large >22 versus small ≤22)	1.6	0.42	6.30	ns

The higher the odds ratio the stronger the predictor of chicken feed shortages. CI: confidence interval. ns not significant (P>0.05), * P<0.05.

Table 6. Seasonal availability (%) of non-conventional animal protein (NCAP) sources in the study area.

Non-conventional animal protein sources	Hot dry	Rainy
Earthworms	21.9	99.6
Fly maggots	10.4	99.2
Termites	32.9	99.2
Locusts	18.3	96.7
Snails	3.3	98.8
Caterpillar	5.8	98.8
Cockroaches	21.3	95.8

Values indicate highest availability of NCAP, relative to low availability in the same season.

feed to chickens.

DISCUSSION

Females are liable for any homestead related activities (Halima et al., 2007; Tarwireyi and Fanadzo, 2013). Most females in rural areas are over-burdened with a wide range of activities, tasks and responsibilities, in agriculture, animal husbandry and in the household (Guèye, 2003). The finding that households major source of income was through receiving old age pension and government grant agrees with Nyoni and Masika (2012). Extensive production system is the common management of scavenging chickens in Africa (Halima et al., 2007; Mtileni et al., 2013; Muchadeyi et al., 2004). Village chickens have a potential to alleviate protein shortages in rural households because they are ubiquitous as they are kept by almost every household (Mtileni et al., 2013). The mean flock size of 21.6 was higher than 17 and 10.9 of reported earlier (Nyoni and Masika, 2012; Mtileni et al., 2013). Production practices, flora and fauna in the locality, disease outbreaks, predation and feed shortages were the major reasons for losses from flocks (Kuit et al., 1986) could explain these flock size differences.

The observed hen to cock ratio obtained is similar to observations by Yakubu (2010) in Nigeria. Cocks are usually slaughtered to keep reasonable ratios of cock to hen, meanwhile providing meat. High proportion of hens in flock indicates that they are reared to produce eggs and chicks. Low proportion of chicks in the flock is a result of high disease prevalence, feed shortages, predation which hampers the growth and production of chicks (Gondwe and Wollny, 2007). Chicks are the weaker group in flock, non- preferential feeding could, therefore, explain the low number of chicks in the flock (Dessie and Ogle, 2001).

The high ranking of chickens for human consumption agrees with Mwale and Masika (2009) who reported that the purpose of keeping chicken was mainly for meat in Centane district, Eastern Cape. Village chickens can be slaughtered easily and can be consumed in one meal without need for refrigeration. Msinga local municipality is one of the most undernourished rural areas of KwaZulu-Natal, which could explain why farmers consider using chicken for meat consumption rather than selling. The observation that the ownership and management of chickens were predominantly by females agrees with Halima et al. (2007). Village chicken production could be a sustainable resource for rural women empowerment. Higher proportion of adult males than youths in owning

Table 7. Farmer perceptions on the potential of using NCAP sources as feed for scavenging chickens.

Predictor	Odds ratio	Lower CI	Upper CI	Significance
Age (youth ≤35 versus adults >35 years)	0.6	0.30	1.36	ns
Gender (female versus male)	2.8	0.78	9.94	ns
Marital status (single versus married)	1.7	0.46	6.35	ns
Religion (tradition versus christian)	1.1	0.31	3.66	ns
Household size (large >6 versus small ≤6 members)	2.4	0.65	8.98	ns
Production system (extensive versus semi-extensive)	0.2	0.03	1.77	ns
Chicken housing (no versus yes)	1.2	0.25	5.46	ns
Chicken flock size (large >22 versus small ≤22)	4.5	1.06	20.43	*

The higher the odds ratio the stronger the probability of farmer being aware of the potential of using NCAP as feed resources. CI: confidence interval. ns: not significant, * P<0.05.

chickens agrees with Muchadeyi et al. (2004) who reported that men owned 36% of the chickens and boys and girls owned 6 and 6%, respectively.

The finding that households were largely challenged by diseases agrees with previous reports (Aboe et al., 2006; Okeno et al., 2012). Extension officers of the municipality highlighted that Newcastle disease was the commonest disease that can kill the almost entire flock (Naidoo, 2003). Government extension officers, in conjunction with local non-government organizations and farmers, need to collaborate when attempting to purge prevalence of Newcastle disease. When the management or combating of Newcastle disease has been done, it would promote investment in chickens (Aboe et al., 2006). Chicken theft necessitates appropriate chicken housing with security features. Chicken feed shortages to rural households could be because harvested maize has many needs in the household. For example, females mostly threshed maize to make maize meal and porridge. Female, male and youth-headed households have different household resources and priorities, these differences are considered to affect the interest of household scale of operation, management strategies and knowledge of poultry (Aklilu et al., 2008).

The finding that most chickens were not provided with overnight housing suggests that farmers do not invest much into their chickens. The interviewed farmers and prominent livestock farmers in the municipality argued that providing overnight housing invites predators, such as snakes. Political leaders, school head masters and farmers also added a plausible explanation that females were responsible for chickens whilst chicken house construction is generally done by males which could also explain minority of households who provide overnight housing. The main reason for providing housing is to protect birds from predation and theft (Gondwe and Wollny, 2007). The major predators were snakes, mongooses, dogs, hawks and wild cats. Harmfulness of hawks to chicks indicates that chicks need to be restricted from scavenging by enclosing them to their house. Dogs prefer eggs more than chickens, probably because they are not fed on balanced diets. Active non-governmental non-profit organisations revealed that high incidence of snakes killing chickens are related with the current study area that is rocky and therefore, provides a good habitat for snakes which are often found underneath the rocks.

The materials used for houses and nests could increase infestation for external parasites such as fowl ticks, mites and fleas which spend most of their lives hiding in cracks and crevices in building (McAinsh et al., 2004). Housing also delays birds to come out and keep them away from the fields during this time of the year (Muchadeyi et al., 2004). Farmers who provided housing at night resorted to cheap and locally available materials such as wood, mud and metal sheets, combination of plank timber and nets and using bricks, as also reported earlier (Mtileni et al., 2013). Farmers should be encouraged and trained to construct appropriate houses for chickens to reduce predation, parasites infestation and improve productivity.

One major constraint to the increase in chicken productivity is feed availability and quality. Unground rotten maize grain was the main supplementary feed given to chicken as also observed in other parts of South Africa (Naidoo, 2003; Mwale and Masika, 2009; Nyoni and Masika 2012). Maize is available in large quantities during harvesting and threshing periods (Mtileni et al., 2013). Although maize grain is rich in energy, aflotoxins and mycotoxins are usually a huge challenge. Supplementing with maize grain could only sort out energy requirements issues but not protein. Therefore, scavenging chickens have to use their ability to hunt for protein-rich feed resources, such as earthworms around the surroundings to meet protein needs. As a result, they are vulnerable to theft and predation. Furthermore, they interact with other neighbouring flock which makes them vulnerable to disease (Kitalyi, 1998). Water supply to birds is useful by reducing hunting responsibility for water in niches where they are susceptible to predation, theft and disease. Supplying of water to birds is likely to promote scavenging for feed resources, thus improve

feed intake and growth.

African traditional religious farmers stored remainders of sorghum to feed chickens after making traditional beer for ritual ceremonies. This could explain why they had less likelihood of facing chicken feed shortages. Sorghum is, however, deficient in protein content. Negligible feeding input to chickens raised under extensive production system could be related to farmers not affording feed that is sold in the market. Youths largely relied on child support grants and casual occupations for income generation. Unstable occupation and meagre income could be the reason young farmers face feed shortages for chickens. Youths have limited access to resources such as credits, agricultural inputs, technologies (Kitalyi, 1998).

Unfamiliarity of farmers with the use of NCAP to chickens calls for training of farmers about importance of NCAP sources to chickens for sustainable feeding system and improvement of chicken productivity. Training should include possible propagating and harvesting techniques using locally available resources to produce these protein sources. Existence of termites during the dry season has been reported by farmers. Termites are known to thrive under dry conditions and recycle to contribute to ecosystem by feeding on dead plants such as wood, leaf litter and animal dung (Okeno et al., 2012). Feeding termites to chicken would be, therefore, a way of converting unusable materials to food for rural people.

Farmers indicated that NCAP sources are available even during the hot dry season could those who are residing in village situated near Tugela river where there is wide alluvial plain. Along the river, there are swampy areas where NCAP sources such earthwoms and flies mostly found. Seasonal availability of NCAP sources necessitates innovative methods that need to be implemented to produce these novel sources at all times to supply birds with protein sources throughout the year. The method of producing these protein sources should be inexpensive and complement the living standards of smallholder farmers by using locally available materials. For example, possible methods of producing and harvesting earthworms are through enclosing them into fresh sludge. Cattle dung provides sources of NCAP sources such as earthworms and cut worms and is used as a media of production (Goromela et al., 2007). Combination of fermented blood mixture, rumen contents and cattle dung can be used to produce maggot larvae (Smith, 1990).

Earthworms, termites and locusts are potential protein sources to birds. They are a natural food source for poultry and are highly palatable to chickens. They are used for human consumption in other countries (Paoletti et al., 2000). Using them as feed to chickens can increase productivity of chickens while maintaining low input cost of production. These protein-rich feed resources have a beneficial effect when included into the poultry diet (Tiroesele and Moreki, 2012). Interviewed famers, prominent livestock owners and local traditional indicated that unlike fly maggots and snails, these protein sources are not disgusting, meaning that they could consume a chicken being supplemented with earthworms, termites, locusts. The farmers and key informants highlighted that they are prepared and willing to adopt technologies that can increase the availability and supply of earthworms, locusts and termites as feed for chickens. Earthworms are easy to produce, since some of the farmers are aware of the concept of vermicomposting which utilises crop residues, detritus material such as kitchen wastes. There is need to determine the digestibility, nitrogen retention, absorption and utilization of these protein sources in village chickens. Although locusts are commonly consumed by chickens, the farmers and active non-government non-profit organizations felt that propagation and production of locusts seems difficult. Consumer unacceptance of feed derived from maggots and snails could limit their use. The unacceptability of maggots and snails is based of cultural beliefs and negative perceptions about them.

Presence of NCAP sources has been reported in crop contents of birds (Goromela et al., 2007). There are variety of reservoirs of NCAP such as river banks, crop fields, cattle dung, and wetlands. Farmers with large chicken flock sizes are likely to have more attention on chicken husbandry, thereby aware of the potential of NCAP as potential protein feed source for chickens. Woman involvement on chicken management and production explains why they are likely to understand the potential of NCAP as feed to chickens. Nutritional value of NCAP supplements need to be determined. Nutritional status of scavenging chicken is also a prerequisite in different locations, seasons, and farming systems. This will help to determine how much of NCAP sources need to be supplemented.

Conclusions

Challenges to chicken production varied with gender of the head of household. Feed shortages were among the major challenges to chicken production. Chicken housing and religion highly influenced the household's probability to experience feed shortages. Farmers who supplemented chickens with NCAP were few and were mostly women. Farmers were aware that these NCAP sources have a potential of being used as chicken feed. Odds ratio estimates showed that farmers with large chicken flock sizes were likely to be aware of NCAP as potential feed to chickens. Availability of these animal protein sources is seasonal. NCAP were the main sources of proteins that chickens scavenge on.

Conflict of interest

No conflicts of interest exist between the authors and the

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REFERENCES

Aboe PAT, Boa-Amponsem K, Okantah SA, Butler EA, Dorward PT, Bryant MJ (2006). Free-range village chickens on the Accra Plains, Ghana: Their husbandry and productivity. Trop. Anim. Health Prod. 38:235-248.

Aklilu HA, Udo HMJ, Almekinders CJM, Van der Zijpp AJ (2008). How resource poor households value and access poultry?: Village poultry keeping in Tigray, Ethiopia. Agric. Syst. 96:175-183.

Dessie T, Ogle B (2001). Village poultry production system in the Central Highlands of Ethiopia. Trop. Anim. Health Prod. 33:521-537

Gondwe TN, Wollny CBA (2007). Local chicken production system in Malawi: Household flock structure, dynamics, management and health. Trop. Anim. Health Prod. 39:103-113.

Goromela EH, Kwakkel RP, Verstegen MWA, Katule AM (2007). Identification, characterisation and composition of scavengeable feed resources for rural poultry production in Central Tanzania. Afr. J. Agric. Res. 2:380-393.

Goromela EH, Kwakkel RP, Verstegen MWA, Katule AM (2006). Strategies to optimize the use of scavengeable feed resource base by smallholders in traditional poultry production systems in Africa: A review. Afr. J. Agric. Res. 1:91-100.

Guèye EF (2003). Gender issues in family poultry production systems in low-income food-deficit countries. Am. J. Altern. Agric. 18:185-195.

Halima H, Neser FWC, Van Marle-Koster E, De Kock A (2007). Village-based indigenous chicken production system in north-west Ethiopia. Trop. Anim. Health Prod. 39:189-197.

Kitalyi A (1998). Village chicken production systems in rural Africa. Household food and gender issues, FAO of the United Nations: Rome Italy P. 81.

Kuit HG, Traore A, Wilson RT (1986). Livestock production in central Mali: Ownership, management and productivity of poultry in traditional sector. Trop. Anim. Health Prod. 18:222-231.

McAinsh CV, Kusina J, Madsen J, Nyoni O (2004). Traditional chicken production in Zimbabwe. World Poultry Sci. J. 60:233-246

Mtileni BJ, Muchadeyi FC, Maiwashe A, Chimonyo M, Mapiye C, Dzama K (2013). Influence of socioeconomic factors on production constraints faced by indigenous chicken producers in South Africa. Trop. Anim. Health Prod. 45:67-74.

Muchadeyi FC, Sibanda S, Kusina NT, Kusina J, Makuza S (2004). The village chicken production system in Rushinga District of Zimbabwe. Livest. Res. Rural Dev. P. 16.

Mwale M, Masika PJ (2009). Ethno-veterinary control of parasites, management and role of village chickens in rural households of Centane district in the Eastern Cape, South Africa. Trop. Anim. Health Prod. 41:1685-1693.

Mwalusanya NA, Katule AM, Mutayoba SK, Mtambo MMA, Olsen JE, Minga UM (2001). Productivity of local chickens under village management conditions. Trop. Anim. Health Prod. 34:405-416.

Naidoo M (2003). Indigenous poultry production systems in Northern KwaZulu-Natal, South Africa, Paper presented at the 1st National Workshop on Indigenous Poultry Development, Nature and Development Group of Africa (NGO Registration No. 026-851-NPO). pp. 66-73.

Nyoni NMB, Masika PJ (2012). Village chicken production practices in the Amatola Basin of the Eastern Cape Province, South Africa. Afr. J. Agric. Res. 7:2647-2652.

Okeno TO, Kahi AK, Peters KJ (2012). Characterization of indigenous chicken production systems in Kenya. Trop. Anim. Health Prod. 44:601-608.

Paoletti MG, Buscardo E, Dufour DL (2000). Edible invertebrates among Amazonian Indians: A critical review of disappearing knowledge. Environ. Dev. Sustain. 2:195-225.

SAS (2003). Statistical Analysis System Users Guide, Version 9, SAS Institute Inc., Raleigh, North Carolina, USA.

Smith AJ (1990). The tropical agriculturalist - poultry CTA-Macmillan press London. pp. 47-64.

Sonaiya EB (2004). Direct assessment of nutrient resources in free range and scavenging systems. World's Poultry Sci. J. 60:523-535.

Tarwireyi L, Fanadzo M (2013). Production of indigenous chickens for household food security in rural KwaZulu-Natal, South Africa: A situation analysis. Afr. J. Agric. Res. 8:5832-5840.

Tiroesele B, Moreki BTJC (2012). Termites and earthworms as potential alternative sources of protein for poultry. Int. J. Agro. Vet. Med. Sci. 6:368-376.

Yakubu A (2010). Indigenous chicken flocks of Nasarawa State, north central Nigeria: Their characteristics, husbandry and productivity. Trop. Subtrop. Agroecosyst. 12:69-76.

Zindove TJ, Chimonyo M (2015). Comparison of trait preferences of Nguni farmers located in semi-arid and sub-humid environments. Trop. Anim. Health Prod. 47:607-611.

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Lead (Pb) and Cadmium (Cd) levels in fresh and smoke-dried grasscutter (*Thryonomys swinderianus* Temminck) meat

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Muscle, liver and kidney of wild and domesticated grasscutters were analyzed in order to determine the Lead (Pb) and Cadmium (Cd) concentrations. Meat samples were obtained from four different origin. A total of twenty-four (24) samples were used. An atomic absorption spectrophotometer (AAS) was used for the analysis after wet digestion of the samples with 1:3 Perchloric acid and Nitric acid. Data obtained were statistically analyzed using SAS. A randomized complete block design was used, with all treatments arranged in a 4×3×2 factorial. The levels (mg/kg, d.w.) of Pb and Cd obtained in (fresh and smoke-dried) muscle, liver and kidney samples were as follows: Pb (ND-0.513) (ND-0.154 and Cd (0.186-7.516) (0.277-2.723). Heavy metal concentrations were higher ($p < 0.05$) in fresh and smoke-dried muscle, liver and kidney of wild grasscutters than those levels found in domesticated grasscutters. However, Pb and Cd levels in fresh grasscutter samples were higher ($p < 0.05$) than those levels found in smoke-dried grasscutter samples. Cd concentration levels were higher than the recommended limits set by international regulations. Pb was not detected in domesticated grasscutters. Proper knowledge of public and health workers regarding hygienic and safe handling of bush meat as well as cutting off of infected parts, are highly recommended.

Key words: Cadmium, trace element, domestication, grasscutter, heavy metal, lead.

INTRODUCTION

Issues of food security and poverty have since been recognized as necessary conditions for the creation of a stable socio-political environment for sustainable economic development (Jibrin, 2004). Human nutrition requires that food be combined in an unbound, reduced and wholesome form so as to facilitate digestion, absorption and excretion as well as promote good health and not constitute any form of health hazard or such nutrition disorders as obesity, underweight, iron deficiency, dental caries and allergies (Mahan and Escott-Stump, 2004). Food borne-diseases pose more risk than vector-borne diseases (malaria, yellow-fever, plague, etc), water contact diseases (leptosipirosis, schistosomiasis), aerosolized or soil contact diseases (Lassa fever), respiratory diseases (meningococcal meningitis), and animal contact diseases such as rabies

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(Cia, 2011).

A common source of meat in West Africa is grasscutter, which is obtained from the wild. The grasscutter is a wild hystricomorphic rodent, widely distributed in most areas and constitutes an important source of animal protein (Asibey, 1974a, b; NRC, 1991). It is the second largest rodent after the porcupine. The average adult weight is 3 kg for females and 4.5 kg for males (Eben, 2004). The grasscutter has thickset body, measuring 40 to 60 cm in addition to a 20 to 25 cm tail (Fitzinger, 1995). Its furs comprise a mixture of brown-reddish and grey hairs that vary depending on its habitat (Jori and Chardonnet, 2001). Some other authors reported that the skin and hair (fur) as well as limbs and tails are easily torn out (Kingdom, 1974). This makes the animal very difficult to catch and even more difficult to handle after capture. The grasscutter is also a prominent and steady source of alternative dietary animal protein in many rural areas in Nigeria and other West African countries like Benin, Ghana, Togo, Cote D'voire (Ogunsanmi et al., 2002; Tettey et al., 2004; Ebabhamiegbebho and Ohanaka, 2012; Owen and Dike, 2012). However, the method of hunting is becoming a growing concern due to bullets containing Pb and other metals (Irschik et al., 2012; Doborowolska and Melosik, 2008; Hunt et al., 2009; ASEAN, 2012).

Heavy metals are ubiquitous in soil, water and air. These elements can be transferred into the food chain, thus presenting a risk for human health. Trace elements are toxic depending on dosis, and meat can be contaminated as those chemicals can be accumulated in tissues, such as Cd (Beiglbock et al., 2001) and Pb (Karita et al., 2000). Pb is a metabolic poison and a neurotoxin that binds to essential enzymes and several other cellular components and inactivates them (Cunningham and Saigo, 1997). Toxic effects of Pb are seen on haemopoietic, nervous, gastrointestinal and renal systems (Baykov et al., 1996). In humans, high Pb concentrations can cause damage to internal organs, especially to the central nervous system (CNS), and can even reduce the ability to create new blood cells. In adults, the kidneys are the most sensitive to chronic exposure to Pb. In children up to the age of 7, the nervous system is the most sensitive and such a threat is even greater in infants and small children (BfR, 2011). Humans can be exposed to Pb through foods, water, the air and dust but food is the main source of exposure (ASEAN, 2012). Cd levels have also been implicated in renal tube dysfunction (WHO, 2003). The accumulation of Cd and Pb increases with age and very high values have been registered in the muscle over surrounding the bullet pathway (Falandysz et al., 2005).

Determining heavy metal contamination in wild animal tissue, such as Pb and Cd can be the first step on knowing the levels of these elements, both in overall population and specific groups, such as hunters and their families. Consumption of meat from hunted wild ungulates is strictly associated with the issue of quality and hygiene assurance. The steps from hunting to market to assure meat quality are very difficult to control (Casoli et al., 2005; Coburn et al., 2005). Again, literatures on the level of these heavy metals in organs of various bush meat species particularly in Nigeria are largely unavailable. The consumers of grasscutter meat are unaware of the level of heavy metal occurrence. Moreover, heavy metal concentrations in grasscutter is a critical issue for consumer safety. The toxicity of heavy metal is one of the major environment problems and is potentially dangerous to animal and human health, because metals are able to bioaccumulate through food chain (Aycicek et al., 2008; Aschner, 2002). There is also the possibility that the heavy metal concentration levels in grasscutter varies with location/origin and thus requires investigation. Again, studies on heavy metals levels in organs of various bush meat species are scarce, particularly in Nigeria, and more research is required moreover, concentration of heavy metals in grasscutter is important for consumer safety as many grasscutter consumers are unaware of the level of heavy metal contamination in them. There is the need to improve the grasscutter meat to the status of a viable industry capable of meeting both local and foreign demands without depending largely on hunting from the wild (Adomah, 2009). The objective of this study was to evaluate the levels of Pb and Cd in muscle, liver and kidney of fresh and smoke-dried carcasses of wild and domesticated grasscutters.

MATERIALS AND METHODS

Sample collection

Fresh and smoke-dried meats of wild grasscutter were obtained from game meat processing centres in Uwa, New Benin and Arbico markets at Benin City, Edo State, Nigeria. Moreover, fresh and smoke-dried meats of domesticated grasscutters were obtained from Makarios graduate grasscutter farmers society at Benin City, Edo State, Nigeria. The sex, age and feeding habits of grasscutter were not taken into consideration during the sampling. A total of 24 random grasscutter samples were collected between March and September 2013. A randomized complete block design was used in a 4×3×2 factorial arrangement to assess whether heavy metals varied significantly between liver, kidney and muscle of the animal. The samples were collected in separate polyethylene bags and transported to the laboratory for their analysis.

Sample preparation

All collected samples were decomposed by means of wet digestion. About 1 g of the samples (liver, kidney and muscle) was weighed into the digestion flask. Then, 5 ml of 0.1 N perchloric acid and 15 ml of 0.1 N concentrated HNO_3 in a ratio 1:3 were added and then heated in an electric plate until clarification. Following, 5 ml of 20% HCl (0.1 N) was added to the content. The content of the flask was filtered using Whatman filter NO 42 paper into a 100 ml volumetric flask and was made up to the mark with a distilled water and then stored in a plastic reagent bottle, ready for Atomic Absorption Spectroscopy (AAS) analysis. Parallelly, 5 g of the fresh and smoke-dried samples (liver, kidney and muscle) was also weighed

Table 1. Mean concentrations of lead (mg/kg) in fresh kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
	DC	ND	ND	ND	ND
	NB	$0.165 \pm 0.005^{b}_{C}$	$0.369 \pm 0.002^{a}_{B}$	$0.119 \pm 0.008^{c}_{B}$	0.0000146
Pb	U	$0.276 \pm 0.005^{a}_{B}$	$0.214 \pm 0.006^{b}_{C}$	$0.089 \pm 0.002^{c}_{C}$	0.00000989
	AB	$0.326 \pm 0.107^{b}_{A}$	$0.513 \pm 0.022^{a}_{A}$	$0.141 \pm 0.008^{c}_{A}$	0.0000929
	SEM	0.0000108	0.0000608	0.000016	

ND: Not Detected; Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market (AB) Arbico Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within columns indicated locations are statistically significant; Mean with different scripts are significantly different at $P < 0.05$; Mean with the same scripts are not significantly different at $P > 0.05$.

in separate beakers and oven dried at 100°C to constant weight for determination of moisture content.

Sample analysis

Pb and Cd in fresh and smoke-dried liver, kidney and muscle samples were analyzed by using an atomic absorption spectrophotometer (AAS) MODEL-SOLAAR 969 UNICAM Series () at 217 nm for Pb and 228.8 nm for Cd. The allowed amounts of heavy metals (HMs) in food vary from country to country and are based on the World Health Organization (WHO) recommendations and local requirements (Oluyemi and Olabanji, 2011).

Statistical analysis

Data obtained from this study were statistically analyzed using Genstat software (2009) to establish the results. Data are shown as the mean ± standard error. Metal concentrations in fresh and smoke-dried samples were analyzed with respect to locations/origin. A statistical level of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Lead (Pb)

The results obtained in Table 1 showed that Pb concentration was significantly higher in the liver of fresh grasscutters from Arbico market, than those levels from New Benin and Uwa markets. On the other hand, Pb concentration (mg/kg) in kidney of smoke-dried grasscutters was higher ($P<0.05$) at Uwa market than Pb levels at Arbico and New Benin markets (Table 2). Pb concentration was significantly higher in fresh liver, kidney and muscle than in the smoke-dried samples irrespective of the market location.

The highest Pb levels (0.513 and 0.154 mg/kg) were observed in fresh liver and smoke-dried kidney at Arbico and Uwa market, respectively. The lowest concentrations in fresh and smoke-dried muscle were found at Uwa (0.089 mg/kg) and New Benin (0.043 mg/kg) market. This finding indicates that Pb concentrations in fresh and smoke-dried samples from all markets were lower than the permissible limit (1 mg/kg) set by the Australia-New Zealand Food Authority (ANZFA, 2001). Pb levels showed that fresh and smoke-dried samples were also lower than 1 mg/kg reported by Galadima and Garba (2011), and approved by the Federal Environmental Protection Agency of Nigeria (FEPA). Similar Pb values were also observed by Falandysz (1994) in liver and kidney, ranging from 0.090 to 0.240 mg/kg and 0.080 to 0.360 mg/kg, respectively. In agreement, Jarzynska and Falandysz (2011) reported Pb contents in liver and kidney of deer as 0.17 mg/kg and 0.30 mg/kg (dry weight), respectively. An earlier study by Doganoc and Gacnik (1995) reported similar Pb levels (0.16 mg/kg) in liver of roe, fallow deer, deer, pheasant, wild duck and hare in Slovenia.

On the contrary, Martelli (2005) found higher Pb levels in liver of wild boars as exceeding regulatory limit, even though he did not observe high values in kidney. Meanwhile, Piskorova et al. (2003) observed higher Pb levels in kidney than Pb levels in liver (0.39 and 0.24 mg/kg, respectively) of wild hunted boars in the Slovak Republic. Martelli (2005) observed similar Pb concentrations in liver (0.302 to 0.674 mg/kg, w.w.) and in kidney (0.401 to 0.774 mg/kg, w.w.) of wild boars reared in Pisa, Italy. Similarly, Flanjak and Lee (1979) reported Pb concentrations up to 0.85 mg/kg and 2.25 mg/kg in liver and kidney of cattle at New South Wales, Australia. An earlier study showed that Pb levels in liver of birds (turkey, pheasant and partridge) were as high as 25 mg/kg (Kreager et al., 2008). Similarly, Maldonado et al. (1996) studied Pb related to its intestinal mobilization during lactation in rats and found significantly higher Pb levels in liver and kidney.

In agreement with that, Mariam et al. (2004) reported Pb levels of 2.18, 4.25 and 3.15 mg/kg in beef, mutton and poultry, respectively. Hunt et al. (2009) stated that high Pb levels could be related to the metal dispersion

Table 2. Mean concentrations of lead (mg/kg) in dried kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
	DC	ND	ND	ND	ND
	NB	$0.111 \pm 0.003^{b}_{B}$	$0.129 \pm 0.002^{a}_{C}$	$0.043 \pm 0.003^{c}_{C}$	0.00000246
Pb	U	$0.154 \pm 0.004^{a}_{A}$	$0.146 \pm 0.006^{a}_{B}$	$0.068 \pm 0.003^{b}_{A}$	0.0000085
	AB	$0.098 \pm 0.002^{b}_{C}$	$0.153 \pm 0.003^{a}_{A}$	$0.057 \pm 0.002^{c}_{B}$	0.00000219
	SEM	0.00000255	0.0000047	0.0000025	

ND: Not Detected; Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market; (AB) Arbico Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within columns indicated locations are statistically significant; Mean with different scripts are significantly different at $P < 0.05$; Mean with the same scripts are not significantly different at $P > 0.05$.

Table 3. Mean concentrations of cadmium (mg/kg) in fresh kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
	DC	$0.477 \pm 0.035^{a}_{D}$	$0.360 \pm 0.021^{b}_{D}$	$0.186 \pm 0.016^{c}_{D}$	0.00029
	NB	$1.071 \pm 0.026^{b}_{C}$	$3.923 \pm 0.071^{a}_{B}$	$0.636 \pm 0.055^{c}_{C}$	0.00137
Cd	U	$4.045 \pm 0.074^{a}_{A}$	$3.452 \pm 0.059^{b}_{C}$	$1.873 \pm 0.031^{c}_{B}$	0.00156
	AB	$3.592 \pm 0.020^{b}_{B}$	$7.516 \pm 0.079^{a}_{A}$	$2.459 \pm 0.041^{c}_{A}$	0.00131
	SEM	0.000917	0.00179	0.000694	

Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market; (AB) Arbicu Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within locations indicated locations are statistically significant; Mean with different scripts are significantly different at $P < 0.05$; Mean with the same scripts are not significantly different at $P > 0.05$.

after bullet fragmentation into animal body. The levels of Pb in animals depends on various factors such as the frequency of the quantity of meat consumed, the degree of leaded-bullet fragmentation, the path that the ammunition has taken and the level of care taken to remove the flesh surrounding the wounds (Hunt et al., 2009). The results revealed that Pb levels in fresh and smoke-dried kidney, liver and meat of grasscutters were at safe levels and were of no danger to health (ANZFA, 2001). As shown in Tables 1 and 2, Pb concentrations were significantly higher in fresh tissues than in smoke-dried tissues, in agreement with the findings of Ajani et al. (2013) and Eboh et al. (2006).

Cadmium (Cd)

As shown in Table 3, Cd concentrations were significantly higher in liver, kidney and muscle of fresh grasscutters at Arbico, Uwa, New Benin and Domesticated. On the contrary, Cd concentrations were lower ($P<0.05$) in liver, kidney and muscle of smoke-dried grasscutters at Arbico, Uwa, New Benin and Domesticated location (Table 4). Cd concentration was the highest in fresh liver at Arbico market, whereas Cd level was the highest in smoke-dried muscle at Uwa market. In general, Cd levels in samples of kidney, liver and muscle of fresh grasscutters were significantly higher than Cd levels detected in samples of smoke-dried grasscutters.

The highest Cd concentrations were observed in fresh liver (7.516 mg/kg) and smoke-dried muscle (2.723 mg/kg) from Arbico and Uwa market. Those Cd levels are above the permissible limit (0.5 mg/kg) stated in meat and liver (FAO/WHO, 2000). On the contrary, the lowest Cd concentrations were found in fresh muscle (0.186 mg/kg) and smoke-dried muscle from domesticated samples. The highest Cd levels in fresh kidney (4.045 mg/kg) and smoke-dried kidney (1.75 mg/kg) were detected in samples from Uwa market. These Cd values in kidneys are above the limit (1.0 mg/kg) accepted (FAO/WHO, 2000). Aranha et al. (1994) and Roga-Franc et al. (1996) found also Cd levels above threshold values (FAO/WHO, 2000) in liver and kidney of cattle in Poland. Doganoc (1996) found Cd levels in liver and kidney of chicken above official tolerance levels.

In contrast, Mariam et al. (2004) reported Cd

Table 4. Mean concentrations of cadmium (mg/kg) in smoke-dried kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
Cd	DC	$0.795 \pm 0.032^{a}_{C}$	$0.636 \pm 0.019^{b}_{D}$	$0.277 \pm 0.019^{c}_{D}$	0.00027
	NB	$1.002 \pm 0.016^{b}_{B}$	$1.957 \pm 0.022^{a}_{B}$	$0.331 \pm 0.017^{c}_{C}$	0.000157
	U	$1.750 \pm 0.053^{b}_{A}$	$1.690 \pm 0.017^{b}_{C}$	$2.723 \pm 0.040^{a}_{A}$	0.000744
	AB	$1.060 \pm 0.011^{b}_{B}$	$2.205 \pm 0.033^{a}_{A}$	$0.871 \pm 0.007^{c}_{B}$	0.00019
	SEM	0.00049	0.000258	0.00027	

Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market; (AB) Arbicu Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within columns indicated locations are statistically significant; Mean with different scripts are significantly different at $P < 0.05$ and Mean with the same scripts are not significantly different at $P > 0.05$.

Table 5. Mean concentrations of heavy metals (mg/kg) in fresh kidney, liver and muscle of grasscutters (dry matter basis).

Organ	**Pb**	**Cd**	**SEM**
Kidney	0.1918^{b}	2.2965^{a}	0.1838
Liver	0.2738^{b}	3.8127^{a}	0.299
Muscle	0.0873^{bc}	1.2886^{a}	0.1319

ND: Not detected; SEM: Standard Error of Mean; Means with different superscripts (a, b, c) within rows are significantly different at $p < 0.05$

concentrations of 0.33, 0.37and 0.31 mg/kg in lean meat of beef, mutton and poultry, respectively. Previously, a study conducted at Poland by Zmudzki and Szkoda (1995) found 0.146 mg Cd/kg in liver and 0.580 mg Cd/kg in kidney of young cattle, while in older animals Cd levels were 0.204 and 0.829 mg/kg in liver and kidney, respectively. Similarly, Falandysz (1994) reported 0.10 mg Cd/kg and 0.450 mg Cd/kg in liver and kidney of cattle in northern Poland. Tahvonen and Kumpulainen (1994) reported in cattle liver, values of 0.061 mg Cd/kg from Finland, 0.070 mg Cd/kg from Sweden and 0.105 mg Cd/kg from Netherlands. Those values probably are reflecting a major environmental pollution, as also suggested by Gasparik et al. (2012), because Cd is one of the most toxic elements to humans and animals (Naja and Volesky, 2009). Cd is toxic virtually to every system in the normal body. The high levels of Cd found in both fresh and smoke-dried tissues are significantly higher than the limits set by FAO/WHO (2000). Chemically, the consumption of grasscutter meat could present a risk for human health.

Interactions

The levels of Pb and Cd found in the fresh and smoke-dried liver samples from the different locations were generally higher than the concentration levels of these heavy metals in the fresh and smoke-dried kidney and muscle samples (Tables, 5, 6 and 7). However, Cd levels were lower in muscle samples than kidney and liver levels in both fresh and smoke-dried samples. This is an indication that these heavy metals accumulate more in the liver than in the kidney and muscle. However, there are a few exceptions, where the concentrations of these heavy metals are higher in the kidney and in the muscle.

It is a favorable condition that Pb was not detected in all the samples of the domesticated grasscutter location. This may probably be attributed to method of capture or capture procedure, good monitoring and uncontaminated feed or drinking water as well as low environmental pollution near the breeding location or area. Overall, the results of this study show that the levels of (Pb) was generally low compared to Cd in the kidney, liver and muscle of fresh and smoke-dried grasscutters studied. Generally low Pb and Cd concentrations observed in the smoke-dried kidney, liver and muscle reduced. This agrees with Ahmed et al. (2011) who suggested heavy metal as being water borne which could explain a dripping deposition resulting from the washing of the meat prior to smoking. The drop in concentration of heavy metal in the smoked samples was also corroborated by Ajani et al. (2013) and Eboh et al. (2006) who attributed it to heat having a significant effect on heavy metals and a possibility of the heavy metal being converted to other compounds.

Table 6. Mean concentrations of heavy metals (mg/kg) in smoke-dried kidney, liver and muscle of grasscutters dry matter basis).

Organ	Pb	Cd	SEM
Kidney	0.0909^{bc}	1.1517^{a}	0.0427
Liver	0.1071^{a}	1.5532^{a}	0.0773
Muscle	0.0419^{b}	1.0508^{a}	0.1248

ND: Not detected; SEM: Standard Error of Mean; Means with different superscripts (a, b, c) within rows are significantly different at $p < 0.05$

Table 7. Interactive comparisons of heavy metal concentrations (mg/kg) between fresh and smoke-dried tissues of grasscutters (dry matter basis).

	Fresh		Dried		
Organ	**Pb**	**Cd**	**Pb**	**Cd**	**SEM**
Kidney	0.1918^{c}	2.2965^{a}	0.0909^{c}	1.1517^{b}	0.240
Liver	0.2738^{c}	3.8127^{a}	0.1071^{c}	1.5532^{b}	0.394
Muscle	0.0876^{b}	1.2886^{a}	0.0419^{b}	1.0508^{a}	0.204

ND: Not Detected, SEM: Standard Error of Mean; Means with different superscripts (a, b, c) within rows are significantly different at $p < 0.05$.

It is imperative to enforce actions to ensure an adequate health education to people, public health personnel and bush meat handlers through media. Grasscutter meat must be safe and suitable for human consumption, therefore all stakeholders including scientists, government, industries and consumers have a role in achieving this outcome. Sadly, West Africa has depleted stocks of wildlife, and farmers have to be financially supported by both public and private sectors toward the domestication of grasscutter. These measures could guaranty a continuous provision of meat of good quality to the growing population. In the meantime, it is highly recommended to cut off animal parts which have bullet wounds before meat processing.

Conclusion

This study has shown that Pb levels were significantly higher in liver, kidney and muscle of fresh grasscutters than smoke-dried grasscutters. Pb was not detected in fresh and smoke-dried organs of domesticated grasscutter samples. Cd levels were highest in fresh liver and smoke-dried muscles of meat obtained from local markets even though Cd levels were lower from samples of domesticated grasscutters. Education of people and public health officials on proper bush meat handling is also highly required.

Conflict of Interest

We declare that we have no conflict of interest.

REFERENCES

Adomah YJC (2009). Features and profitability of domestic grasscutter production in the brong Ahafo region. A thesis submitted to the school of graduate studies, Kwame Nkrumah, University of Science and Technology, Kumasi-Ghana. P. 8.

AESAN (2012). Scientific Committee Risk associated with the Presence of lead in wild game meat in Spain. Report approved by the Scientific Committee on plenary session, February 22nd, 2012. Reference Number: AESAN 2012-002.

Ahmed A, Dodo A, Bouba AM, Clement S, Dzudie T (2011). Influence of Traditional Drying and Smoke-drying on the Quality of Three fish Species (*Tilapia nilotica, Silurus glanis* and *Arius parkii*) from Lagdo lake, Cameroon. J. Anim. Vet. Adv.10(3):301-306.

Ajani F, Adetuaji VO, Oyedokun JO (2013). Biophysiochemical changes that occur in fish during different stages of traditional processing. Department of Animal Science and Fisheries Management, Bowen University, Iwo, Nigeria. Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria. 13(3) June 13.

ANZFA (Australia New Zealand Food Authority) (2001). Wellington NZ 6036 May, 2001. Retrieved from: http://www.anzfa.gov.au

Aranha K (1994). Environmental Chemistry. 3rd Edn. New Age International Ltd. Publisher, New Delhi, pp. 213-219.

Aranha S, Nishkawa AM, Taka T, Salioni EMC (1994). Cadmium and lead levels in cattle's liver and kidney. Rev. Inst. Adolfo Lutz. 54:16-20.

Aschner M (2002). Neurotoxic mechanism of fish-bone methylmetry. Environ. Toxicol. Phamacol. 12:101-102.

Asibey EOA (1974(a)). Wildlife as a source of protein in Africa South of the Sahara. Biol. Conserv. 6(1):32-39.

Asibey EOA (1974b). The Grasscutter (*Thryonomys swinderianus Temminck*, in Ghana. Symp. Zool. Soc. Lond. 34:161-170.

Aycicek M, Kaplan M, Yarman M (2008). Effect of cadmium on germination, seedling growth and metal contents of sunflower (*Helianthus annus* L). Asian J. Chem. 20:2663-2672.

Baykov BD, Stoyanov MP, Gugova ML (1996). Cadmium and lead bioaccumulation in male chickens for high food concentrations. Toxicol. Environ. Chem. 54:155-159.

Beiglbock C, Steineck T, Tataruch F, Ruf T (2001). Environmental candium induces histopathological changes in kidneys of roe deer. Environ. Toxicol. Chem. 21:1811-1816.

BfR (2011). Bundesinstitut für Risikobewerfung. Lead fragments in game meat can be added health risk for certain consumer groups.
Casoli C, Duranti E, Cambiotti F, Avellini P (2005). Wild ungulate slaughtering and meat inspection. Vet. Res. Commun. 29:89-95.
Cia (2011). World Factbook. Major Infectious diseases. http//www.indexmundi.com/Nigeria.
Coburn HL, Snary EL, Kelly LA, Wooldridge M (2005). Qualitative risk assessment of the hazards and risks from wild game. Vet. Rec. 157:321-322.
Cunningham WP, Saigo BW (1997). Environmental Science a Global Concern. 4th Edition, WMC Brown Publisher, New York, P. 389.
Doborowolska A, Melosik M (2008). Bullet derived lead in tissues of the wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). Eur. J. wildlife Res. 54:231-235.
Doganoc DZ (1996). Distribution of lead, cadmium, and zinc in tissues of hens and chickens from Slovenia. Bull. Environ. Contam. Toxicol. 57:932-937.
Doganoc DZ, Gacnik KS (1995). Lead and cadmium in meat and organs of game in Slovenia. Bull. Environ. Contam. Toxicol. 54:166-170.
Ebabhamiegbebho PA, Ohanaka MC (2012). Consumer preferences for different types of Bush meat sold in Benin City, Nigeria. J. Appl. Sci. 30:105-110.
Eben AB (2004). Grasscutter: Importance, Habitat, Characteristics, Feed and Feeding, breeding and diseases. Centre for Biodiversity Utilization and Development (CBUD,) Kumasi Ghana, pp. 1-4.
Eboh L, Mepba HD, Ekpo MB (2006). Heavy metal contaminants and processing effects on the composition, storage stability and fatty acid profiles of five common commercially available fish species in Oron Local Government, Nigeria. Food Chem. 97:490-497.
Falandysz J (1994). Some toxic and trace metals in big game hunted in the northern part of Poland in 1987-1991. Sci. Total Environ. 141:59-73.
Falandysz J, Szymczyk-Kobrzyska K, Brzostowski A, Zalewski K, Zasadowski A (2005). Concentrations of heavy metals in the tissues of red deer (*Cervus elaphus*) from the region of Warmia and Mazury, Poland. Food Addit. Contam. 22:141-149.
FAO/WHO (2000). Report of the 32nd Session of the codex committee of the food additives Contaminants. Beijing People's Republic of China, 20-24 March.
Fitzinger F (1995). Cane rats. In: Nowak, R, (ed.) Walker's Mammals of the World. The John Hopkins University Press, pp. 1650-1651.
Flanjak J, Lee HY (1979). Trace metals content of livers and kidneys of cattle. J. Sci. Food Agric. 30:503-507.
Galadima A, Garba ZN (2011). Recent Issues in Environemntal Science. "Including incidences and reports from Nigeria". Lap Lambert Academic Publishers, Germany, ISBN: 978-3-8454-2915-1, P.100.
Gasparik J, Dobias M, Capcarova M, Smehyl P, Slamecka J, Bujko J (2012). Concentration of cadmium, mercury, zinc, copper and cobalt in the tissues of wild boar (*Sus scrofa*) hunted in the western Slovakia. J. Environ. Sci. Health A. 47:1212-1216.
GENSTAT (2009) Pc and Windows Vista Package, Law Agricultural Trust, Rothamsted Experimental station. ISN International Ltd. Version PL20.1.
Hunt WG, Watson RT, Oaks LJ, Parish CN, Burnham KK, Tucker RL, Belthoff JR, Hart G (2009). Lead Bullet Fragments in Venison from Rifle-Killed Deer: Potential for Human Dietary Exposure. PLoS ONE 4(4):e5330- e5330.
Irschik I, Bauer F, Sager M, Paulsen P (2012). Copper residues in meat from wild artiodactyls hunted with two types of rifle bullets manufactured from copper. Eur. J. Wildlife Res. doi:10.1007/s10344-012-0656-9.
Jarzynska G, Falandysz J (2011). Selenium and other 17 largely essential and toxic metals in muscle and organ meats of Red Deer (*Cervus elaphus*)-Consequences to human health. Environ. Int. 37:882-888.
Jibrin A (2004). Global food security. Excerpt of Obasanjo's speech. In Obasanjo and the New Face of Nigeria's Foreign Policy. M.O.D. Press and publishers, Kaduna.
Jori F, Chardonnet P (2001). Cane rat farming in Gabon. Status and Perspective. Paper presented at the 5th Iritemational wildlife Ranching Symposium, Pretoria, South Africa. March, pp. 33-51.
Karita K, Shinozaki T, Yano E, Amari N (2000). Blood lead levels in copper smelter workers in Japan. Ind. Health 38:57-61.
Kreager N, Wainman BC, Jayasinghe RK, Tsuji LJS (2008). Lead Pellet Ingestion and Liver-Lead Concentrations in Upland Game Birds from Southern Ontario, Canada. Arch. Environ. Contam. Toxicol. 54:331-336.
Mahan LK, Escott-Stump S (2004). Krause's Food, nutrition and diet therapy, 11th Edition Elsevier, USA.
Maldonado VM, Cerbon SJ, Albores AM, Hernandez LC, Calderonsalinas JV (1996). Lead intestinal absorption and bone mobilization during lactation. Hum. Exp. Toxicol. 15:872-877.
Mariam I, Iqbal S, Magra S (2004). Distribution of some trace and macrominerals in beef, mutton and poultry. Agric. Biol. 6(5):816-820.
Martelli P (2005). Controllo ispettivo qualitative di carni di cinghiale regolarmente macellati nella provincia di Siena. Degree Diss., Università di Pisa, Italy.
Naja GM, Volesky B (2009). Toxicity and sources of Pb, Cd, Hg, Cr, As and radionuclides in the environment. Available at http://biosorption.mcgill.ca/publication/HandB-Ch2.pdf. Accessed on March10, 2015.
National Research Council (NRC) (1991). Micro livestock. Luthe-Kwnown Small Animals (grasscutter) with promising Economic future. National Academy Press, Washington, D.C., P. 449.
Ogunsanmi AO, Ozegbe PC, Ogunjobi D, Taiwo VO, Adu JO (2002). Haematology, plasma biochemistry and whole blood minerals of the captive adult African grasscutter (*Thryonomys swinderianus* Temminck). Trop. Vet. 20:27-35.
Oluyemi EA, Olabanji IO (2011). Heavy Metals Determination in Some Species of Frozen Fish sold at Ile-Ife Main Market, South West Nigeria. Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria. Ife J. Sci. 13(2) ISSN 0794-4896.
Owen OJ, Dike VA (2012). Grasscutter (*Thryonomys swinderianus*) Husbandry in Nigeria. A Review of the potentialities, opportunities and challenges. Department of Animal science, Rivers State University of Science and Technology, Port Harcourt, Nigeria. J. Environ. Issues Agric. Dev. Ctries. 4(1). April 2012.
Piskorová L, Vasilková Z., Krupicer I (2003). Heavy metal residues in tissues of wild boar (*Sus scrofa*) and red fox (*Vulpes vulpes*) in the Central Zemplin region of the Slovak Republic. Czech J. Anim. Sci. 48:134-138.
Roga-Franc M, Kosla T, Rokicki E (1996). Cadmium concentration in organs of dairy cows depending on that element contents in meadow cover. Mengen-Spurenelem. Arbeistag. P. 393-397.
Tahvonen R, Kumpulainen J (1994). Lead and Cadmium contents in pork, beef and chicken and in pigs and cow liver in Finland during 1991. Food Addit. Contam. 11(4):415 426.
Tettey ECT, Osei-Yaw A, Hodari-Okae M (2004). Studies on the quality of traditionally smoked-dry snail meat (*Achatina achatina*) in Ghana. Ghan. J. Agric. Sci. 30:145-150.
Watanabe M (1997). Phytoremediantion on the brink of commercialization. Environ. Sci. Technol. 3:182-186.
WHO (2003). World Health Organization. Evaluation of certain food additives and contaminants. Sixty-first report of the Joint FAO/WHO Expert Committee on food Additives. Rome, 10-19th June 2003.
Zmudzki J, Szkoda J (1995). Zawartose Kadmuw zywnosci pochodzenia zwierze. Cego Medycyna Pracy 5(5):71-76.

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Full Length Research Paper

Seeds germination and early development of beggartick on extracted soil solution from an area with cover crops

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Plants produce and store many products of secondary metabolism that are released into the environment and can influence direct or indirectly on nearby organisms. Thus, this study investigated the allelopathic potential of extracted soil solution from an area with cover crops according to germination and early development of beggartick. Soil solution was extracted in an area where black oats, turnip and hairy vetch were grown. The concentrated solution was tested in sand substrate while the dilutions were tested in 100; 200; 300 and 400 ml L^{-1} germitest paper substrate, plus the control treatment on beggartick. Germination was tested for 10 days and the early development for 20 days. Twenty seeds or seedlings were distributed in gerbox, with three replications and the experimental design was completely randomized (CRD). Soil solution showed considerable changes on beggartick germination only in the last collections, mainly with extracted solution in an area cropped with turnip and hairy vetch. The results, in this case, were more significant when germitest was used than with sand substrate. Therefore, it is recommended these cover crops in crop rotation, aiming to reduce beggartick infestation in a long term.

Key words: Allelopathy, Weeds, crop rotation, secondary metabolites.

INTRODUCTION

Secondary metabolites had been considered, wrong, without function, except in storing extra carbon that was fixed during photosynthesis process, but it is known that they play a fundamental role in the process of plants development and their interaction with the environment (Kutchan, 2001). Plants produce and store many products of secondary metabolism, which are released into environment. They may suffer direct or indirectly influence of a number of factors such as quality and light intensity, day length, mineral deficiency, age of plant organs, genetic, pathogens and predators, among others. These factors can influence both allelochemical production in donor species and their susceptibility to the same receptor species (Pedrol et al., 2006).

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On soil, these substances can interact strongly with colloids and also suffer microbial transformations, responsible for their disappearance or deactivation. Microorganisms have the ability to convert, synthesize, eliminate or use secondary metabolites as carbon and energy sources. The allelochemicals can be immobilized on solid phase, can establish equilibrium with soil solution and can undergo degradation or even leaching, mainly on sandy soils (Moreira and Siqueira, 2006; Colozzi Filho; Andrade, 2006).

A lot of work done has been allelopathic effects on weeds in recent years (Isik et al., 2009; Souza et al., 2010; Mauli et al., 2011; Rosa et al., 2011), however, many things still need to be studied and what new information will be added to science via this research. Knowledge of occurrence and activity of microorganisms and natural compounds in soil processes underlies the design of crop rotation and residue management, in order that they get more efficient and conservationist (Pedrol et al., 2006). Allelopathic effects and action mechanisms of many substances are also important to understand plants interactions in both natural and agricultural systems (Ferreira and Aquila, 2000).

In this context, this study aimed to evaluate the allelopathic potential of extracted soil solution in an area with black oats (*Avena strigosa* Schreb.), turnip (*Vicia villosa* Roth) and hairy vetch (*Raphanus sativus* L.) as cover crops on seed germination and early development of beggartick (*Bidens pilos*a), weed affecting commercial crops.

MATERIALS AND METHODS

The trials with soil solution were carried out at Seeds and Plants Evaluation Laboratory, at Western Paraná State University, *Campus* of Cascavel-PR, Brazil. The solutions were collected with soil solution extractors and it was used a 20 cm pipe with a ceramic tip of 1 Bar to be applied on soils of intermediate and high hardness, model EX-20-TR, brand Tracom®, according to Filizola (2006). The extractors were set in the cropped area with black oats, turnip and hairy vetch, 20 cm depth, in Catanduvas-PR countryside.

The study was carried out under field conditions in farm, whose geographical location is 25° 18' 16" S latitude, 53° 11' 34.1" W longitude and 465 m altitude. The climate is subtropical, mesothermal and super-humid, with 1,800 mm average annual rainfall, hot summers, with rainfall concentrations without defined dry season and little frequent frosts. The annual average temperature is around 20°C and average relative humidity is 75% (IAPAR, 1998). The local soil is classified as dystrophic Oxisol Udic (USA, 1998) or red distroferric latosol with a soft relief and clayey soil, according to EMBRAPA (2006). Soil solution samples were obtained in 15, 30; 45 and 60 days after the management of the three evaluated cover crops.

The soil solutions were collected and tested on germination and early plant development of beggartick weed. Concentrated solutions were directly tested on seed germination, in gerbox, with approximately 1 cm of sand. It was moistened with distilled water and subsequently added 3 ml of pure soil solution plus the control treatment with only distilled water. Dilutions were also obtained with water as solvent from the pure solution to 100, 200, 300 and 400 ml L^{-1} and the control treatment, which were applied in germitest substrate about 20 beggartick seeds.

Germination evaluation was daily obtained during 10 days in growth chamber for 12 h of light and 25°C, while germinated seeds were removed. The seeds with 2.00 mm root extension were considered as germinated. The germination speed index (GSI) was calculated according to Maguire (1962) and germination speed (GS) was obtained according to Edmond and Drapalla (1958).Ten pre-germinated seedlings, with three days, were used to obtain the early development of plants. They were taken to growth chamber for 24 h light photoperiod at 25°C. After 20 days, the plants were separated into roots and shoots and the fresh mass (g per plant) was determined on a 0.001 g precision scale. They were dried at 65°C until constant mass so, dry mass was determined. The experiment was a completely randomized design (CRD) with three replications per treatment. The results of seed germination were presented as percentage of inhibition in relation to the control. Qualitative data were submitted to analysis of variance and to regression analysis at 5% probability to identify the significant model of the highest degree of regression with some adjustments to the studied data. Quantitative data were submitted to averages comparison by Tukey test at 5% probability.

Data variability evaluation was initially performed to verify data normality and homogeneity of variances and if necessary their transformation; these analyses were performed according to Pimentel Gomes (2000) and Banzattto and Kronka (2000) with Minitab® 14 and SISVAR software (Ferreira, 2008). Data in percentage were transformed by arcsen$\sqrt{}$ (x / 100) and those who were not normally distributed underwent transformation $\sqrt{x}$. A trend line and a regression equation were added for significant data according to the model. On the other hand, the average plotted values were presented for non-significant data.

RESULTS AND DISCUSSION

The SGI of beggartick seeds under soil solution, in an area cropped with turnip, collected 15 days after cover crop management, showed significant difference, with a decrease on germinated seeds number per day as soil solution concentration increased. Germination inhibition percentage and SG showed no changes when submitted to black oats, turnip and hairy vetch (Figure 1). The allelopathic effect sometimes does not occur on germination percentage, but on other parameters of this process such as germination speed (Ferreira and Borghetti, 2004), and this answer was observed in this trial.

The soil solution collected 30 days after cover crops management did not influence on germination percentage with black oats, turnip and hairy vetch. There was a decrease on GSI of beggartick seeds when concentration of soil solution (extracted from areas cropped with the three studied species) was increased (Figure 2). The germination speed of beggartick seeds was influenced by soil solution with hairy vetch. Thus, the number of days for seeds germination was increased as soil solution concentration increased.

Allelopathy is a mechanism by which certain plants interfere on the others development. It can become crop management by the plants use that control some undesired species, consequently, more efficient production systems are obtained (Goldfarb et al., 2009).

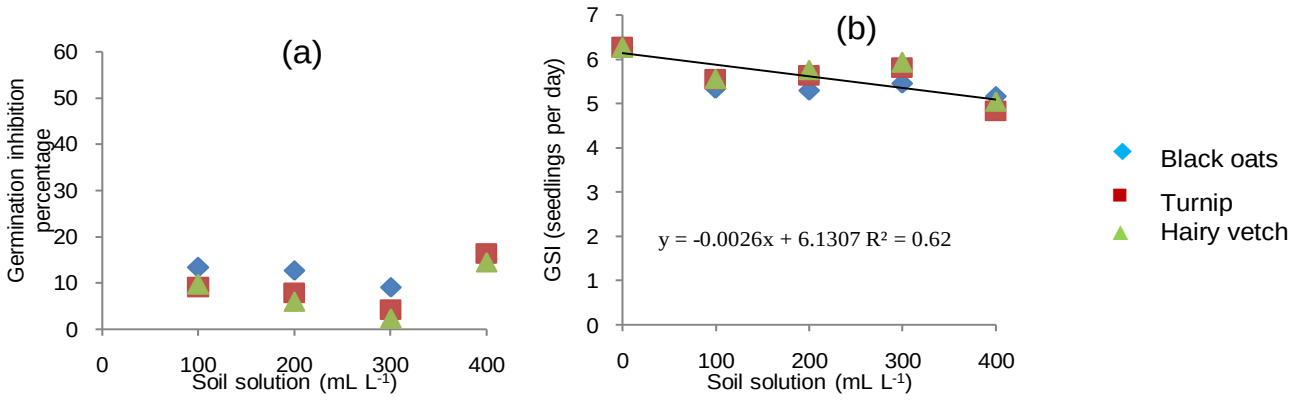

Figure 1. (a) Germination inhibition percentage and (b) germination speed index (GSI), of beggartick seeds moistened with soil solution in 0, 100, 200, 300 and 400 mL L^{-1} ratios, collected 15 days after management of black oats, turnip and hairy vetch as cover crops.

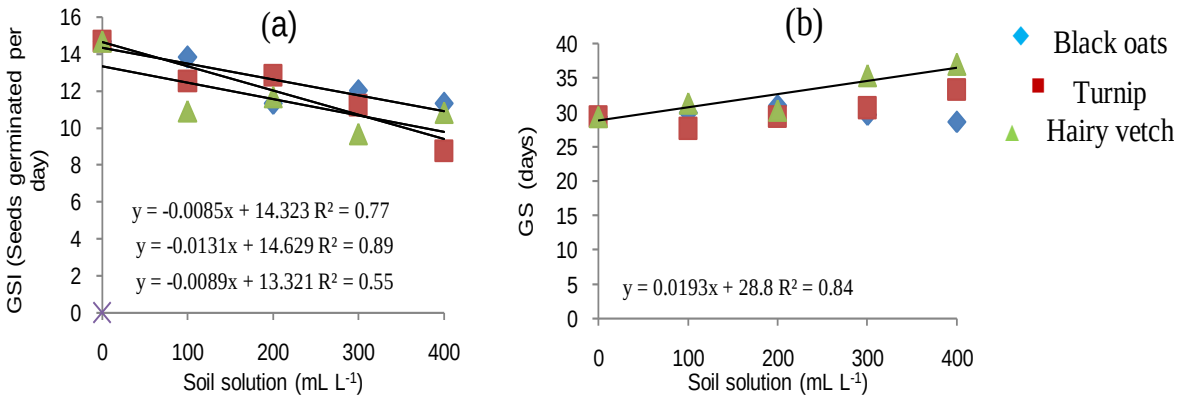

Figure 2. (a) Germination speed index (GSI) and (b) germination speed (GS) of beggartick seeds moistened with soil solution in 0, 100, 200, 300 and 400 ml L^{-1} ratios, collected 30 days after management of black oats, turnip and hairy vetch as cover crops.

There was no significant difference in the following parameters: germination inhibition percentage, germination speed index and germination speed when soil solution extracted from cropped area with the three studied cover crops was applied. This occured in the third collection 45 days after the management.

Martins et al. (2006) have already studied the soil solution in an area cropped with *Brachiaria brizantha* cv. Marandu for five years. The authors found out that there was no influence of soil solution on germination speed index and normal seedlings percentage of *Sida rhombifolia*. This trial is in accordance with these results with beggartick weed. One of the possible causes of non-fact is that plant substances, when in soil, interact strongly with colloids and may also suffer microbial transformations responsible for disabling or even degradation (Moreira and Siqueira, 2006).

The speed germination index of seeds submitted to the last collection of soil solution (Figure 3), 60 days after the management of cover crops, showed significant difference under extracted soil solution from a black oat cropped area. There was some decrease up to 200 ml L^{-1} concentrations and there was an increase in the number of germinated seeds per day when this value increased. Martins et al. (2006) studied the soil solution from an area with *B. brizantha* cv. Marandu (Poaceae), cropped along five years and found out that, when compared to distilled water, the soil solution decreased the germination speed index of *Panicum maximum* cv. Tanzania, as it happened in this experiment with black oats (Poaceae) (Figure 3).

The germination speed also showed statistically significant differences in soil solution extracted in an area cropped with black oats. Thus, it reduced the number of days the seeds took to germinate, as concentration increased. It was observed that the shoot dry mass of beggartick plants that were moistened with soil solution, extracted from an area cropped with turnip and collected 15 days after management of cover crops, showed

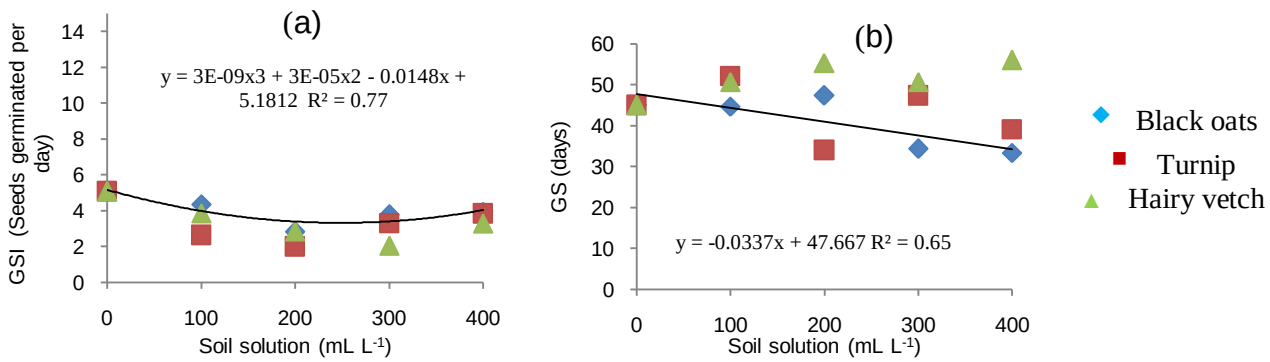

Figure 3. (a) Germination speed index (GSI) and (b) germination speed (GS) of beggartick seeds moistened with soil solution in 0, 100, 200, 300 and 400 ml L^{-1} ratios, collected 60 days after management of black oats, turnip and hairy vetch as cover crops.

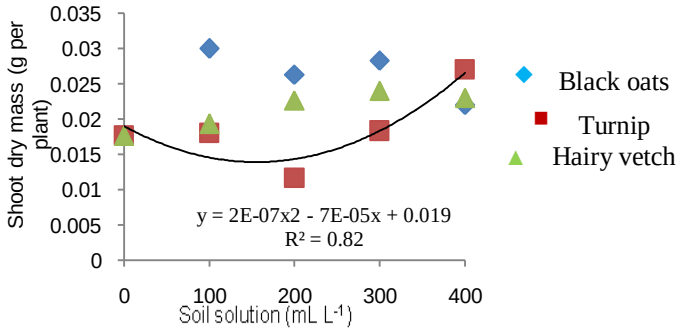

Figure 4. Shoot dry mass (g per plant) of beggartick, moistened with 0, 100, 200, 300 and 400 ml L^{-1} soil solution, collected 15 days after management of black oats, turnip and hairy vetch as cover crops.

decreases in 100 and 200 ml L^{-1} concentrations and increased in the other concentrations (Figure 4). Parameters such as shoot and root fresh mass and root dry mass did not show significant statistical changes.

The allelochemical substances can have no effect at low concentrations, positive effects as the concentration increases, which can reach from adverse to lethal effects to the plants. Therefore, the intrinsic power and some bioavailability of these substances in soil should be considered (Moreira and Siqueira, 2006). Thus, based on these characteristics, it can be defended the obtained results in this trial.

The shoot dry mass of beggartick plants under soil solution collected from an area cropped with hairy vetch, 30 days after their management, showed a linear decrease as the solution concentration increased (Figure 5). There was no significant effect on the following parameters: shoot and root fresh mass and root dry mass.

Parameters as shoot and root fresh mass and root dry mass of beggartick plants showed no significant effect when grew on soil solution collected 45 days after management of the three studied cover crops (Figure 6). Only the shoot dry mass under soil solution extracted from an area cropped with hairy vetch showed a linear decrease as solution concentration increased. The shoot fresh mass of beggartick plants under soil solution extracted from an area cropped with turnip, 60 days after the cover crop management, increased as soil solution concentration increased (Figure 7). The root fresh mass of beggartick plants also had a significant increase up to 200 ml L^{-1} solution, from an area cropped with turnip plants with subsequent decrease. While the shoot dry mass of beggartick plants showed a decrease on its mass up to 200 ml L^{-1} with the subsequent increase. Just the opposite of the following answers, whose root dry mass under soil solution of turnip and hairy vetch had an initial decrease (100 ml L^{-1}) with a subsequent increase for turnip, but a linear increase as soil solution concentration increased for hairy vetch.

However, the nutritional effect provided by the soil solution should be considered as there are some ions like: Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Fe^{+2}, Cl^{-}, NO_3^{-}, SO_4^{-2}, and $Si(OH)_4$. It is known that the root plants absorb ions by mass flow or diffusion, whether it is in soil solution (Meurer, 2010). This would explain the highest values found for the initial development of beggartick plants and in some cases in the presence of soil solution. The values of germination percentage, germination speed index and germination speed of beggartick seeds under soil solution extracted 15 days after cover crop management for tests using sand as substrate are shown on Table 1. Soil solution collected at 15 days and extracted from an area cropped with the three studied plants was applied with a pure solution on beggartick seeds on sand substrate and did not influence on the analyzed parameters. The allelochemicals interact in the environment, as well as herbicides, and are submitted to degradation processes such as microbial degradation, photolysis, oxidation and removal or transfer processes,

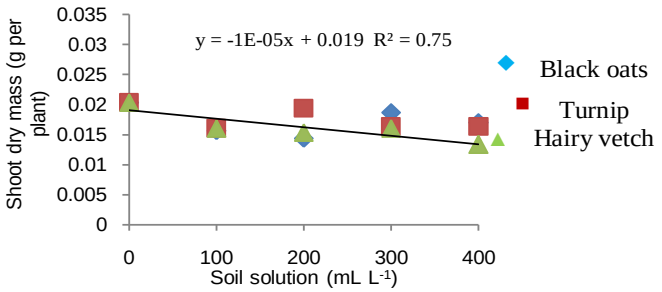

Figure 5. Shoot dry mass (g per plant) of beggartick, moistened with 0, 100, 200, 300 and 400 ml L^{-1} soil solution, collected 30 days after management of black oats, turnip and hairy vetch as cover crops.

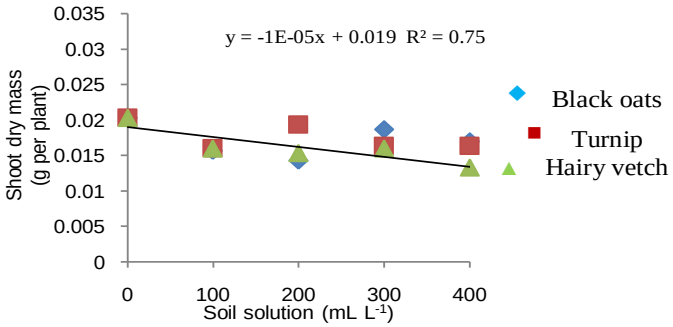

Figure 6. Shoot dry mass (g per plant) of beggartick, moistened with 0, 100, 200, 300 and 400 ml L^{-1} soil solution, collected 45 days after management of black oats, turnip and hairy vetch as cover crops.

as volatilization and adsorption (Vidal; Bauman, 1997). This fact can justify some possible causes of non-effects that were observed in soil solution collection during this experiment.

Analyzing germination parameters on beggartick seeds, submitted to soil solution and collected 30 and 45 days after cover crops management (second and third collection), there was no significant change (Tables 2 and 3).

Soil solution, in the last sample, 60 days after cover crops management, caused no change in germination percentage of beggartick seeds (Table 4). However, it was observed that seeds in soil solution extracted from an area with hairy vetch modified in other germination processes: as germination speed index and germination speed. Speed germination has been changed under soil solution extracted from the area with black oats. These answers are in accordance with Borghetti and Ferreira (2004), who stated that the allelopathic effect can occur on other parameters of this process, such as germination speed. This may be due to hairy vetch has shown faster decomposition when compared to the others. It has also provided allelopathic substances more quickly in soil solution.

According to Crusciol et al. (2008), 53 days after black oats management, there was still a remain of 33.6% of initial dry matter in soil, in order to highlight the low residue of grass decomposition. Perhaps, the collection period for this experiment was short to evaluate the availability of these substances, since the most important changes were recorded in the indicating parameters 60 days after cover crops management, which was also observed in Figure 8. Values of shoot and root for fresh and dry mass of beggartick plants are shown on Table 5. They were submitted to soil solution extracted from the area cropped with black oats, turnip and hairy vetch at 15 days (first collection). There was a significant difference only for beggartick shoot fresh mass submitted to soil solution extracted from the area with turnip. When beggartick plants received soil solution, their mass was higher when compared to the control, so, the other parameters did not show any significant change.

Martins et al. (2006) found out that the soil solution collected in grazing area with *B. brizantha* cv. Marandu (Poaceae), cropped for more than five years, increased shoot and root length of *Sida rhombifolia* weed. This result was different from the one obtained in this experiment. Soil solution collected 30 days after cover crops management, applied on a substrate where beggartick plants were cropped, caused no significant change (Table 6).

The shoot fresh mass of beggartick plants submitted to soil solution extracted at 45 days in an area cropped with black oats was significant (Table 7). The plants mass increased due to soil solution presence when compared to the control. There was no significant result for soil solution treatments extracted from turnip and hairy vetch crops neither on the other studied parameters. It was found that root fresh mass increased significantly under extracted soil solution in a cropped area with hairy vetch when compared to the control (Table 8). On the other hand, the other studied parameters showed no significant changes 60 days after the cover crops management.

One of the main analyzed variables in allelopathic tests is germination; however, this is less sensitive to allelochemicals than seedling development (Souza Filho et al., 2010). Therefore, this did not happened in these tests, in which extracted soil solutions in area with turnip and hairy vetch changed seeds germination patterns of beggartick and did not affect its initial development. When sand was used as substrate, under concentrated soil solution, the effects were lower than when germitest paper was used.

In soils, allelochemicals may have limited activity in space and time. The space limitations occur because, even though all allelochemicals could be present in plant residues and released at once, microbial degradation and adsorption were interrupted, the natural chemicals performance would be spatially limited concerning the seeds closed to the donor plant or residues under decomposition. The time limitation happens because allelochemicals are not released at once from residues

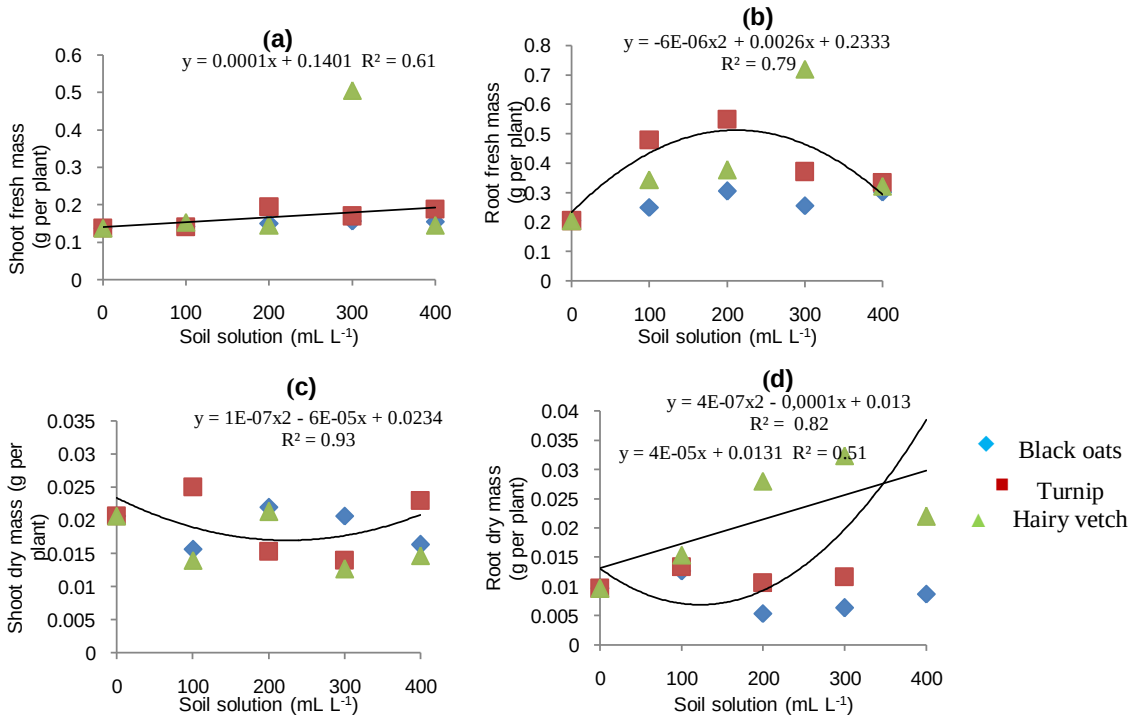

Figure 7. (a) Shoot fresh mass (b) root fresh mass (c) shoot dry mass and (d) root dry mass (g per plant) of beggartick, moistened with 0, 100, 200, 300 and 400 ml L^{-1} soil solution, collected 60 days after management of black oats, turnip and hairy vetch as cover crops.

Table 1. Germination percentage, germination speed index and germination speed of beggartick seeds in moistened sand substrate with soil solution collected 15 days after management of black oats, turnip and hairy vetch as cover crops.

	Black oats	Turnip	Hairy vetch
	% germination		
Control	90.66	90.66	90.66
Soil solution	80.66	90.33	90.20
Average	85.66	90.49	90.43
CV	19.30	7.72	7.70
F value	0.59^{ns}	0.06^{ns}	0.05^{ns}
	Germination speed index (seeds per day)		
Control	3.73	3.84	3.86
Soil solution	2.82	3.73	3.71
Average	3.28	3.78	10.68
CV	18.10	10.84	10.68
F value	0.89^{ns}	0.11^{ns}	0.13^{ns}
	Germination speed (days)		
Control	31.33	31.30	31.33
Soil solution	26.00	27.66	27.00
Average	28.66	29.50	22.53
CV	17.79	17.99	11.19
F value	0.45^{ns}	0.72^{ns}	0.65^{ns}

Averages followed by lettes, in collun, differ among thenselves by Tukey test at 5% probability. ns = none significant; * = significant, CV = coefficient variation.

Table 2. Germination percentage, germination speed index and germination speed of beggartick seeds in moistened sand substrate with soil solution collected 30 days after management of black oats, turnip and hairy vetch as cover crops.

	% germination		
	Black oats	**Turnip**	**Hairy vetch**
Control	100.00	100.00	100.00
Soil solution	90.00	90.67	90.66
Averages	95.00	95.33	95.34
CV	14.22	7.48	7.20
F value	2.46^{ns}	2.30^{ns}	0.42^{ns}
	Germination speed index (seeds per day)		
Control	7.41	7.41	7.47
Soil solution	5.36	7.22	7.41
Average	6.38	7.31	7.44
CV	21.25	14.11	19.14
F value	0.85^{ns}	0.05^{ns}	0.002^{ns}
	Germination speed (days)		
Control	26.66	26.66	28.66
Soil solution	23.33	25.66	26.66
Average	24.99	26.16	27.66
CV	15.78	14.04	11.71
F value	6.12^{ns}	0.11^{ns}	0.57^{ns}

Averages followed by lettes, in collun, differ among thenselves by Tukey test at 5% probability. ns = none significant; * = significant, CV = coefficient variation.

Table 3. Germination percentage, germination speed index and germination speed of beggartick seeds in moistened sand substrate with soil solution collected 45 days after management of black oats, turnip and hairy vetch as cover crops.

	% germination		
	Black oats	**Turnip**	**Hairy vetch**
Control	100.00	100.00	100.00
Soil solution	90.00	91.66	91.33
Average	95.00	95.83	95.66
CV	7.72	5.44	6.60
F value	4.00^{ns}	1.00^{ns}	0.07^{ns}
	Germination speed index (seeds per day)		
Control	5.39	6.77	5.39
Soil solution	4.52	5.38	5.11
Average	4.95	6.08	5.25
CV	14.58	14.39	9.19
F value	2.13^{ns}	3.74^{ns}	0.50^{ns}
	Germination speed (Days)		
Control	34.66	34.66	34.66
Soil solution	26.33	31.00	33.33
Average	30.50	32.83	34.00
CV	17.06	10.77	18.10
F value	1.08^{ns}	0.45^{ns}	0.02^{ns}

Averages followed by lettes, in collun, differ among thenselves by Tukey test at 5% probability. ns = none significant; * = significant, CV = coefficient variation.

Table 4. Germination percentage, germination speed index and germination speed of beggartick seeds in moistened sand substrate with soil solution collected 60 days after management of black oats, turnip and hairy vetch as cover crops.

% germination			
	Black oats	**Turnip**	**Hairy vetch**
Control	100.00	100.00	100.00
Soil solution	90.00	90.66	90.33
Average	95.00	95.33	95.16
CV	25.67	12.46	2.09
F value	1.47^{ns}	3.94^{ns}	64.00^{ns}
Germination speed index (seeds per day			
Control	5.83	5.83	5.83 ^{a}
Soil solution	4.17	4.84	3.83 ^{b}
Average	5.00	5.33	4.83
CV	15.28	12.32	7.40
Valor F	1.61^{ns}	16.70^{ns}	47.25*
Germination speed (Days)			
Control	69.66 ^{a}	69.66	69.66 ^{a}
Soil solution	38.00 ^{b}	41.33	38.66 ^{b}
Average	53.83	55.50	54.16
CV	27.42	20.78	5.02
F value	1.53*	2.05^{ns}	47.26*

Averages followed by lettes, in collun, differ among thenselves by Tukey test at 5% probability. ns = none significant; * = significant, CV = coefficient variation.

Table 5. Shoot and root for fresh and dry mass of beggartick plants (g per plant) in sand, moistened with soil solution collected 15 days after black oats, turnip and hairy vetch as cover crops management.

Shoot fresh mass (g per plant)			
	Black oats	**Turnip**	**Hairy vetch**
Control	0.11	0.11 ^{b}	0.12
Soil solution	0.16	0.18 ^{a}	0.10
Average	0.14	0.15	0.12
CV	29.16	9.98	5.93
F value	1.96^{ns}	18.75*	0.03^{ns}
Root fresh mass (g per plant)			
Control	0.13	0.13	0.12
Soil solution	0.06	0.07	0.09
Average	0.09	0.10	0.09
CV	7.61	8.14	5.71
F value	1.03^{ns}	0.61^{ns}	1.78^{ns}
Shoot dry mass (g per plant)			
Control	0.01	0.02	0.02
Soil solution	0.02	0.03	0.02
Average	0.02	0.03	0.02
CV	1.03	0.79	0.40
F value	0.14^{ns}	1.00^{ns}	1.00^{ns}

Table 5. Contd.

Root dry mass (g per plant)			
Control	0.02	0.03	0.02
Soil solution	0.04	0.05	0.04
Average	0.03	0.04	0.03
CV	2.65	4.08	2.50
F value	0.33^{ns}	0.074^{ns}	1.48^{ns}

Averages followed by lettes, in collun, differ among thenselves by Tukey test at 5% probability. ns = none significant; * = significant, CV = coefficient variation.

Table 6. Shoot and root for fresh and dry mass of beggartick plants (g per plant) in sand, moistened with soil solution collected 30 days after black oats, turnip and hairy vetch as cover crops management.

Shoot fresh mass (g per plant)			
	Black oats	**Turnip**	**Hairy vetch**
Control	0.11	0.12	0.12
Soil solution	0.14	0.14	0.09
Average	0.13	0.12	0.10
CV	25.58	28.27	35.08
F value	0.57^{ns}	0.62^{ns}	0.43^{ns}
Root fresh mass (g per plant)			
Control	0.14	0.13	0.14
Soil solution	0.07	0.08	0.09
Average	0.10	0.11	0.11
CV	8.43	8.16	5.84
F value	0.63^{ns}	0.48^{ns}	3.16^{ns}
Shoot dry mass (g per plant)			
Control	0.03	0.02	0.02
Soil solution	0.02	0.05	0.02
Average	0.02	0.03	0.02
CV	1.04	1.75	0.39
F value	0.14^{ns}	3.85^{ns}	4.00^{ns}
Root dry mass (g per plant)			
Control	0.04	0.03	0.04
Soil solution	0.02	0.04	0.01
Average	0.03	0.04	0.03
CV	2.65	4.08	2.50
F value	0.33^{ns}	0.77^{ns}	1.48^{ns}

under decomposition, and also because the process degradation and removal may reduce the available concentration in soil solution (Vidal and Bauman, 1997) and this may have occurred in these experiments.

Uchinho et al. (2012) studied the effect of cover crops on weeds in soybeans and concluded that, hairy vetch and rye can suppress effectively these plants without yield decrease of major crops in organic farming systems.

Conclusion

According to the observed changes, the possible allelophatic effects were more evident in the last collection of soil solution 60 days after the cover crops management. This evidence was greater on germination when compared to the early development of the studied plants. There is an indication of longer periods under

Table 7. Shoot and root for fresh and dry mass of beggartick plants (g per plant) in sand, moistened with soil solution collected 45 days after black oats, turnip and hairy vetch as cover crops management.

Shoot fresh mass (g per plant)			
	Black oats	**Turnip**	**Hairy vetch**
Control	0.11 [b]	0.12	0.12
Soil solution	0.17 [a]	0.10	0.09
Average	0.14	0.11	0.10
CV	4.99	2.97	3.25
F value	75.00*	0.20^{ns}	0.38^{ns}
Root fresh mass (g per plant)			
Control	0.14	0.13	0.14
Soil solution	0.07	0.09	0.10
Average	0.10	0.11	0.12
CV	4.81	5.52	4.79
F value	1.66^{ns}	3.98^{ns}	0.66^{ns}
Shoot dry mass (g per planta)			
Control	0.03	0.02	0.02
Soil solution	0.02	0.04	0.03
Average	0.02	0.03	0.02
CV	0.39	0.66	0.79
F value	4.00^{ns}	12.00^{ns}	0.25^{ns}
Root dry mass (g per plant)			
Control	0.04	0.04	0.04
Soil solution	0.02	0.02	0.03
Average	0.03	0.03	0.04
CV	2.65	2.11	1.00
F value	0.32^{ns}	1.58^{ns}	0.57^{ns}

Averages followed by lettes, in collun, differ among thenselves by Tukey test at 5% probability. ns = none significant; * = significant, CV = coefficient variation.

Table 8. Shoot and root for fresh and dry mass of beggartick plants (g per plant) in sand, moistened with soil solution collected 60 days after black oats, turnip and hairy vetch as cover crops management.

Shoot fresh mass (g per plant)			
	Black oats	**Turnip**	**Hairy vetch**
Control	0.12	0.12	0.13
Soil solution	0.18	0.11	0.12
Average	0.15	0.11	0.12
CV	4.48	2.87	4.65
F value	1.81^{ns}	0.01^{ns}	0.02^{ns}
Root fresh mass (g per plant)			
Control	0.15	0.14	0.15 [b]
Soil solution	0.36	0.28	0.52 [a]
Average	0.25	0.22	0.33
CV	10.77	12.48	6.08
F value	9.57^{ns}	0.73^{ns}	17.56*

Table 8. Contd.

Shoot dry mass (g per plant)			
Control	0.02	0.02	0.02
Soil solution	0.04	0.03	0.03
Average	0.03	0.02	0.02
CV	1.02	1.17	0.39
F value	9.14^{ns}	1.00^{ns}	16.00^{ns}
Root dry mass (g per plant)			
Control	0.04	0.04	0.04
Soil solution	0.10	0.03	0.08
Average	0.07	0.03	0.06
CV	2.27	3.62	5.49
F value	8.30^{ns}	0.17^{ns}	0.61^{ns}

Averages followed by lettes, in collun, differ among thenselves by Tukey test at 5% probability. ns = none significant; * = significant, CV = coefficient variation.

observation to evaluate green decomposition to release allelochemicals.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Banzattto DA, Kronka SN (2006). Experimentação agrícola. Jaboticabal: FUNEP. 4 ed. P. 237.

Colozzi Filho A, Andrade DS (2006). Organismos do solo e atividade microbiana no plantio direto. In: Casão Junior R; Siqueria R; Mehta YR; Passini JJ. Sistema plantio direto com qualidade. Londrina: IAPAR, ITAIPU Binacional. 4:39-53.

Crusciol CAC, Moro E, Lima EV, Andreotti M (2008). Taxa de decomposição e liberação de macronutrientes da palhada de aveia preta em plantio direto. Bragantia Campinas 67:2:481-489. http://dx.doi.org/10.1590/S0006-87052008000200024

Edmond JB, Drapalla WJ (1958). The effects of temperature, sana and soil, and acetone on germination oj okra seed. Proceed. Am. Soc. Horticutic. Sci. Alexandria 71(2):428-443.

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) (2006). Sistema brasileiro de classificação de solos. Rio de Janeiro: Embrapa solos, 2. ed., P. 306.

Ferreira DF (2008). Sisvar: um programa para análise e ensino de estatística. Rev. Cient. Symposium, Lavras 6(2):36-41.

Ferreira A, Aquila MEA (2000). Alelopatia: uma área emergente da ecofisiologia. Rev. Bras. Fisiol. Veg. Bras. 12:175-204.

Ferreira AG, Borghetti F (2004). Germinação: do básico ao aplicado. Porto Alegre: ARTMED, P. 323.

Filizola HF (2006). Manual de procedimentos de coleta de amostras em áreas agrícolas para análise da qualidade ambiental: solo, água e sedimentos. Jaguariúna: Embrapa Meio Ambiente P. 169.

Goldfarb M, Pimentel LW, Pimentel NW (2009). Alelopatia: relações nos agroecossistemas. Tecnol. Ciênc. Agrop. João Pessoa 3(1):23-28.

IAPAR (Instituto agronômico do Paraná) (1998). Cartas climáticas do Estado do Paraná. Londrina, IAPAR.

Kutchan TM (2001). Ecological arsenal anddevelopmentaldispatcher. The paradigm of secondary metabolism. Plant Physiol. Maryland, 125(1):58–60. http://dx.doi.org/10.1104/pp.125.1.58

Maguire JD (1962). Seeds of germination-aid selection and evaluation seedling emergence and vigor. Crop Sci. Madison 2(1):176-177. http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x

Martins D, Martins CC, Costa NV (2006). Potencial alelopático de soluções de solo cultivado com Brachiariabrizantha: Efeitos sobre a germinação de gramíneas forrageiras e plantas daninhas de pastagens. Planta Daninha, Viçosa 24(1):61-67.

Meurer EJ (2010). Fundamentos da química do solo. 4 ed Porto Alegre: Evangraf P. 266.

Moreira FMS, Siqueira JO (2006). Microbiologia e bioquímica do solo. Lavras: Editora UFLA. P. 729.

Pedrol N, Gonzalez L, Reigosa M (2006). Allelopathy and abiotic stress.Cap. 9. In: Reigosa, M. J.; Pedrol, N.; Gonzalez, L. (Eds). Allelopathy: A physiological process with ecological implications. Springer, Dordrecht, Holanda P. 637. http://dx.doi.org/10.1007/1-4020-4280-9_9

Pimentel Gomes F (2000). Curso de estatística experimental. 14. ed. Piracicaba: Degaspari, P. 477.

Souza Filho APS, Guilhon GMSP, Santos LS (2010). Metodologias empregadas em estudos de avaliação da atividade alelopática em condições de laboratório – Revisão crítica. Planta Daninha, Viçosa 28(3):689-697.

USA (1998). Department of Agriculture. Natural Resources Conservation Service. Soil Survey Staff. Keys to soil taxonomy. 8.ed. Washington, USA: Department of Agriculture.

Uchinho H, Iwama K, Jitsuyama Y, Ichiyama K, Sugiura E, Yudatea T, Nakamura S, Gopal J (2012). Effect of interseeding cover crops and fertilization on weed suppression under an organic and rotational cropping system .1. Stability of weed suppression over years and main crops of potato, maize and soybean. Field Crops Res. 127(8):9–16. http://dx.doi.org/10.1016/j.fcr.2011.10.007

Vidal RA, Bauman TT (1997). Fate of allelochemicals in the soil. Ciênc. Rural Santa Maria 27(2):351-357. http://dx.doi.org/10.1590/S0103-84781997000200032

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Genetic divergence of corn cultivars in relation to grain productivity, crude protein content and amino acid profile

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The aim of this study was to investigate the genetic variability among early-maturing and extremely early-maturing corn cultivars in relation to grain productivity, crude protein content and amino acid profile to obtain future crosses of cultivars with improved nutritional characteristics. Data from two experiments, which were both conducted in randomized block design and three replicates. Thirty-six early-maturing and 22 extremely early-maturing cultivars were evaluated of the experiments. Grain productivity, crude protein, lysine, methionine, cysteine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, histidine and arginine were evaluated. Analysis of variance was performed for each variable and means were compared by Scott-Knott test. Mahalanobis generalized distance matrix was calculated, and the cultivars were grouped using the unweighted pair group method with arithmetic mean (UPGMA). A dendrogram was then constructed, and the cophenetic correlation coefficient was calculated. The group means were compared using the t-test for independent samples. Genetic variability was detected in 13 variables for both maturity groups. For the early-maturing and extremely early-maturing cultivars, five and four groupings were obtained, respectively, based on grain productivity, crude protein content and amino acid profile, indicating that the crossbreeding of these cultivars may be used to improve the grain protein content and amino acid profile.

Key words: *Zea mays* L., genetic variability, nutritional variability, clustering analysis, near-infrared reflectance spectroscopy.

INTRODUCTION

Studies on the variability of the nutritional composition of corn are important because they contribute to increase nutrient use efficiency and minimize environmental damage. Although corn is a cereal used for energy

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purposes, studies on its protein contribution, particularly those related to its profile of essential amino acids are needed. Studies in this area are relevant because corn is the most expensive nutrient in the feed formulation (Jompuk et al., 2011), resulting in significant effects on production costs. In this direction, Pollak and Scott (2005) emphasized that breeding for grain quality provides end users with grain better suited to their needs and the improved amino acid balance lowers the cost of animal feed.

In recent decades, genetic improvement in corn has mainly focused on increasing grain productivity (PROD), which may have diminished the nutritional value of grain. For instance, Idikut et al. (2009) and Aliu et al. (2012) demonstrated that hybrids and corn populations show an inverse relationship between PROD and the crude proteins (CP) content of grain, and from 1930 to 1991, the level of this nutrient decreased 0.3% per decade (Duvick, 2005). Consequently, studies have been conducted to improve the protein quality and amino acid profile of corn grains. A review performed by Prasanna et al. (2001) shows that the QPM (Quality Protein Maize) cultivars with high quality protein provide benefits for human nutrition due to a significant increase on lysine and tryptophan levels compared to conventional corn cultivars. In order to improve the amino acid composition, Shewry (2007) demonstrates the possibility of using genetic engineering strategies to increase both the total protein content and composition of essential amino acids or even for exploitation of natural high lysine mutants.

Study done by Mittelmann et al. (2011), evaluating ten Brazilian corn populations under three environments, showed that these populations differ in relation to most of the nutritional traits evaluated, indicating the possibility of selecting genotypes with improved nutritional quality. In the study developed by Kil et al. (2014), with yellow corn from the United States and with yellow and white corn from South Africa, was observed that the concentrations of CP and AA (amino acids) varied among corn from different origins. Also, in a study of genetic divergence among modern inbred lines, teosinte accessions, and landraces, Flint-Garcia et al. (2009) verified that teosinte has very small seeds, but twice the protein content of landraces and inbred lines and a lower carbohydrate content, being considered a source of genetic variability for maize breeding programs.

Another important characteristic to be considered in a breeding program for corn is the texture of the grain, which is characteristic of fundamental importance to the industry, producers and processors of grain. Once the proteins, grain texture and hardness grains are inter-related, it is worth considering these features in a breeding program in order to address the storage pest resistance in maize (Mwololo et al., 2013). From the viewpoint of processing to feed grains, grains hard texture consume more energy in milling process and the difficulties in obtaining uniform grain size can affect the digestion of swine (Lawrence, 1972). In developing countries, is commonly used hard and dent grain, but the emergence of mutants with alterations in protein synthesis, thus causing changes in the texture of the grains, which is not always desirable (Sofi et al., 2009).

The study of genetic diversity is the basis of genetic improvement programs for corn plants (Subramanian and Subbaraman, 2010; Cruz and Carneiro, 2006). Clustering analysis using hierarchical methods is among the most commonly applied techniques for the description of genetic diversity patterns. By this technique, it is possible to classify individuals into distinct groups according to the variables of interest (Cruz and Carneiro, 2006) and to identify individuals that can be crossbred to maximize the gain in heterosis or hybrid vigor in the F_1 generation.

To estimate the distance between cultivars in replicated experiments, the Mahalanobis generalized distance (D^2), which takes into account the correlations between characters, is the most appropriate (Singh, 1981). These distances can be represented later in a dendrogram generated by different methods, such as the average linkage between groups clustering method [unweighted pair group method with arithmetic mean (UPGMA)], which is considered appropriate for the grouping of corn cultivars (Cargnelutti Filho and Guadagnin, 2011). This clustering method was used by Alves et al. (2014) to assess the variability of grain productivity and energy profile of maize cultivars. They indicated that it is possible to plan crossings between groups of genotypes in terms of productivity, ethereal extract and amylose, with the goal of maximizing heterosis. The UPGMA clustering method was also used by Osorno and Carena (2008) to determine the genetic diversity in early maturity maize, based on quality (percentage of protein, oil, and starch) and agronomic traits.

Despite the large number of genotypes, hybrids, cultivars and strains of corn grown, there is still missing data concerning their genetic variability in relation to PROD and nutritional characteristics. The aim of the present study was to investigate the genetic variability among early-maturing and extremely early-maturing cultivars of corn in relation to PROD and the CP content and the amino acid profiles of grain, thereby providing a basis for obtaining cultivars with improved nutritional characteristics in future crossbreeding studies.

MATERIALS AND METHODS

Field experiments

Two experiments were conducted with corn (*Zea mays* L.) cultivated during the 2009/2010 agricultural year in the experimental area of the Department of Plant Science, in the Federal University of Santa Maria, Santa Maria, Rio Grande do Sul State, at 29°42'S, 53°49'W and an altitude of 95 m. The

experimental area is included in the physiographic region of the Central Depression of the state of Rio Grande do Sul, Brazil. Fundamentally, the region is characterized by a humid subtropical climate, type Cfa according to the Köppen classification, and the soil is classified as Paleudalf (Embrapa, 2006), with a sandy loam surface texture.

The first experiment included 36 early-maturing corn cultivars, and the second included 22 extremely early-maturing corn cultivars. The cultivars used in each of the experiments (early-maturing and extremely early-maturing) belonged to the maize cultivar competition testing of the State of Rio Grande do Sul, Brazil. The relation of the cultivars included in each of the experiments was determined by the Agriculture Research State Foundation (Fundação Estadual de Pesquisa Agropecuária - FEPAGRO), which coordinates the maize cultivars evaluation testing in Rio Grande do Sul, based on information provided by the companies responsible for these cultivars, with respect to the cycle (early-maturing and extremely early-maturing). Further details of each cultivar, as commercial name, genetic basis, company and grain texture are presented in Table 1.

In both experiments, a randomized block design with three replications was used. The experimental plots consisted of two rows each 5 m long and spaced 0.8 m apart. Sowing was performed manually on October 26, 2009, with basic fertilization at a rate of 37.5 kg·ha^{-1} of nitrogen (N), 150 kg·ha^{-1} of phosphorus (P_2O_5) and 150 kg·ha^{-1} of potassium (K_2O). At the time of sowing, two seeds were placed every 0.20 m in each row, and after emergence, the plants were thinned to adjust the population to 62,500 plants ha^{-1}. The plants emerged between days 01 and 03 of November 2009 and were harvested on March 15, 2010. N fertilizer was applied as a top-dressing when plants were at the 3, 5 and 10 leaves, totaling 200 kg·ha^{-1} of N (Fancelli and Dourado Neto, 2004). All other cultivation practices were performed as locally recommended for the corn crop. During the experimental period, rainfall totaled 1,374 mm, and the average daily air temperature was 23.9°C [data collected at a conventional weather station belonging to the 8th District of Meteorology of the National Meteorological Institute (8° DISME/INMET), located 50 m from the experimental area] (Figure 1).

Measurement of variables and laboratory procedures

After the corn cob harvest, PROD was expressed in t·ha^{-1} for each repetition of each cultivar at 13% humidity. A sample of 500 g was collected, wrapped in a paper bag and maintained in a forced-air incubator (50°C) until 10% moisture was attained. After drying, the grain samples were ground in a micromill (model MA-630, Marconi, Piracicaba, São Paulo, Brazil) to obtain a particle size between 0.3 and 0.5 mm. Based on the percentage of raw material (% RM) in each milled sample, crude protein (CP) and the amino acids lysine (Lys), methionine (Met), cysteine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg) were quantified using near infrared reflectance spectroscopy (NIR - Near Infrared reflectance), with specific methodology in equipment manual. The analyses were performed by the Laboratory CEAN - ADISSEO using the XDS EQUIPMENT FOSS® 2.5002, with wavelength range from 400 nm to 2500 nm, with calibration adjusted by analytical procedure CEAN 010 Adisseo Brazil SA.

Verification of assumptions, analysis of variance and comparison of means

For each variable measured in each experiment, analysis of variance was performed according to the mathematical model of a randomized complete block design given by: $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$, where Y_{ij} is the observed value of the response variable in the plot $_{ij}$, μ is the overall mean, τ_i is the effect of cultivar, β_j is the effect of block and ε_{ij} is the effect of experimental error (Storck et al., 2011). To ensure the reliability of the analysis of variance results, the assumptions of the mathematical model were verified. For this, the Kolmogorov-Smirnov test for verifying the normality of the errors (Campos, 1983) and Levene's test for checking the homogeneity of residual variances among the treatments (Steel et al., 1997) were performed. In addition, the selective accuracy (SA) was estimated by the equation $SA = (1\ (1 / Fc))^{0.5}$ and used to evaluate the experimental accuracy, in accordance to the limits postulated by Cargnelutti Filho and Storck (2009). SA can alternatively be obtained by square root of the heritability and accuracy is considered very high when the SA≥0.90, high when 0.70≤SA<0.90, moderate when 0.50≤SA<0.70 and low when the SA<0.50 (Cargnelutti Filho and Storck, 2009). After, the means of the cultivars were compared using the Scott-Knott test (1974) at the 5% probability level.

Genotypic correlation matrix and diagnosis of multicollinearity

In each experiment (early and extremely early maturing cultivars), a genotypic correlation matrix between the 13 variables was calculated, and the multicollinearity was diagnosed to identify and eliminate highly correlated variables. The degree of multicollinearity of the genotypic correlation matrix was evaluated based on the condition number (CN). CN an efficiente procedure for the diagnosis of multicollinearity should directly reflect the intensity of its effects and allow the identification of the independent variables involved whit this problem (Montgomery and Peck, 1982). The decision criterion used was based on the classification proposed by Montgomery and Peck (1982) and described by Cruz and Carneiro (2006).

Analysis and validation of clusters

For the cluster analysis, a Mahalanobis generalized distance matrix (D^2) between the cultivars of each experiment was determined first. Then, the D^2 matrix was used on a relative scale as the dissimilarity measure for cluster analysis of the cultivars by the average linkage between groups hierarchical method (UPGMA) (Cruz and Carneiro, 2006). Next, a dendrogram was constructed, and the cophenetic correlation coefficient (CCC) was calculated. Thereafter, using 50% dissimilarity (Cruz, 1990) as the cutoff criterion for the dendrogram, the cultivars were identified for each group. This percentage was set at 50%, because groups formed with more than 50% of genetic variability would present little practicality, due to the large genetic differentiation between genotypes considered in homogeneous groups (Barroso and Arts, 2003). The mean of each variable was then estimated within each group, and subsequently for grouping validation, the group means were compared two by two with Student's t test for independent samples. Next, these means were transformed into vectors, and the highest mean was considered equal to one. For each experiment, the group means were represented on a graph of means profiles (Barroso and Artes, 2003). In addition, multivariate analysis of variance (MANOVA) at a 5% probability level was used to test for differences between the profiles of group means using Wilk's criterion. The statistical analysis was performed using Genes (Cruz, 2013) and Bioestat 5.0 (Ayres et al., 2007) software and the Microsoft Office Excel application.

Table 1. Cultivar number, commercial name, genetic basis, company and grain texture for each of the 36 cultivars tested in the early-maturing experiment and for the 22 cultivars tested in the extremely early-maturing experiment, both conducted during the 2009/2010 harvest season.

Experiment early-maturing				
Cultivar number	**Commercial name**	**Genetic basis**	**Company**	**Grain texture**
1	20A55	Triple hydrid	Agromen	Semi flint
2	30A91	Simple hydrid	Agromen	Semi flint
3	ATL 200	Triple hydrid	Atlantica	Semi dent
4	BM 207	Double hydrid	Biomatrix	Semi flint
5	BM 822	Simple hydrid	Biomatrix	Semi dent
6	CD 321	Simple hydrid	Coodetec	Semi dent
7	CO 327	Simple modified hydrid	Coodetec	Flint
8	CO 388	Double hydrid	Coodetec	Semi dent
9	DKB 245	Simple hydrid	Dekalb	Flint
10	Dx 510	Triple hydrid	Delta	Semi flint
11	2B655	Triple hydrid	Dow	Semi flint
12	2B688	Triple hydrid	Dow	Semi flint
13	PMS 0219A54	Triple hydrid	Embrapa	Semi flint
14	FTH 404	Double hydrid	FT Sementes	Semi flint
15	FTH 900	Triple hydrid	FT Sementes	Semi flint
16	CEP M 128	Simple hydrid	Fundacep	Semi flint
17	CEP M 130	Simple hydrid	Fundacep	Semi flint
18	CEP M 143	Simple hydrid	Fundacep	Flint
19	GNZ 2005	Triple hydrid	Geneze Sementes	Semi flint
20	GNZ 2728	Double hydrid	Geneze Sementes	Semi flint
21	GNZX 0744	Double hydrid	Geneze Sementes	Semi flint
22	KSP 1356	Simple hydrid	KSP Sementes	Semi flint
23	KSP 3246	Triple hydrid	KSP Sementes	Semi flint
24	BX 945	Simple hydrid	Nidera	Semi dent
25	BG 7060	Triple hydrid	Pioneer	Semi flint
26	P 30B39	Simple modified hydrid	Pioneer	Semi flint
27	SHX 5121	Triple hydrid	Santa Helena	Flint
28	SHX 7222	Simple hydrid	Santa Helena	Flint
29	SHX 7323	Simple hydrid	Santa Helena	Flint
30	XB 6012	Simple hydrid	Semeali	Semi dent
31	XBX 70202	Simple hydrid	Semeali	Semi dent
32	AG 8025	Simple hydrid	Agroceres	Semi flint
33	AG 9040	Simple hydrid	Agroceres	Semi flint
34	P 30R50	Simple hydrid	Pioneer	Semi flint
35	AG 5011	Triple hydrid	Agroceres	Semi dent
36	AG 2020	Double hydrid	Agroceres	Semi flint
Experiment extremely early-maturing				
Cultivar number	**Commercial name**	**Genetic basis**	**Company**	**Grain texture**
1	BM 911	Simple hydrid	Biomatrix	Semi dent
2	Dx 915	Simple modified hydrid	Delta	Semi flint
3	2B433	Triple hydrid	Dow	Semi dent
4	PMS 3919	Simple hydrid	Embrapa	Semi flint
5	PMS 1635A08	Triple hydrid	Embrapa	Semi flint
6	FTH 960	Triple hydrid	FT Sementes	Flint
7	GNZ 0729	Simple hydrid	Geneze Sementes	Semi flint
8	GNZ 9505	Simple hydrid	Geneze Sementes	Semi flint

Table 1. Contd.

9	Bx 898	Simple hydrid	Nidera	Semi dent
10	HS 79707	Simple hydrid	Nidera	Semi dent
11	PRE 12S12	Simple hydrid	Prezzotto	Semi flint
12	PRE 22D11	Double hydrid	Prezzotto	Semi flint
13	PRE 22S11	Simple hydrid	Prezzotto	Semi flint
14	PRE 22T10	Triple hydrid	Prezzotto	Semi flint
15	RBX 79	Simple hydrid	Riber	Semi dent
16	SHS 7090	Simple hydrid	Santa Helena	Semi flint
17	SHX 7111	Simple hydrid	Santa Helena	Semi flint
18	AG 9045	Simple hydrid	Agroceres	Semi flint
19	BALU 7690	Simple hydrid	Sementes Balu	Flint
20	SG 6302	Triple hydrid	Sementes Guerra	Semi flint
21	AG 9020	Simple hydrid	Agroceres	Semi dent
22	BG 7060	Triple hydrid	Pioneer	Semi flint

Source: The relation of the cultivars included in each of the experiments was determined by the Agriculture Research State Foundation (Fundação Estadual de Pesquisa Agropecuária - FEPAGRO), which coordinates the maize cultivars evaluation testing in Rio Grande do Sul, for the 2009/2010 harvest season.

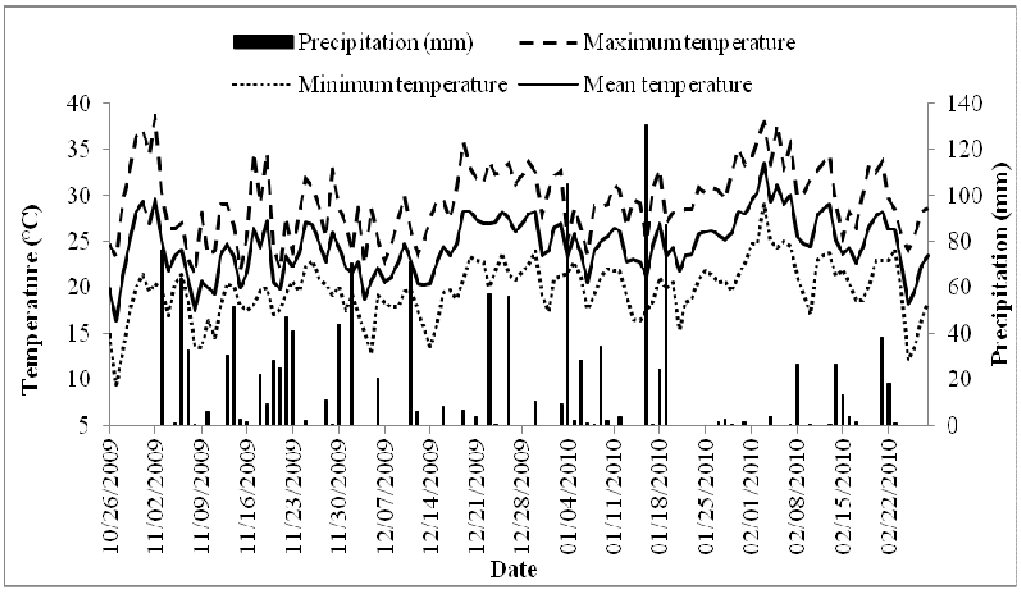

Figure 1. Minimum temperature, maximum temperature, mean daily air temperature (°C) and precipitation (mm).

RESULTS

Assumptions verification and analysis of variance

In the experiment with the early maturing cultivars, the errors in nine of the 13 variables (69.23%) conformed to a normal distribution based on the Kolmogorov-Smirnov test. In the experiment with the extremely early-maturing cultivars, the errors in 12 variables (92.31%) conformed to the normal distribution (Table 2). In both experiments, the residual variances of the variables were homogeneous by Levene's test. Therefore, in general, the assumptions of normality of errors and homogeneity of variances were met, which offered credibility to the results of the analysis of variance.

The high values for selective accuracy (SA) indicated that the experimental precision was high at 7.69% (one variable) and 15.38% (two variables) for the 13 variables

Table 2. Summary of the analysis of variance [number of degrees of freedom (DF) and the mean square for the sources of variation for blocks, cultivars and error], mean, experimental coefficient of variation (CV%), selective accuracy (SA), experimental precision, normality of errors by the Kolmogorov-Smirnov test and homogeneity of residual variances by Levene's test in one experiment with 36 early-maturing corn cultivars and another experiment with 22 extremely early-maturing corn cultivars, both conducted during the 2009/2010 harvest season.

Experiment early-maturing									
Variable[a]	Blocks (DF = 2)	Cultivars (DF = 35)	Error (DF = 70)	Mean	CV(%)	SA	Precision[b]	Normality	Homogeneity
PROD	1.420.030	3.614059*	0.443559	5	13.32	0.94	VH	Normal	Homogeneous
CP	0.115833	0.264911*	0.101864	7.54	4.24	0.78	H	Normal	Homogeneous
Lys	0.000036	0.000136*	0.000026	0.21	2.4	0.9	VH	Non-Normal	Homogeneous
Met	0.00004	0.000067*	0.000011	0.14	2.37	0.91	VH	Non-Normal	Homogeneous
Cys	0.000011	0.000104*	0.00002	0.16	2.81	0.91	VH	Non-Normal	Homogeneous
Thr	0.000118	0.000551*	0.000107	0.21	4.89	0.9	VH	Normal	Homogeneous
Trp	0.000004	0.000071*	0.00001	0.05	6.49	0.93	VH	Non-Normal	Homogeneous
Val	0.000169	0.000965*	0.000087	0.3	3.12	0.95	VH	Normal	Homogeneous
Ile	0.000181	0.000543*	0.000063	0.18	4.4	0.94	VH	Normal	Homogeneous
Leu	0.000381	0.007932*	0.000901	0.79	3.78	0.94	VH	Normal	Homogeneous
Phe	0.000311	0.001372*	0.00012	0.27	4.02	0.96	VH	Normal	Homogeneous
His	0.000045	0.000351*	0.000033	0.19	3.02	0.95	VH	Normal	Homogeneous
Arg	0.000073	0.000611*	0.000063	0.33	2.4	0.95	VH	Normal	Homogeneous
Experiment extremely early-maturing									
Variable[a]	Blocks (DF = 2)	Cultivars (DF = 35)	Error (DF = 70)	Mean	CV(%)	SA	Precision[b]	Normality	Homogeneity
PROD	0.703974	3.557506*	0.39626	5.92	10.64	0.94	VH	Normal	Homogeneous
CP	0.18138	0.217603*	0.051776	7.31	3.11	0.87	H	Normal	Homogeneous
Lys	0.000006	0.000217*	0.000043	0.22	3.02	0.9	VH	Normal	Homogeneous
Met	0.000041	0.000089*	0.000024	0.15	3.34	0.86	H	Normal	Homogeneous
Cys	0.000002	0.000158*	0.000021	0.16	2.92	0.93	VH	Normal	Homogeneous
Thr	0.000024	0.000866*	0.000162	0.22	5.77	0.9	VH	Normal	Homogeneous
Trp	0.000011	0.000097*	0.000019	0.05	8.4	0.9	VH	Non-Normal	Homogeneous
Val	0.000156	0.001091*	0.000143	0.31	3.81	0.93	VH	Normal	Homogeneous
Ile	0.000056	0.000657*	0.000069	0.19	4.37	0.95	VH	Normal	Homogeneous
Leu	0.001156	0.006512*	0.000902	0.84	3.56	0.93	VH	Normal	Homogeneous
Phe	0.000505	0.001380*	0.000152	0.29	4.33	0.94	VH	Normal	Homogeneous
His	0.000018	0.000505*	0.000033	0.19	3.11	0.97	VH	Normal	Homogeneous
Arg	0.000029	0.000945*	0.000079	0.33	2.67	0.96	VH	Normal	Homogeneous

[a]PROD, grain productivity in $t \cdot ha^{-1}$; CP, crude protein as a percentage of raw materials (% RM); Lys, lysine as % RM; Met, methionine as % RM; Cys, cysteine as % RM; Thr, threonine as % RM; Trp, tryptophan as % RM; Val, valine as % RM; Ile, isoleucine as % RM; Leu, leucine as % RM; Phe, phenylalanine as % RM; His, histidine as % RM; and Arg, arginine as % RM. *Significant difference by the F test at the 5% probability of error. [b]Class limits established by Resende and Duarte (2007), with VH being very high (SA ≥ 0.90), H high (0.70 ≤ SA < 0.90), M moderate (0.50 ≤ SA < 0.70) and L low (SA < 0.50).

measured in the experiments with the early and extremely early-maturing cultivars, respectively (Table 2). Likewise, the precision was quite high at 92.31% and 84.62% for the variables measured in the experiments with the early and extremely early-maturing cultivars, respectively (Resende and Duarte, 2007; Cargnelutti Filho and Storck, 2009). Furthermore, the coefficient of variation (CV) was low for all the variables measured in the two experiments (CV ≤ 13.32%).

The effect of cultivar was significant for all the variables measured in the two experiments (Table 2), which indicated that there was genetic variability among the early and extremely early-maturing cultivars for PROD and for the grain CP and amino acid levels. Considering that the assumptions of the model were met, the experimental precisions were high, and there was variability among the cultivars for all the variables, it can be inferred that this database is suitable for genetic diversity studies among cultivars by clustering analysis.

For the experiments with the early and extremely early-maturing cultivars, the PROD averaged 5.00 and 5.92 $t \cdot ha^{-1}$, respectively, and the CV (experimental coefficient of variation) was 13.32% and 10.64% (Table 2). Despite the higher CV for the PROD in relation to the other

variables, the SA was 0.94 for the two experiments, which indicated quite high experimental precision in the measurement of PROD (Resende and Duarte, 2007; Cargnelutti Filho and Storck, 2009). The PROD ranged between 2.7581 and 7.1936 $t \cdot ha^{-1}$ for the early-maturing cultivars (Table 3) and between 3.8391 and 7.8526 $t \cdot ha^{-1}$ for the extremely early-maturing cultivars (Table 4). These results indicated wide variability in the PROD values of the cultivars evaluated in both experiments.

The mean CP content for the early and extremely early-maturing cultivars was 7.54 and 7.31%, respectively (Table 2). The values ranged between 7.0467% and 8.5667% for the early-maturing cultivars (Table 3) and between 6.7600% and 7.9933% for the extremely early-maturing cultivars (Table 4). In both experiments, the experimental precision was high (SA = 0.78 and 0.87) according to the criteria of Resende and Duarte (2007) and Cargnelutti Filho and Storck (2009) (Table 2).

In the experiment with the early-maturing cultivars, the mean % RM value for each amino acid was 0.21 for Lys, 0.14 for Met, 0.16 for Cys, 0.21 for Thr, 0.05 for Trp, 0.30 for Val, 0.18 for Ile, 0.79 for Leu, 0.27 for Phe, 0.19 for His and 0.33 for Arg. In the experiment with the extremely early-maturing cultivars, the mean % RM value for each amino acid was 0.22 for Lys, 0.15 for Met, 0.16 for Cys, 0.22 for Thr, 0.05 for Trp, 0.31 for Val, 0.19 for Ile, 0.84 for Leu, 0.29 for Phe, 0.19 for His and 0.33 for Arg (Table 2). Thus, the mean percentage for each amino acid was found to be similar between the early and extremely early-maturing cultivars. However, these values fluctuated widely (variability) among the early-maturing cultivars (Table 3) and among the extremely early-maturing cultivars (Table 4).

The CV values associated with the amino acid measurements ranged from 2.37 to 6.49% in the early-maturing cultivars, resulting from the Met and Trp variables, respectively, and from 2.67 to 8.40% in the extremely early-maturing cultivars, resulting from the Arg and Trp variables, respectively (Table 2). The SA in the experiment with the early-maturing cultivars ranged between 0.90 and 0.96, indicating that the experimental precision was quite high for all the variables. In the experiment with the extremely early-maturing cultivars, the SA varied between 0.86 for the Met variable and 0.97 for the His variable, indicating that for most of the amino acids (90.9%), the experimental precision was quite high according to the criteria established by Resende and Duarte (2007) and Cargnelutti Filho and Storck (2009).

From the Scott-Knott means comparison test, two or more groupings of the cultivars were obtained for all the variables in both experiments (Tables 3 and 4). These results confirmed the above inference that there was genetic variability among the cultivars of the same plant maturity class for PROD and for grain CP and amino acid levels. Thus, it is possible to investigate the genetic variability among cultivars through cluster analysis.

Diagnosis of multicollinearity and elimination of variables

The diagnosis of multicollinearity based on the matrices of genetic correlations between the 13 variables resulted in a CN of 40,261 and 2,220, respectively, for the early and extremely early-maturing cultivars. Therefore, in both experiments, multicollinearity was severe according to the criteria of Montgomery and Peck (1982), and clustering analysis is not recommended under this condition because the multicollinear variables contribute greater weight in the clustering process (Cruz and Carneiro, 2006). With the elimination of the highly correlated variables (Cys, Thr, Trp, Ile, Phe, His and Arg for the early-maturing cultivars and Thr, Trp, Val, Ile, Phe, His and Arg for the extremely early-maturing cultivars), the CN was reduced to 86 and 93, respectively, which indicated weak collinearity and therefore enabled the clustering analysis to proceed satisfactorily.

Analysis and validation of clusters

In both experiments, the dendrogram was generated by the average linkage between groups method of grouping (UPGMA) using D^2 as a measure of dissimilarity. For the 36 early-maturing cultivars (Figure 3A), five groupings were obtained based on the PROD, CP, Lys, Met, Val and Leu variables, using 50% as the criterion of dissimilarity. The number of cultivars in Groups 1 through 5 was 21, 10, 1, 3 and 1, respectively. The 22 extremely early-maturing cultivars were divided into four groups (Figure 2B) based on the PROD, CP, Lys, Met, Cys and Leu variables and using 50% dissimilarity as the criterion for defining the groups. The number of cultivars in Groups 1 through 4 was 13, 2, 6 and 1, respectively. The CCCs of 0.67 for the early-maturing cultivar groups (Figure 2A) and of 0.58 for the extremely early-maturing cultivar groups (Figures 2B), were statistically significant, which indicated good relationships between the D^2 matrix and the graphical distance matrix and, consequently, good group consistency.

The means of the six variables used in the clustering of the early-maturing cultivars and of the six variables used in the clustering of the extremely early-maturing cultivars differed between the groups, which revealed contrasting groups (Table 5). For the early-maturing cultivars, Groups 2 and 3 had the highest mean PROD, followed by Groups 1, 4 and 5. Group 5 had the highest mean CP. The highest mean Lys values were observed in Groups 3, 4 and 5, followed by Groups 1 and 2. Met showed the highest means in groups 3 and 4, followed by Group 1 and then Groups 2 and 5. Group 4 showed the highest mean Val value and Group 2, the lowest. For Leu, Groups 4 and 5 showed the highest mean values, followed by Group 1 and then Groups 2 and 3.

Table 3. Means for grain productivity (PROD) in t·ha^{-1}, crude protein (CP) as a percentage of raw material, lysine (Lys), methionine (Met), cysteine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg) in 36 early-maturing corn cultivars evaluated during the 2009/2010 harvest.

Nº	Cultivar	PROD	CP	Lys	Met	Cys	Thr	Trp
1	20A55	5.2847^{ba}	7.2767^{b}	0.2100^{c}	0.1400^{b}	0.1600^{b}	0.2133^{b}	0.0500^{c}
2	30A91	4.8165^{b}	8.0000^{a}	0.2133^{b}	0.1400^{b}	0.1600^{b}	0.2167^{b}	0.0500^{c}
3	ATL 200	4.3258^{c}	7.6167^{b}	0.2133^{b}	0.1467^{a}	0.1633^{b}	0.2133^{b}	0.0533^{b}
4	BM 207	5.0614^{b}	7.3833^{b}	0.2100^{c}	0.1467^{a}	0.1567^{c}	0.2133^{b}	0.0500^{c}
5	BM 822	6.5684^{a}	7.3333^{b}	0.2067^{c}	0.1433^{b}	0.1533^{c}	0.1967^{c}	0.0400^{d}
6	CD 321	3.1912^{d}	7.4467^{b}	0.2000^{d}	0.1400^{b}	0.1500^{c}	0.2000^{c}	0.0433^{d}
7	CO 327	3.8575^{c}	7.5333^{b}	0.2133^{b}	0.1400^{b}	0.1600^{b}	0.2200^{b}	0.0500^{c}
8	CO 388	3.4642^{d}	7.4333^{b}	0.2133^{b}	0.1433^{b}	0.1567^{c}	0.2267^{a}	0.0500^{c}
9	DKB 245	6.0506^{a}	7.0467^{b}	0.2033^{d}	0.1400^{b}	0.1500^{c}	0.1933^{c}	0.0400^{d}
10	Dx 510	3.4902^{d}	7.4733^{b}	0.2300^{a}	0.1533^{a}	0.1733^{a}	0.2400^{a}	0.0600^{a}
11	2B655	5.1640^{b}	7.1667^{b}	0.2133^{b}	0.1400^{b}	0.1600^{b}	0.2100^{b}	0.0500^{c}
12	2B688	5.8645^{b}	7.3767^{b}	0.2100^{c}	0.1400^{b}	0.1533^{c}	0.2100^{b}	0.0500^{c}
13	PMS0219A54	4.5828^{c}	7.5567^{b}	0.2133^{b}	0.1400^{b}	0.1533^{c}	0.2133^{b}	0.0467^{c}
14	FTH 404	4.5645^{c}	7.3033^{b}	0.2100^{c}	0.1400^{b}	0.1567^{c}	0.2067^{c}	0.0500^{c}
15	FTH 900	4.5317^{c}	7.5367^{b}	0.2167^{b}	0.1433^{b}	0.1600^{b}	0.2200^{b}	0.0500^{c}
16	CEPM128	5.2400^{b}	7.5100^{b}	0.2133^{b}	0.1500^{a}	0.1600^{b}	0.2333^{a}	0.0533^{b}
17	CEPM130	5.1388^{b}	7.5733^{b}	0.2100^{c}	0.1467^{a}	0.1600^{b}	0.2167^{b}	0.0500^{c}
18	CEPM143	3.8565^{c}	7.4100^{b}	0.2100^{c}	0.1367^{b}	0.1567^{c}	0.2133^{b}	0.0500^{c}
19	GNZ 2005	5.2137^{b}	7.4367^{b}	0.2200^{a}	0.1500^{a}	0.1633^{b}	0.2267^{a}	0.0500^{c}
20	GNZ 2728	3.3693^{d}	7.6467^{b}	0.2233^{a}	0.1500^{a}	0.1667^{a}	0.2233^{a}	0.0533^{b}
21	GNZX0744	2.7581^{d}	7.5133^{b}	0.2200^{a}	0.1533^{a}	0.1700^{a}	0.2367^{a}	0.0567^{a}
22	KSP 1356	5.3924^{b}	7.1033^{b}	0.2000^{d}	0.1367^{b}	0.1500^{c}	0.1900^{c}	0.0400^{d}
23	KSP 3246	6.7217^{a}	7.5400^{b}	0.2067^{c}	0.1400^{b}	0.1500^{c}	0.2000^{c}	0.0433^{d}
24	BX 945	5.7612^{b}	7.3767^{b}	0.2033^{d}	0.1400^{b}	0.1600^{b}	0.1933^{c}	0.0433^{d}
25	BG 7060	5.1401^{b}	8.0933^{a}	0.2167^{b}	0.1400^{b}	0.1633^{b}	0.2067^{c}	0.0500^{c}
26	P30B39	3.4087^{d}	7.5800^{b}	0.2167^{b}	0.1433^{b}	0.1633^{b}	0.2233^{a}	0.0500^{c}
27	SHX 5121	5.1536^{b}	7.8400^{a}	0.2067^{c}	0.1400^{b}	0.1600^{b}	0.2000^{c}	0.0500^{c}
28	SHX 7222	5.6476^{b}	7.6000^{b}	0.2033^{d}	0.1367^{b}	0.1533^{c}	0.1833^{c}	0.0400^{d}
29	SHX 7323	4.1077^{c}	8.5667^{a}	0.2200^{a}	0.1400^{b}	0.1700^{a}	0.2267^{a}	0.0533^{b}
30	XB 6012	5.9336^{b}	8.0867^{a}	0.2067^{c}	0.1400^{b}	0.1633^{b}	0.2167^{b}	0.0500^{c}
31	XBX 70202	5.7330^{b}	7.6933^{b}	0.2200^{a}	0.1500^{a}	0.1600^{b}	0.2167^{b}	0.0500^{c}
32	AG 8025	7.1936^{a}	7.5867^{b}	0.2000^{d}	0.1400^{b}	0.1567^{c}	0.1933^{c}	0.0400^{d}
33	AG 9040	6.4125^{a}	7.6200^{b}	0.2100^{c}	0.1400^{b}	0.1500^{c}	0.2000^{c}	0.0433^{d}
34	P30R50	6.5827^{a}	7.5300^{b}	0.2133^{b}	0.1433^{b}	0.1633^{b}	0.2067^{c}	0.0500^{c}
35	AG 5011	5.1031^{b}	7.3233^{b}	0.2133^{b}	0.1500^{a}	0.1600^{b}	0.2233^{a}	0.0533^{b}
36	AG 2020	5.3447^{b}	7.1467^{b}	0.2100^{c}	0.1400^{b}	0.1633^{b}	0.2033^{c}	0.0500^{c}
Nº	Groups	4	2	4	2	3	3	4

Nº	Cultivar	Val	Ile	Leu	Phe	His	Arg
1	20A55	0.3100$^{b\,a}$	0.1867^{b}	0.8500^{b}	0.2833^{c}	0.1933^{b}	0.3267^{c}
2	30A91	0.3067^{b}	0.1833^{b}	0.8367^{b}	0.2867^{c}	0.1867^{c}	0.3267^{c}
3	ATL200	0.3200^{a}	0.1900^{b}	0.8367^{b}	0.2900^{c}	0.2000^{b}	0.3400^{b}
4	BM 207	0.2933^{c}	0.1733^{c}	0.7733^{c}	0.2733^{c}	0.1867^{c}	0.3267^{c}
5	BM 822	0.2800^{d}	0.1700^{c}	0.7667^{c}	0.2633^{d}	0.1700^{d}	0.3133^{d}
6	CD 321	0.2800^{d}	0.1733^{c}	0.7700^{c}	0.2533^{d}	0.1800^{d}	0.3133^{d}
7	CO327	0.3033^{b}	0.1867^{b}	0.8000^{c}	0.2767^{c}	0.1933^{b}	0.3300^{c}
8	CO388	0.3000^{c}	0.1867^{b}	0.8033^{c}	0.2767^{c}	0.1867^{c}	0.3367^{b}

Table 3. Contd.

9	DKB245	0.2667[d]	0.1533[d]	0.7167[d]	0.2367[e]	0.1733[d]	0.3100[d]
10	Dx5 10	0.3400[a]	0.2100[a]	0.8933[a]	0.3200[a]	0.2133[a]	0.3567[a]
11	2B655	0.2967[c]	0.1800[c]	0.7833[c]	0.2700[d]	0.1867[c]	0.3200[c]
12	2B688	0.2900[c]	0.1733[c]	0.7900[c]	0.2667[d]	0.1833[c]	0.3133[d]
13	PMS0219A54	0.2833[d]	0.1700[c]	0.7200[d]	0.2533[d]	0.1800[d]	0.3267[c]
14	FTH 404	0.2867[c]	0.1700[c]	0.7800[c]	0.2633[d]	0.1867[c]	0.3267[c]
15	FTH 900	0.2967[c]	0.1833[b]	0.7633[c]	0.2767[c]	0.1933[b]	0.3367[b]
16	CEPM128	0.3233[a]	0.2033[a]	0.8500[b]	0.3033[b]	0.2000[b]	0.3467[a]
17	CEPM130	0.3067[b]	0.1867[b]	0.8067[c]	0.2833[c]	0.1967[b]	0.3367[b]
18	CEPM143	0.2933[c]	0.1767[c]	0.7933[c]	0.2633[d]	0.1867[c]	0.3267[c]
19	GNZ 2005	0.3200[a]	0.1900[b]	0.8567[b]	0.3000[b]	0.2000[b]	0.3500[a]
20	GNZ 2728	0.3267[a]	0.1933[b]	0.8700[a]	0.3067[b]	0.2033[b]	0.3533[a]
21	GNZX 0744	0.3300[a]	0.2100[a]	0.8933[a]	0.3167[a]	0.2100[a]	0.3567[a]
22	KSP 1356	0.2700[d]	0.1600[d]	0.7000[d]	0.2367[e]	0.1733[d]	0.3100[d]
23	KSP 3246	0.2833[d]	0.1700[c]	0.7833[c]	0.2533[d]	0.1800[d]	0.3233[c]
24	BX 945	0.2833[d]	0.1733[c]	0.7600[c]	0.2567[d]	0.1800[d]	0.3100[d]
25	BG 7060	0.3067[b]	0.1800[c]	0.8167[b]	0.2767[c]	0.2033[b]	0.3467[a]
26	P30B39	0.3100[b]	0.1867[b]	0.7800[c]	0.2767[c]	0.2000[b]	0.3433[b]
27	SHX 5121	0.2933[c]	0.1700[c]	0.8100[c]	0.2667[d]	0.1900[c]	0.3267[c]
28	SHX 7222	0.2733[d]	0.1600[d]	0.7567[c]	0.2400[e]	0.1800[d]	0.3100[d]
29	SHX 7323	0.3167[b]	0.1867[b]	0.8967[a]	0.2967[b]	0.2100[a]	0.3567[a]
30	XB 6012	0.3133[b]	0.1900[b]	0.8000[c]	0.2767[c]	0.2000[b]	0.3400[b]
31	XBX 70202	0.2933[c]	0.1800[c]	0.7433[d]	0.2633[d]	0.1867[c]	0.3367[b]
32	AG 8025	0.2867[c]	0.1667[c]	0.7300[d]	0.2500[e]	0.1867[c]	0.3200[c]
33	AG 9040	0.2800[d]	0.1600[d]	0.7133[d]	0.2400[e]	0.1767[d]	0.3167[d]
34	P30R50	0.2900[c]	0.1733[c]	0.7633[c]	0.2633[d]	0.1867[c]	0.3300[c]
35	AG 5011	0.3200[a]	0.2000[a]	0.8233[b]	0.2933[b]	0.1833[c]	0.3333[b]
36	AG 2020	0.2967[c]	0.1800[c]	0.7733[c]	0.2667[d]	0.1900[c]	0.3267[c]
Nº	Groups	4	4	4	5	4	4

[a]Means followed by the same letter do not differ by the Scott-Knott test at the 5% level of probability.

Table 4. Means for grain productivity (PROD) in $t \cdot ha^{-1}$, crude protein (CP) as a percentage of raw material, lysine (Lys), methionine (Met), cysteine (Cys), threonine (Thr), tryptophan (Trp), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His) and arginine (Arg) in 22 extremely early-maturing corn cultivars evaluated during the 2009/2010 harvest.

Nº	Cultivar	PROD	CP	Lys	Met	Cys	Thr	Trp
1	BM 911	7.2870[a a]	7.7833[a]	0.2167[b]	0.1433[b]	0.1600[b]	0.2200[a]	0.0500[b]
2	Dx 915	5.3894[c]	7.4633[b]	0.2267[a]	0.1500[a]	0.1667[a]	0.2400[a]	0.0567[a]
3	2B433	6.6780[b]	7.4233[b]	0.2233[a]	0.1433[b]	0.1567[c]	0.2300[a]	0.0533[a]
4	PMS 3919	6.9009[a]	7.2167[c]	0.2167[b]	0.1500[a]	0.1500[c]	0.2067[b]	0.0467[b]
5	PMS 1635A08	5.7010[c]	7.2733[c]	0.2033[c]	0.1433[b]	0.1500[c]	0.2067[b]	0.0467[b]
6	FTH 960	6.1784[b]	7.1033[c]	0.2100[c]	0.1433[b]	0.1567[c]	0.2167[b]	0.0533[a]
7	GNZ 0729	4.4088[d]	7.9933[a]	0.2300[a]	0.1567[a]	0.1733[a]	0.2467[a]	0.0600[a]
8	GNZ 9505	5.1994[c]	7.4800[b]	0.2333[a]	0.1500[a]	0.1633[b]	0.2433[a]	0.0600[a]
9	Bx 898	7.0347[a]	7.2600[c]	0.2000[c]	0.1333[b]	0.1500[c]	0.1833[b]	0.0400[c]
10	HS 79707	6.3075[b]	7.2633[c]	0.2100[c]	0.1367[b]	0.1533[c]	0.2033[b]	0.0500[b]
11	PRE 12S12	3.8391[d]	7.0567[c]	0.2200[a]	0.1467[a]	0.1633[b]	0.2300[a]	0.0567[a]
12	PRE 22D11	4.3221[d]	7.4433[b]	0.2233[a]	0.1500[a]	0.1667[a]	0.2400[a]	0.0567[a]

Table 4. Contd.

13	PRE 22S11	5.9081[b]	7.3967[b]	0.2167[b]	0.1433[b]	0.1600[b]	0.2267[a]	0.0500[b]
14	PRE 22T10	5.1617[c]	7.4400[b]	0.2233[a]	0.1467[a]	0.1633[b]	0.2267[a]	0.0567[a]
15	RBX 79	7.3071[a]	6.7600[d]	0.2067[c]	0.1433[b]	0.1467[c]	0.2000[b]	0.0400[c]
16	SHS 7090	5.1798[c]	7.3833[b]	0.2167[b]	0.1500[a]	0.1600[b]	0.2300[a]	0.0567[a]
17	SHX 7111	6.1691[b]	7.1933[c]	0.2133[b]	0.1400[b]	0.1533[c]	0.2000[b]	0.0500[b]
18	AG 9045	7.8526[a]	7.3567[b]	0.2100[c]	0.1433[b]	0.1567[c]	0.2100[b]	0.0500[b]
19	BALU 7690	5.7573[c]	7.1533[c]	0.2133[b]	0.1433[b]	0.1533[c]	0.2100[b]	0.0467[b]
20	SG 6302	4.4419[d]	7.3900[b]	0.2267[a]	0.1533[a]	0.1700[a]	0.2433[a]	0.0567[a]
21	AG 9020	6.3993[b]	6.8167[d]	0.2167[b]	0.1500[a]	0.1500[c]	0.2133[b]	0.0500[b]
22	BG 7060	6.7783[a]	7.2367[c]	0.2200[a]	0.1500[a]	0.1633[b]	0.2267[a]	0.0567[a]
Nº	Groups	4	4	3	2	3	2	3

Nº	Cultivar	Val	Ile	Leu	Phe	His	Arg
1	BM 911	0.3067[ba]	0.1867[c]	0.7967[d]	0.2767[c]	0.1900[b]	0.3367[b]
2	Dx 915	0.3300[a]	0.2000[b]	0.8700[b]	0.3000[b]	0.1967[a]	0.3533[a]
3	2B433	0.3100[b]	0.1867[c]	0.8167[c]	0.2800[c]	0.1833[c]	0.3300[c]
4	PMS 3919	0.3067[b]	0.1800[c]	0.8367[c]	0.2733[c]	0.1833[c]	0.3267[c]
5	PMS 1635A08	0.3033[b]	0.1867[c]	0.8300[c]	0.2733[c]	0.1733[d]	0.3133[d]
6	FTH 960	0.3033[b]	0.1833[c]	0.8200[c]	0.2733[c]	0.1867[b]	0.3300[c]
7	GNZ 0729	0.3467[a]	0.2200[a]	0.9267[a]	0.3233[a]	0.2067[a]	0.3600[a]
8	GNZ 9505	0.3400[a]	0.2067[b]	0.9100[a]	0.3133[a]	0.1933[b]	0.3533[a]
9	Bx 898	0.2767[c]	0.1567[d]	0.7800[d]	0.2467[d]	0.1667[d]	0.3000[d]
10	HS 79707	0.3000[b]	0.1833[c]	0.8367[c]	0.2733[c]	0.1700[d]	0.3067[d]
11	PRE 12S12	0.3300[a]	0.2000[b]	0.8667[b]	0.2967[b]	0.1967[a]	0.3433[b]
12	PRE 22D11	0.3367[a]	0.2067[b]	0.9100[a]	0.3133[a]	0.2000[a]	0.3500[a]
13	PRE 22S11	0.3067[b]	0.1900[c]	0.8433[c]	0.2833[c]	0.1867[b]	0.3300[c]
14	PRE 22T10	0.3267[a]	0.1967[b]	0.8767[b]	0.2967[b]	0.1967[a]	0.3400[b]
15	RBX 79	0.2867[c]	0.1700[d]	0.7667[d]	0.2500[d]	0.1667[d]	0.3067[d]
16	SHS 7090	0.3167[b]	0.1933[b]	0.8500[c]	0.2967[b]	0.1900[b]	0.3433[b]
17	SHX 7111	0.2967[b]	0.1767[c]	0.8233[c]	0.2700[c]	0.1733[d]	0.3167[d]
18	AG 9045	0.3033[b]	0.1833[c]	0.8233[c]	0.2767[c]	0.1833[c]	0.3233[c]
19	BALU 7690	0.2967[b]	0.1767[c]	0.7667[d]	0.2567[d]	0.1700[d]	0.3133[d]
20	SG 6302	0.3467[a]	0.2167[a]	0.9233[a]	0.3200[a]	0.2067[a]	0.3567[a]
21	AG 9020	0.3100[b]	0.1900[c]	0.8267[c]	0.2767[c]	0.1700[d]	0.3233[c]
22	BG 7060	0.3260[a]	0.1967[b]	0.8633[b]	0.3000[b]	0.2000[a]	0.3467[b]
Nº	Groups	2	4	4	4	4	4

[a]Means followed by the same letter do not differ by the Scott-Knott test at the 5% level of probability.

For the extremely early-maturing cultivars, group 2 had the highest mean PROD, followed by Group 1 and then Groups 3 and 4 (Table 5). The mean CP value was highest in Group 4, followed by Groups 1 and 3, and was lowest in Group 2. The mean Lys value was highest in Groups 4 and 5, followed by Group 1, and was lowest in Group 2. For the Met, Cys and Leu variables, the highest means were in Group 4, followed by Group 3, then Group 1 and finally Group 2, with the lowest mean.

In the early-maturing (PROD, CP, Lys, Met, Val and Leu variables) and extremely early-maturing (PROD, CP, Lys, Met, Leu and Cys variables) cultivars after the completion of the MANOVA using Wilk's criterion, the group mean vectors were found to differ. In the experiment with the early-maturing cultivars, considering the results from the comparison tests of the group means (Table 5) and the vectors of the means (Figure 3A), Group 2 was found to have higher PROD values and, in general, the smallest values of CP, Lys, Met, Val and Leu compared with the other groups. In addition, Groups 4 and 5 showed lower mean PROD and, generally, higher mean CP, Lys, Met, Val and Leu values.

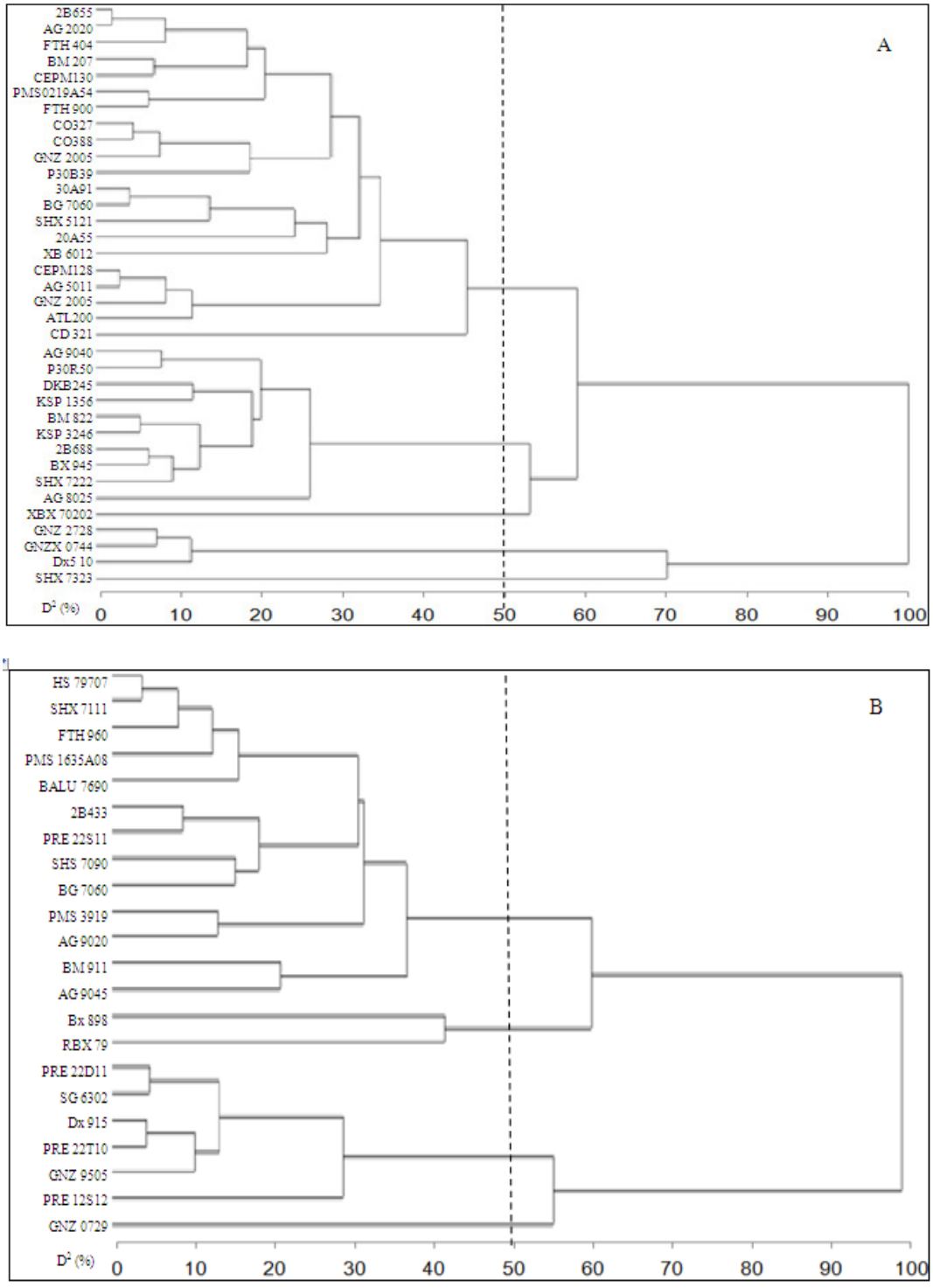

Figure 2. Dendrogram obtained by the average linkage between groups hierarchical clustering method (UPGMA) from the Mahalanobis generalized distance for 36 early-maturing corn cultivars. The variables used in the cluster were grain productivity, crude protein, lysine, methionine, valine and leucine. The cophenetic correlation coefficient was 0.67, which was significant at the 5% level of probability (A). Dendrogram obtained by the average linkage between groups hierarchical clustering method (UPGMA) from the Mahalanobis generalized distance for 22 extremely early-maturing corn cultivars. The variables used in the cluster were grain productivity, crude protein, lysine, methionine, cysteine and leucine. The cophenetic correlation coefficient was 0.58, which was significant at the 5% level of probability (B).

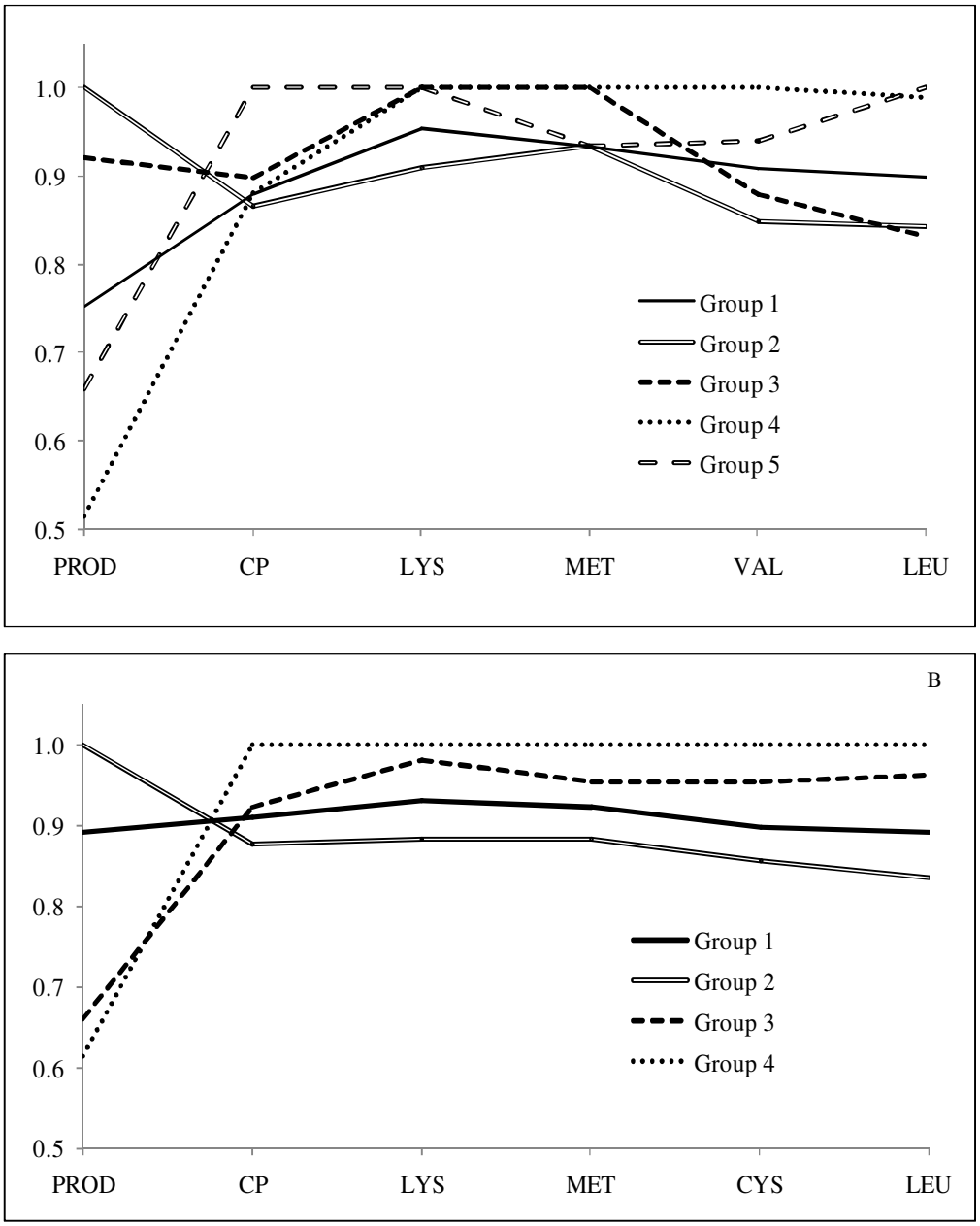

Figure 3. Profile of means for grain productivity (PROD), crude protein (CP), lysine (Lys), methionine (Met), valine (Val) and leucine (Leu) in early-maturing corn cultivars for each of the five groups (A). Profile of means for PROD, CP, Lys, Met, cysteine (Cys) and Leu in extremely early-maturing corn cultivars for each of the four groups (B).

For the experiment with the extremely early-maturing cultivars, Group 2, formed by the cultivars with higher PROD values, was found to have the lowest CP and amino acid levels (Figure 3B). By contrast, Group 4, formed by the cultivars with lower PROD values, showed higher CP, Lys, Met, Leu and Cys levels. Behavior similar to that of Group 4 was also observed for Group 3.

However, the cultivars of Group 1 showed intermediate values for all the variables measured. Thus, based on the results of the experiment with the early-maturing cultivars (Table 4 and Figure 3A) and, especially, the results of the experiment with the extremely early-maturing cultivars (Table 4 and Figure 3B), as the PROD of a cultivar group increased, the group protein and essential amino acid

Table 5. Comparison of means between groups for grain productivity (PROD) in t·ha^{-1}, crude protein (CP) as a percentage of raw material, lysine (Lys), methionine (Met), valine (Val) as and leucine (Leu) in 36 early-maturing corn cultivars allocated to five groups formed by the UPGMA. Comparison of means between groups for PROD, CP, Lys, Met, cysteine (Cys) and Leu 22 extremely early-maturing corn cultivars allocated to five groups formed by the UPGMA.

Experiment early-maturing							
Numbers cultivars	**Group***	**PROD**	**CP**	**Lys**	**Met**	**Val**	**Leu**
21	1	4.68462^{b}	7.53587^{b}	0.21190^{b}	0.14270^{b}	0.30286^{c}	0.80127^{b}
10	2	6.21951^{a}	7.41133^{b}	0.20567^{c}	0.14000^{c}	0.28033^{d}	0.74800^{c}
1	3	5.73301^{a}	7.69333^{b}	0.22000^{a}	0.15000^{a}	0.29333^{c}	0.74333^{c}
3	4	3.20584^{b}	7.54444^{b}	0.22444^{a}	0.15222^{a}	0.33222^{a}	0.88556^{a}
1	5	4.10773^{b}	8.56667^{a}	0.22000^{a}	0.14000^{c}	0.31667^{b}	0.89667^{a}
Experiment extremely early-maturing							
Numbers cultivars	**Group***	**PROD**	**CP**	**Lys**	**Met**	**Cys**	**Leu**
13	1	6.39209^{b}	7.27692^{b}	0.21436^{b}	0.14462^{c}	0.15564^{c}	0.82564^{c}
2	2	7.17089^{a}	7.01000^{c}	0.20333^{c}	0.13833^{d}	0.14833^{d}	0.77333^{d}
6	3	4.72558^{c}	7.37889^{b}	0.22556^{a}	0.14944^{b}	0.16556^{b}	0.89278^{b}
1	4	4.40876^{c}	7.99333^{a}	0.23000^{a}	0.15667^{a}	0.17333^{a}	0.92667^{a}

*For each variable, group means within columns followed by different letters differed using a t test at the 5% probability level.

levels declined.

DISCUSSION

Assumptions and analysis of variance

In both experiments, the high proportion of variables with normally distributed errors and the homogeneity observed among the residual variances for all the variables indicated that the database was suitable for performing the analysis of variance and complementary studies. The analysis of variance based on the 13 measured variables showed that the early and extremely early-maturing cultivars were genetically variable. The existence of genetic variability is essential in a breeding program, as highlighted by Vilela et al. (2008), and is a requirement for performing clustering analysis. Furthermore, the experimental precision based on the SA values was found to be high or extremely high in the present study. Accordingly, Cargnelutti Filho and Storck (2009) noted that SA is more suitable than the coefficient of variation (CV) for the verification of experimental precision in genetic studies. In addition, according to Resende and Duarte (2007), an SA value exceeding 0.90 (exceptionally high experimental precision) would be ideal for safer statistical inference.

The Scott-Knott means comparison test confirmed the existence of genetic variability, forming two or more distinct groupings of cultivars for all the variables measured (Tables 3 and 4). The mean PROD of the early and extremely early-maturing cultivars was 5.00 and 5.92 t·ha^{-1}, respectively, which surpassed the 4.46 t·ha^{-1} mean productivity of the state of Rio Grande do Sul, Brazil for the 2009/2010 crop (CONAB, 2010). However, some of the 36 early maturing and 22 extremely early-maturing cultivars showed PROD values that were below the state average. The variations occurred for each variable in both early and extremely early maturing cultivars are related to the genetic characteristics of each cultivar, once all the genotypes were exposed to similar growing conditions, indicating the existence of variability. According to Zhu and Khan (2001), the genotype, the environment and the interaction between these factors influence the protein and amino acid composition in grain. Although, the protein content of corn is less expressive than its carbohydrates content, variations in the content and amino acid composition of this nutrient can cause significant effects on animal metabolism, which uses this cereal as a major ingredient of the diet.

CP is the major variable in the chemical composition of food to be determined to adequately balance animal feed (Vieira et al., 2007). In the experiments with the early-maturing and extremely early-maturing corn cultivars, the grain CP content averaged 7.54 and 7.31% RM, respectively. These values were lower than those observed by Has et al. (2010), which ranged from 11.2 to 15.6%, and also lower than those described by Prasanna et al. (2001), which ranged from 8.9 to 10.2%. Aliu et al. (2012) found CP values between 11.02 and 13.02%. However, Vieira et al. (2007) reported levels between 6.73% and 10.04%, similar to those obtained in the present study. Moore et al. (2008) evaluated six maize hybrids, found an average value PB of 5.16%, lower than

those observed in this study. Although these results suggested that the cultivars used in the present study, which are available for cultivation in Rio Grande do Sul, have low CP contents, some studies have shown that the grain CP content varies with the level of N fertilizer, making it difficult to obtain convergent values (Shewry, 2007). Furthermore, Singh et al. (2005) determined the variability in corn protein by near-infrared transmission and observed CP values between 5.7 and 11.0%, with the highest protein content in plots who received 202 kg ha^{-1} of N, close to those used in this study, of 200 $kg \cdot ha^{-1}$ of N. The protein quality of corn grains depends on their amino acid profile. In this sense, the corn grains were deficient in essential amino acids, such as Lys, Met and Trp (Sofi et al., 2009). The mean values of amino acids obtained in the present study were lower than the levels determined by Abou-Deif et al. (2012), except for Ile, Cys and Trp, which were not quantified by the authors. In a study by Moore et al. (2008), were obtained lower amino acid values that in the present study, with the exception of TRP, ILE and FEN which presented similar values in early genotypes and TRP and ILE in superprecoce genotypes. Values higher than in the present study were reported by Lovatto et al. (2006) for the amino acids LIS, MET, THR, TRP, VAL, IT, READ, FEN, HIS and ARG. Although the corn kernels are use essentially as energetic ingredients in feed, variations in its protein content as well as in its composition in its amino acid profile, can cause significant effects on animal metabolism, which have this cereal as the majority of their diet.

Diagnosis of multicollinearity and elimination of variables

In clustering analysis, the diagnosis of multicollinearity is a basic assumption to be met because the variables will be weighted more heavily in the presence of multicollinearity (Barroso and Artes, 2003; Hair et al., 2009). The degree of multicollinearity of the matrix can be established based on the condition number, which is the ratio between the higher and the smallest eigenvalue of the matrix. Thus, when the number of conditions is less than 100, multicollinearity is weak; between 100 and 1000, multicollinearity is moderate to heavy, and when it is larger than 1000, multicollinearity is severe (Montgomery and Peck, 1982). In the case of the moderately or severe multicollinearity, proceeded to elimination of highly correlated variables.

In the study developed by Alves et al. (2014), was also observed severe multicollinearity among the ethereal extract and nitrogen-corrected apparent metabolizable energy variables in the extremely early-maturing cultivars. In a clustering analysis of corn crops, Cargnelutti Filho and Guadagnin (2011) performed a diagnosis of multicollinearity. These authors found that it was not necessary to discard variables because those used showed a low degree of multicollinearity, whereas certain variables were discarded in the present study because they created multicollinearity.

Analysis and validation of clusters

After variability among the cultivars was verified, the study of genetic diversity in corn was initiated based on a hierarchical method, enabling the classification of cultivars into groups. The formation of groups using hierarchical methods was also utilized by Osorno and Carena (2008), Silva et al. (2009), Subramanian and Subbaraman (2010), Oliboni et al. (2012) and Alves et al. (2014). In clustering analysis, the average linkage between groups hierarchical method (UPGMA), which was considered to be usual and appropriate by Mohammadi et al. (2003) and Cargnelutti Filho and Guadagnin (2011), was used. In the early and extremely early-maturing corn cultivars examined in the present study, five and four groupings, respectively were formed. According to Osorno and Carena (2008), the clustering analysis by UPGMA method showed the presence of five diversity groups based on their breeding origin. Osorno and Carena (2008), used ten early maturity maize improved populations, in a diallel mating design without reciprocals, based on grain quality traits (percentage of protein, oil, and starch) and agronomic traits. They also highlighted that a preliminary classification of the genotypes into groups of genetic diversity could help decide which combinations are the best.

The high and significant values of the CCCs indicated that the dendrograms of the two experiments reported here reliably expressed the similarity of the cultivars within each group and the genetic differences between the groups.

In each experiment, the analysis of variance, the tests to compare means and the cluster analysis indicated genetic variability, in early and extremely early maturing corn cultivars. In the study developed by Kil et al. (2014), significant differences in the coefficients of ileal apparent digestibility of CP and AA (arginine, histidine, lysine, tryptophan, aspartic acid, cysteine, glycine, and proline) among corn sources from different origins were observed. Already Alves et al. (2014), using cluster analysis to assess the variability of grain productivity and energy profile of maize cultivars, observed that groups with more productive genotypes presented lower ethereal extract and higher amylase in grain from early and extremely early-maturing corn cultivars.

Finally, the present study found that the higher the PROD of a particular group of cultivars, the lower the protein and amino acid levels were in these cultivars. This inverse relationship has been described by Duvick

(2005), Idikut et al. (2009) and Aliu et al. (2012). Therefore, it is necessary for researchers involved in corn genetic breeding programs to be attentive to the relationship between PROD and the nutritional composition of the grains. It is important to minimize the reduction of nutrient levels in cultivars of high productive potential or seek the development of cultivars with highly nutritious grain without significantly compromising the productivity of the cultivar.

Conclusions

There was genetic variability for PROD and for grain CP and amino acid levels in the early and extremely early maturing corn cultivars. For the early and extremely early maturing corn cultivars, respectively, five and four groupings were formed based on the PROD, CP content and amino acid profile, indicating the possibility of crossbreeding these cultivars to improve grain protein content and amino acid profiles. Groupings of cultivars with higher grain yields generally exhibited lower CP and amino acid levels.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Abou-Deif MH, Mekki BB, Mostafa EAH, Esmail RM, Khattab SAM (2012). The genetic relationship between proteins, oil and grain yield in some maize hybrids. World J. Agric. Sci. 8:43-50.

Aliu S, Rusinovci I, Fetahu S, Simeonovska E (2012). Genetic diversity and correlation estimates for grain yield and quality traits in Kosovo local maize (*Zea mays* L.) populations. Acta Agric. Slov. 99:121-128.

Alves BM, Cargnelutti Filho A, Toebe M, Burin C, Silva LP (2014). Variability of grain productivity and energy profile of maize (*Zea mays* L.) genotypes. J. Cereal Sci. 60:164-171.

Ayres M, Ayres Jr M, Ayres DL, Santos AS (2007). BioEstat: aplicações estatísticas nas áreas das ciências biomédicas. Belém: Sociedade Civil Mamirauá 364 pp.

Barroso LP, Artes R (2003). Análise multivariada. Lavras: UFLA, 151 pp.

Campos H (1983). Estatística experimental não-paramétrica. 4 ed. Piracicaba: Departamento de Matemática e Estatística - ESALQ, 349 pp.

Cargnelutti Filho A, Guadagnin JP (2011). Consistência do padrão de agrupamento de cultivares de milho. Cien. Rural. 41:1503-1508.

Cargnelutti Filho A, Storck L (2009). Medidas do grau de precisão experimental em ensaios de competição de cultivares de milho. Pesq. agropec. Bras. 44:111-117.

CONAB (Companhia Nacional de Abastecimento) (2010). Acompanhamento da Safra Brasileira de Grãos 2009/2010 – Sexto Levantamento, Março/2010/ Companhia Nacional de Abastecimento. Brasília - DF: CONAB, 2010.

Cruz CD (1990). Aplicação de algumas técnicas multivariadas no melhoramento de plantas. Tese de doutorado. Escola Superior de Agricultura Luiz de Queiroz, Piracicaba 188 pp.

Cruz CD (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35:271-276. http://dx.doi.org/10.4025/actasciagron.v35i3.21251

Cruz CD, Carneiro PCS (2006). Modelos biométricos aplicados ao melhoramento genético.2.ed, v.2. Viçosa: UFV, 585 pp.

Duvick DN (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). Adv Agron. 86:83-145.

EMBRAPA - Empresa Brasileira De Pesquisa Agropecuária (2006). Centro Nacional de Pesquisa de Solos. Sistema Brasileiro de Classificação de Solos. Brasília: Embrapa produção de informação, Rio de Janeiro: Embrapa Solos, 306 pp.

Fancelli AL, Dourado-Neto D (2004). Produção de Milho. Guaíba: 2° Ed. Agropecuária, 360 pp.

Flint-Garcia SA, Bodnar AL, Scott MP (2009). Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. Theor. Appl Genet. 119:1129-1142.

Hair JF, Black WC, Babin BJ, Anderson RE (2009). Multivariate Data Analysis. 7 ed. Prentice Hall, 816 pp.

Has V, Has I, Pamfil D, Copandean A (2010). Characterization of „turda" maize germplasm for the chemical composition of the grain. Rom. Agric. Res. 27:60-67.

Idikut L, Atalay AI, Kara SN, Kamalak A (2009). Effect of hybrid on starch, protein and yields of maize grain. J. Anim. Vet. Adv. 8:1945-1947.

Jompuk C, Cheuchart P, Jompuk P, Apisitwanich S (2011). Improved tryptophan content in maize with opaque-2 gene using marker assisted selection (MAS) in backcross and selfing generations. Kasetsart J. Nat. Sci. 45:666-674.

Kil DY, Park CS, Son AR, Ji SY, Kim BG (2014). Digestibility of crude protein and amino acids in corn grains from different origins for pigs. Anim. Feed Sci. Technol. 196:68-75.

Lawrence TLJ (1972). A review of some aspects on health and performance of variations in the physical form of the diet of the growing pig. I. Cereal processing. Vet. Rec. 91:67-70.

Lovatto PA, Mallmann CA, Ceccantini M, Hauschild L, Storck L, Dilkin P (2006). Relations entre les caractéristiques agronomiques, nutritionnelles et mycotoxicologiques de différents hybrides de maïs. J. Recherche Porcine 38:163-170.

Mittelmann A, Miranda Filho JB, Nass LL, Lima GJMM, Hara-Klein C, Silva RM (2011). Quantitative variation for grain quality in Brazilian maize populations. Sci. Agric. 68:50-56.

Mohammadi SA, Prasanna BM (2003). Analysis of genetic diversity in crop plants - Salient statistical tools and considerations. Crop Sci. 43:1235-1248.

Montgomery DC, Peck EA (1982). Introduction to linear regression analysis. New York: John Wiley & Sons 504 pp.

Moore SM, Stalder KJ, Beitz DC, Stahl CH, Fithian WA, Bregendahl, K (2008). The correlation of chemical and physical corn kernel traits with production performance in broiler chickens and laying hens. Poult. Sci. 87:665–676.

Mwololo JK, Mugo S, Tefera T, Munyiri SW (2013). Evaluation of traits of resistance to postharvest insect pests in tropical maize. Int. J. Agric. Crop Sci. 6:926-933.

Oliboni R, Faria MV, Neumann M, Battistelli GM, Tegoni RG, Resende JTV (2012). Genetic divergence among maize hybrids and

correlations with heterosis and combining ability. Acta Sci. Agron. 34:37-44.
Osorno JM, Carena MJ (2008). Creating groups of maize genetic diversity for grain quality: implications for breeding. Maydica 53:131-141.
Pollak LM, Scott MP (2005). Breeding for grain quality traits. Maydica 50:247-257.
Prasanna BM, Vasal SK, Kassahun B, Singh NN (2001). Quality protein maize. Curr. Sci. 81:1308-1319.
Resende MDV, Duarte JB (2007). Precisão e controle de qualidade em experimentos de avaliação de cultivares. Pesqui. Agropecu. Trop. 37:182-194.
Scott AJ, Knott M (1974). A cluster analysis method for grouping means in the analysis of variance. Biometrics 30:507-512.
Shewry PR (2007). Improving the protein content and composition of cereal grain. J. Cereal Sci. 46:239-250.
Silva TA, Pinto RJB, Scapim CA, Mangolin CA, Machado MFPS (2009). Genetic divergence in popcorn genotypes using microsatellites in bulk genomic DNA. Crop Breed. Appl. Biotechnol. 9:31-36.
Singh D (1981). The relative importance of characters affecting divergence. Indian J. Genet. Plant Breed. 41:237-245.
Singh M, Paulsen MR, Tian L, Yao H (2005). Site-specific study of corn protein, oil, and extractable starch variability using NIT spectroscopy. Appl. Engine. Agric. 21:239-251.
Sofi PA, Wani SA, Rather AG, Wani SH (2009). Review article: Quality protein maize (QPM): Genetic manipulation for the nutritional fortification of maize. J. Plant Breed. Crop Sci. 1:244-253.
Steel RGD, Torrie JH, Dickey DA (1997). Principles and procedures of statistics a biometrical approach, 3rd ed. McGraw-Hill, Nova York.
Storck L, Garcia DC, Lopes SJ, Estefanel V (2011). Experimentação vegetal. Santa Maria: UFSM. 200 pp.
Subramanian A, Subbaraman N (2010). Hierarchical cluster analysis of genetic diversity in Maize germplasm. Electron J. Plant Breed. 1:431-436.
Vieira RO, Rodrigues PB, Freitas RTF, Nascimento GAJN, Silva ELS, Hespanhol R (2007). Composição química e energia metabolizável de híbridos de milho para frangos de corte. R. Bras. Zootec. 36:832-838.
Vilela FO, Júnior ATA, Pereira MG, Scapim CA, Viana AP, Júnior SPF (2008). Effect of recurrent selection on the genetic variability of the UNB-2U popcorn population using RAPD markers. Acta Sci. Agron. 30:25-30.
Zhu J, Khan K (2001). Effects of genotype and environment on glutenin polymers and breadmaking quality. Cereal Chem. 78:125-130.

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Nitrogen availability modulating the growth of improved genotypes of *Coffea canephora*

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Studies quantifying the effect of the nitrogen over the growth of genotypes of Conilon coffee contribute to improve the fertilization management. Thus, this study was developed to evaluate the growth of improved genotypes of *Coffea canephora* Pierre ex Froehner submitted to nitrogen fertilization. Six improved genotypes of *C. canephora*, from different groups of ripening cycles, were grown without nitrogen fertilization or with fertilization at level of 0.625 g kg^{-1} of soil, cultivated in greenhouse, following a completely randomized design, with four replications. The plant growths were evaluated considering the plant height, stem diameter, number of leaves and relative growth rate. Nitrogen fertilization was able to promote higher growth, as well as, in general, a higher relative growth rate of all genotypes. Differentiation among genotypes was observed for their responses to each of the evaluated parameters. Noteworthy are the genotypes 77, in leafiness, and 67 in plant height and stem diameter, which presented higher growth compared to other genotypes, with or without nitrogen fertilization.

Key words: *Coffea canephora*, response, deficiency, mineral nutrition.

INTRODUCTION

Nitrogen (N) is the macronutrient with higher accumulation in the tissues of plants of Conilon coffee (*Coffea canephora* Pierre ex Froehner), corresponding to approximately 38% of all macronutrients distributed among the vegetal organs. It is a highly demanded nutrient and there are records showing increases in the crop yield of near 410%, achieved with the increase of nitrogen supply (Bragança et al., 2008; Bragança et al., 2010; Clemente et al., 2013).

The adequate supply of nitrogen is essential for the

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formation of vegetative structures, such as leaves, stems and roots; as well as for flowering and fruit filling; also interfering with productivity (Quintela et al., 2011; Taiz and Zeiger, 2013).

The growth rate of the aerial part of coffee plants varies seasonally due to environmental factors (Amaral et al., 2006; Ronchi and DaMatta, 2007). For Conilon coffee, studies that provide information on the growth rate are necessary to allow optimization of management practices, especially fertilization, pruning and irrigation.

For Hunt (1990), the growth analysis is a very important tool to study the performance of cultivated species growing in natural or controlled environments. The expression of physiological characteristics and the evaluation of its net production can be observed by growth analysis, since they are products of photosynthesis and result from the assimilatory system performance over a period of time. Intrinsic and extrinsic factors to the plant may influence this performance, causing variation of growth and development of plants (Dardengo et al., 2010).

Due to the large variability existing for many agronomic characteristics of genotypes of Conilon coffee, the breeding programs use the ripening cycle to classify groups of genotypes. It is important to highlight that there is differentiation in growth patterns among genotypes (Fonseca et al., 2006; Rodrigues et al., 2012; Martins et al., 2013b), as well as variation regarding the response to fertilization with nitrogen (Colodetti et al., 2014). Therefore, it is important to characterize the response of genotypes to the fertilization, in order to improve the recommendation and to establish differentiated schemes of fertilization for plantations using improved clonal cultivars.

According to this scenario, this study aims to evaluate the response in growth of improved genotypes of *C. canephora* Pierre ex Froehner submitted to nitrogen fertilization.

MATERIALS AND METHODS

The experiment was conducted in greenhouse, located at the experimental site of the Centro de Ciências Agrárias da Universidade Federal do Espírito Santo (CCA/UFES), in the municipality of Alegre, southern Espírito Santo State, in latitude 20°45'S, longitude 41°33'W and at 136 m of altitude.

The experiment followed a triple factorial scheme 6×2×5, with a completely randomized design and four replications. The first and second factors being, respectively, 6 improved genotypes of Conilon coffee and 2 conditions of nitrogen availability in the soil: no addition of nitrogen and fertilization with 0.625 g kg^{-1} of nitrogen (which, according with preliminary tests, was the level of fertilization that promoted the expression of higher variability for plant growth). The third factor was 5 evaluation periods: 30, 60, 90, 120, and 150 days of cultivation.

The soil used in the experiment was collected at a depth of 40 cm, discarding the first 10 cm of in order to reduce the effect of organic matter present in the superficial layer. A sample of this soil was sent to laboratory for chemical and physical analysis, being characterized as a dystrophic oxisol of clayey texture (Embrapa, 2006). After the characterization, the entire volume of soil was dried in shade and homogenized with a 2.0 mm mesh sieve. It was subsequently separated into samples of 10 dm^3 and accommodated into pots of 14 liters of capacity.

Presenting different ripening cycles, the six genotypes of *C. canephora* tested in this experiment were 67 and 23, of early cycle; 77 and 31, of intermediate cycle; and 76 and 153, of late cycle. These genotypes were developed by the breeding program from the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), featuring desirable agronomic characteristics and adaptation to cultivation in the main producer region of Brazil. These genotypes are available for cultivation, comprising some clonal cultivars that were developed by Incaper.

The fertilization, except for nitrogen, was performed according to the recommendation for nutritional studies in controlled environment (Novais et al., 1991). The nutrients were supplied through solutions prepared with salts (KNO_3, KH_2PO_4, NH_4NO_3, $CaHPO_4$) to establish the nutritional balance of the soil.

The genotypes were multiplied asexually through cuttings and the plantlets were formed in nurseries registered and certified by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA). From the early development of the third pair of leaves, the plantlets were transplanted to the pots and subjected to differential nitrogen fertilizations.

The fertilization with nitrogen was performed in coverage, with application of urea, split in four applications, starting 15 days after replanting and spaced at intervals of 30 days. The plants were grown under conditions of absence of nitrogen fertilization and fertilization at the level of 0.625 g kg^{-1}.

Irrigation was performed by keeping the soil moisture near 60% of the total pore volume during the experiment. The total pore volume was obtained using the particle density and soil density, determined by the test tube method, according to Embrapa (1997). The other cultural practices were performed manually according to the need.

The plants were conducted for 150 days and their growths were monthly evaluated, collecting data about: number of leaves (NL), obtained by counting; plant height (PH), measured with graduated ruler (cm); and stem diameter (SD), measured with digital caliper (millimeters). The relative growth rate (RGR) was calculated based on the temporal variation of the plant height, using the methodology described by Embrapa (2000).

All analyzes considered 5% of probability and were done with the statistical software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

According to the analysis of variance, there was significant effect of different interactions between the factors for each dependent variable (Table 1). For plant height, there was a significant interaction between genotypes and nitrogen fertilization, and between days of cultivation and nitrogen fertilization. The interaction between genotypes and the fertilization favored the study of differential performance among the genotypes, where, in the presence of fertilization with N, all genotypes showed gains in height when compared to the cultivation in absence of fertilization. Provided no nitrogen supply through fertilization, the Genotype 67 presented higher growth of orthotropic stem. With nitrogen supply, the plants from the Genotypes 67, 23 and 76 had higher vertical growth (Table 2).

To quantify the speed and intensity of growth in height of coffee plants is extremely important, since the

Table 1. Mean squares (MS), coefficients of variation (CV) and overall means for plant height, stem diameter and number of leaves of improved genotypes of *C. canephora*, modulated by nitrogen fertilization and days of cultivation.

Parameter	Plant height (cm)	Stem diameter (mm)	Number of leaves
$MS_{(genotype\ x\ nitrogen\ x\ time)}$	3.00^{ns}	0.12^{ns}	13.07*
$MS_{(nitrogen\ x\ time)}$	68.10*	0.23^{ns}	324.47*
$MS_{(genotype\ x\ time)}$	2.24^{ns}	0.10^{ns}	18.66*
$MS_{(genotype\ x\ nitrogen)}$	36.13*	0.88*	48.88*
MS_{time}	927.29*	37.71*	1247.99*
$MS_{nitrogen}$	2704.36*	7.06*	2769.08*
$MS_{genotype}$	59.62*	0.84*	119.72*
CV (%)	7.96	9.28	9.28
Mean	27.30	16.28	16.28

^{ns} non significant or * significant, at 5% of probability, by the F test.

Table 2. Means of plant height of improved genotypes of *C. canephora* modulated by the nitrogen fertilization.

Genotype	Nitrogen supply (g kg^{-1})	
	0.000	0.625
67	26.12^{Ba}	33.68^{Aa}
23	22.92^{Bb}	32.26^{Aa}
31	23.07^{Bb}	29.52^{Ab}
77	22.40^{Bb}	30.14^{Ab}
76	22.03^{Bb}	32.89^{Aa}
153	24.02^{Bb}	28.59^{Ab}

Means followed by the same uppercase letter in the rows do not differ by the Tukey test, and means followed by the same lowercase letter in the columns do not differ by the Scott-Knott test, both at 5% of probability.

promotion of growth and development of orthotropic stems can positively influence the amount of reproductive branches that the plant can develop, these being related to the capacity of sustaining the fruit production (Carvalho et al., 2010).

The plant height of all genotypes increased linearly along the days of cultivation. However, the plants grown in the presence of nitrogen fertilization developed faster, which can be observed in Figure 1A, by the higher slope coefficient in the regression for the plants cultivated with 0.625 g kg^{-1} of nitrogen. It is worth mentioning that, from the first 30 days of cultivation, the plants supplied with nitrogen fertilization already grew significantly higher than those who did not receive fertilization with N, a behavior that was maintained throughout the whole period evaluated in the study (Figure 1B).

Nitrogen is directly involved in the plant metabolism, acting as a component of amino acids, proteins, amines, amides, amino sugars, purines, pyrimidines alkaloids, coenzymes, vitamins and pigments, directly interfering with photosynthesis, which leads to the conclusion that, due to this interference, the lack of this nutrient limits the proper plant growth. Environments with restricted supply of this nutrient cause blockage in cytokinin synthesis, a hormone responsible for the plant growth, which results in losses in size and development of plant organs (Taiz and Zeiger, 2013).

Only the interaction between genotypes and nitrogen fertilization had significant effect for stem diameter (Table 1). The addition of nitrogen promoted the formation of thicker stems in most genotypes, except for the Genotypes 31 and 153, for which the stem grew to statistically similar values in either absence or addition of fertilization with N (Table 3).

The Genotypes 67, 31 and 153 showed the greatest thickening of the stem when cultivated with absence of fertilization with N, while the addition of the nutrient caused the Genotypes 67 and 23 to express superiority in the radial growth of their stems (Table 3).

The growth of the stems along the days of cultivation followed a linear trend, showing good fit to the regression $SD=0.02^{*}D+3.04^{*}$ ($R^2=0.98$), for which SD represents the stem diameter and D is the number of days of cultivation (up to 150).

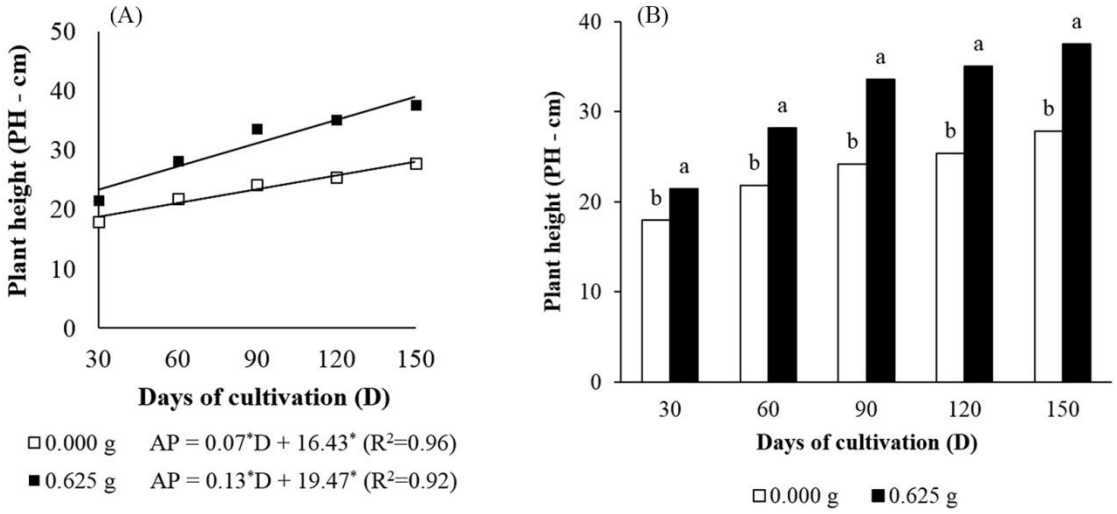

Figure 1. Regression analysis for plant height (A) and means of plant height (B) of improved genotypes of *C. canephora*, modulated by the presence or absence of nitrogen fertilization, along 150 days of cultivation a) *Coefficient is significant by the t-test, at 5% of probability, (b) means following by the same letter in each period of time do not differ by Tukey test, at 5% of probability.

Table 3. Means of stem diameter of improved genotypes of *C. canephora* modulated by the nitrogen fertilization.

Genotype	Nitrogen supply (g kg^{-1})	
	0.000	**0.625**
67	4.88^{Ba}	5.61^{Aa}
23	4.54^{Bb}	5.40^{Aa}
31	4.91^{Aa}	5.01^{Ab}
77	4.61^{Bb}	4.99^{Ab}
76	4.64^{Bb}	4.96^{Ab}
153	5.05^{Aa}	5.07^{Ab}

Means followed by the same uppercase letter in the rows do not differ by the Tukey test, and means followed by the same lowercase letter in the columns do not differ by the Scott-Knott test, both at 5% of probability.

The effect of the differences caused by the genotypes of Conilon coffee, the absence or presence of nitrogen fertilization and the period of cultivation modulated the means for number of leaves, and a triple interaction between these factors was significant (Table 1).

At 30 days of cultivation, there was no difference in the number of leaves among the genotypes, regardless of the management of the nitrogen fertilization (Table 4). At 60 days, it was already possible to notice a lesser leafiness of the plants from Genotypes 23 and 31 due to the lack of nitrogen supply, whereas there was no difference for the other genotypes for the condition or absence or presence of fertilization with N (Table 4).

After 90 days of cultivation (for 90, 120 and 150 days), the leafiness developed by plants cultivated with nitrogen fertilization was superior than the observed for the plants kept in absence of fertilization with this nutrient, emphasizing the importance of nitrogen for the development of new leaves in plants of Conilon coffee (Table 4).

The Genotypes 31 and 77 stood out regarding emission of new leaves at 90 days of cultivation, while the Genotype 67 had lesser leafiness than all others, considering the results obtained with nitrogen fertilization. The Genotype 77 was the only one who stood out in the absence of N fertilization, presenting good leafiness for both conditions of fertilization (Table 4).

With addition on nitrogen, the Genotypes 77 and 76 produced more leaves than the other at 120 days of cultivation, while the Genotypes 67 and 153 had the smaller number of leaves at this time. For the condition of absence of nitrogen fertilization, the Genotype 77 was

Table 4. Means of number of leaves of improved genotypes of *C. canephora* modulated by the nitrogen fertilization, along 150 days of cultivation.

Gen.	Days of cultivation									
	30		60		90		120		150	
	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}
67	8.66^{Aa}	9.66^{Aa}	10.66^{Ab}	12.00^{Ab}	10.66^{Bb}	17.33^{Ac}	10.66^{Bc}	22.00^{Ac}	10.66^{Bd}	34.66^{Aa}
23	8.66^{Aa}	9.66^{Aa}	8.66^{Bb}	12.66^{Ab}	11.33^{Bb}	21.66^{Ab}	12.66^{Bb}	26.33^{Ab}	13.00^{Bc}	28.00^{Ac}
31	7.33^{Aa}	9.33^{Aa}	8.00^{Bb}	15.33^{Aa}	11.33^{Bb}	25.66^{Aa}	13.33^{Bb}	27.00^{Ab}	13.66^{Bc}	31.00^{Ab}
77	8.66^{Aa}	10.66^{Aa}	13.33^{Aa}	13.33^{Ab}	18.66^{Ba}	26.00^{Aa}	22.00^{Ba}	30.66^{Aa}	23.33^{Ba}	35.33^{Aa}
76	7.66^{Aa}	9.33^{Aa}	10.33^{Ab}	10.66^{Ab}	12.00^{Bb}	21.00^{Ab}	13.66^{Bb}	30.00^{Aa}	15.00^{Bb}	31.33^{Ab}
153	8.66^{Aa}	8.66^{Aa}	12.66^{Aa}	12.66^{Ab}	13.33^{Bb}	20.00^{Ab}	15.66^{Bb}	21.33^{Ac}	16.66^{Bb}	23.00^{Ad}

Means followed by the same uppercase letter in the rows (for each period) do not differ by the Tukey test, and means followed by the same lowercase letter in the columns do not differ by the Scott-Knott test, both at 5% of probability.

able to maintain the leafiness less limited, growing a greater number of leaves than the others genotypes; in contrast, the Genotype 67 presented greater limitation, producing a smaller number of leaves (Table 4).

At the end of 150 days of cultivation, after all the parcels of the nitrogen fertilization, the Genotypes 67 and 77 presented greater amount of leaves in response to the fertilization with N, while the Genotype 153 had the smaller number of leaves. The Genotype 77 is highlighted from the group for being able to produce a larger number of leaves even in absence of fertilization (Table 4).

This characteristic of the Genotype 77 is relevant for the breeding programs, since this genotype may be a source of variability to explore the tolerance to cultivation in environments with nutritional limitations or even be studied to seek nutritional efficiency.

The fertilization with N promoted the growth parameters, leading to plants with higher plant height, stem diameter and number of leaves. There is a close relationship between these parameters, as the leafiness is a direct result of the activity of the apical bud of the branches, especially plagiotropics branches. And the growth of these branches is directly related to the growth of orthotropic stem, where the thickening is the result of its secondary growth, playing an important role in plant physical support and in the translocation of solutes between organs. In turn, the leaves are the source of most photosynthetic assimilates essentials to fuel the plant growth (Gomes et al., 2008; Rena and Maestri, 1986).

According to Clement et al. (2013), the nitrogen fertilization mainly influences the characteristics of vegetative growth, and the deficit of this nutrient affects the development of the coffee plant and, consequently, the fruit production.

By regression analysis, it is noted that leafiness increased linearly, for all genotypes, with time when the plants were cultivated with nitrogen fertilization (Figure 2). This same behavior was observed in absence of fertilization, except for the Genotype 67, which was not able to grow new leaves due the nutritional stress (Figure 2A).

The positive effect of the nitrogen fertilization over the emission of leaves of genotypes of Conilon coffee is easily noticeable in Figure 2. The slope coefficients of the regressions makes possible to hypothesize that cultivating the genotypes with nitrogen fertilization near the level of 0.625 g kg^{-1} promoted the leafiness, resulting in plants with higher vigor, faster growth, and, therefore, presenting a better development, which is necessary on the early stages to grant a possible higher production of fruits.

The analysis of relative growth rate is an important tool to study the speed at which the plant is growing. Using this tool, it was observed that plants of improved genotypes of *C. canephora* showed a similar growth trend, where higher growth rates occurred in the first two periods of evaluation (30-60 days and 60-90 days), indicating that during the first months after planting, the Conilon coffee seedlings express higher gains in plant height. After 90 days of cultivation, the plants presented smaller gains in height, matching the period when they start the radial growth of their canopies. This behavior was easier to identify when the plants were fertilized with N, since the nutritional stress caused limitation to the growth rate (Figure 3).

From 90 to 120 days of cultivation, smaller values of RGR were observed for both conditions of nitrogen fertilization (Figure 3). This behavior is related to the start of development of reproductive branches, thereby the metabolism is directed to the lateral growth of the canopy and the rate of growth in height is reduced. This explains why, after this period, the response in RGR of the fertilization seems to be not as expressive as during other periods.

For the fourth period (120-150 days), the absence of nitrogen fertilization promoted a higher relative growth

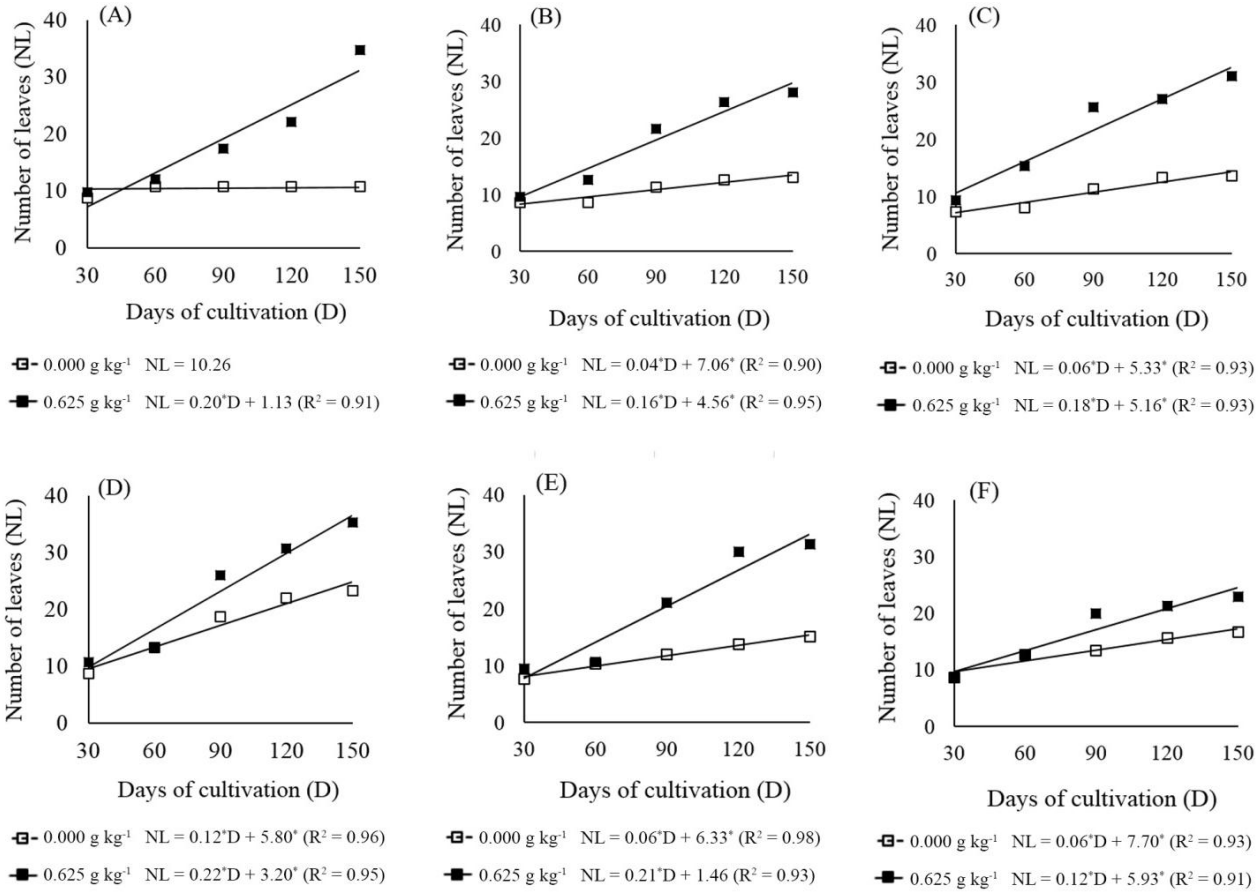

Figure 2. Regression analysis for number of leaves of improved genotypes of *Coffea canephora* (A – 67; B – 23; C – 31; D – 77; E – 76; F – 153), modulated by the presence or absence of nitrogen fertilization, along 150 days of cultivation. *Coefficient is significant by the t-test, at 5% of probability.

rate for the Genotypes 31, 76 and 153, showing that those genotypes were able to grow more in height, relatively to the previous period, in the condition of nutritional stress. This may be a strategy of the plant to accelerate its growth in an adverse environment, aiming to reach further phonologic stages quicker, in order to perpetuate the species.

It is understood that the vegetative growth of Conilon coffee is influenced by the nitrogen supply. The plant growth is a result of photosynthetic process, and it is known that there is a close relationship between the metabolism or carbon and nitrogen. The processes are connected, since the energy required to assimilate nitrogen is directly or indirectly derived from photosynthesis and the photosynthetic capacity itself depends on the nitrogen supply, since this nutrient is largely allocated in leaf proteins involved in photosynthetic processes (Evans, 1983; Evans and Seemann, 1984; Seemann et al., 1987).

The promotion of growth as a function of nitrogen fertilization observed in the genotypes is a result of the proper nutrition promoting the metabolism and the development of plant organs (Marschner, 2012). This growth response can be modulated by the phenotypic expression of different capacities of the genotypes in acquire and utilize the nutrient. Structural and morphological changes as a function of the nutritional efficiency have been already reported in the literature for *C. canephora* genotypes (Martins et al., 2013a; 2013b).

Studies conducted with different genotypes of Conilon coffee have also pointed out the existence of different responses to nitrogen fertilization, such as different accumulation rate of nutrients, accumulation of dry matter, growth, crop yield, nutritional parameters and expression of morphological characteristics (Clemente et al., 2013; Prezotti and Bragança, 2013; Pedrosa et al., 2014, Colodetti et al., 2014).

The combination of this information with the results obtained in this study reinforces the evidence that there is differentiation in the nutritional demands for nitrogen during the formation of seedlings, growth and production of improved genotypes of Conilon coffee with high potential of crop yield. Therefore, it is necessary to modify the management of the fertilization to make sure

that the nutritional demands of those highly productive genotypes are satisfied.

Conclusion

The growth of improved genotypes of *C. canephora* is modulated by nitrogen fertilization, being promoted by the increase of nitrogen supply in the soil. Differentiation among genotypes was observed for their responses to each of the evaluated parameters. Noteworthy are the Genotypes 77, in leafiness, and 67 in plant height and stem diameter, which presented higher growth compared to other genotypes, with or without nitrogen fertilization. The relative growth rate of improved genotypes follows the same general pattern as a function of the nitrogen availability, being promoted by it.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Amaral JAT, Rena AB, Amaral JFT (2006). Seasonal vegetative growth of the coffee plant and its relationship with the photoperiod, fructification, stomatic resistance and photosynthesis. Pesq. Agropec. Bras. 41(3):377-384. [in portuguese]

Bragança SM, Martinez HEP, Leite HG, Santos LP, Lani JA, Sediyama CS, Alvarez VHV (2010). Dry matter accumulation by Conilon coffee. Rev. Ceres. 57(1):48-52. [in portuguese]

Bragança SM, Martinez HEP, Leite HG, Santos LP, Sediyama CS, Alvarez VHV, Lani JA (2008). Accumulation of macronutrients for the conilon coffee tree. J. Plant Nutr. 3(1):103-120.

Carvalho AM, Mendes ANG, Carvalho GR, Botelho CE, Gonçalves FMA, Ferreira AD (2010). Correlation between growth and yield of coffee cultivars in different regions of the state of Minas Gerais, Brazil. Pesq. Agropec. Bras. 45(3):269-275. [in portuguese]

Clemente JA, Martinez HEP, Alves LC, Lara MCR (2013). Effect of N and K doses in nutritive solution on growth, production and coffee bean size. Rev. Ceres 60(2):279-285. [in portuguese]

Colodetti TV, Rodrigues WN, Martins LD, Tomaz MA (2014). Differential tolerance between genotypes of conilon coffee (*Coffea canephora*) to low availability of nitrogen in the soil. Aust. J. Crop Sci. 8(12):1648-1657.

Cruz CD (2013). GENES: a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35:271-276.

Dardengo MCJD, Reis EF, Passos RR (2010). Influence of field capacity on the growth rate of Conilon coffee. Rev. Ceres. 57(1):42-47. [in portuguese]

Embrapa – Empresa Brasileira de Pesquisa Agropecuária (2000). Análise do crescimento de comunidades vegetais. Brasília, Embrapa. 18 pp.

Embrapa – Empresa Brasileira de Pesquisa Agropecuária (1997). Manual de métodos de análises de solo. 2 ed. Rio de Janeiro, Ministério da Agricultura e do Abastecimento 212 p.

Embrapa – Empresa Brasileira de Pesquisa Agropecuária (2006). Sistema brasileiro de classificação de solos. 2 ed. Rio de Janeiro, Centro Nacional de Pesquisa de Solos. 306 pp.

Evans JR (1983). Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). Plant Physiol. 72:297-302.

Evans JR, Seemann JR (1984). Differences between wheat genotypes in specific activity of RuBP carboxilase and the relationship to photosynthesis. Plant Physiol. 74(4):759-765.

Fonseca AFA, Sediyama T, Cruz CD, Sakaiyama NS, Ferrão MAG, Ferrão RG, Bragança SM (2006). Genetic divergence in conilon coffee. Pesq. Agropec. Bras. 41(4):599-605. [in portuguese]

Gomes IAC, Castro EM, Soares AM, Alves JD, Alvarenga MIN, Alves E, Barbosa JPRAD, Fries DD (2008). Morphophysiological alterations in leaves of *Coffea arabica* L. cv. 'Oeiras' shaded by *Acacia mangium* Willd. Ciência Rural. 38(1):109-115. Hunt R (1990). Basic growth analysis. London, Unwin Hyman. 112 p.

Marschner H (2012). Mineral nutrition of higher plants. 3 ed. New York, Academic Press. 651 pp.

Martins LD, Tomaz MA, Amaral JFT, Bragança SM, Rodrigues WN, Reis EF (2013a). Nutritional efficiency in clones of conilon coffee for phosphorus. J. Agric. Sci. 5(1):130-140.

Martins LD, Tomaz MA, Amaral JFT, Christo LF, Rodrigues WN, Colodetti TV, Brinate SVB (2013b). Morphological changes in conilon coffee clones submitted to phosphorus levels. Sci. Plena. 9(4):1-11. [in portuguese]

Novais RF, Neves JCL, Barros NF (1991). Ensaio em ambiente controlado. In: Oliveira AJ, Garrido WE, Araújo JDE, Lourenço S. Métodos de pesquisa em fertilidade do solo. Brasília, Embrapa/SAE. pp.189-254.

Pedrosa AW, Favarin JL, Vasconcelos ALS, Carvalho BV, Oliveira FB, Neves GB (2014). *Brachiaria* residues fertilized with nitrogen in coffee fertilization. Coffee Sci. 9(3):366-373. [in portuguese]

Prezotti LC, Bragança SM (2013). Accumulation of dry mass, n, p and k in different genetic sources of conilon coffee. Coffee Sci. 8(3):284-294. [in portuguese]

Quintela MP, Silva TJA, Bonfim-Silva DM, Silva EFF, Bebé FV (2011). Nutritional parameters of coffee production and submitted in the region of nitrogen Garanhuns. Rev. Caatinga. 24(4):74-79. [in portuguese]

Rena AB, Maestri M (1986). Fisiologia do cafeeiro. In: Rena AB, Malavolta E, Rocha M, Yamada T. Cultura do cafeeiro: fatores que afetam a produtividade. Piracicaba, Associação Brasileira para Pesquisa da Potassa e do Fosfato pp. 13-85.

Ronchi CP, DaMatta FM (2007). Aspectos fisiológicos do café conilon. In: Ferrão RG, Fonseca AFA, Bragança SM, Ferrão MAG, De Muner LH. Café conilon. Vitória, Incaper. pp. 93-120.

Rodrigues WN, Tomaz MA, Ferrão RG, Ferrão MAG, Fonseca AFA, Miranda FD (2012). Genetic parameters estimation in groups of conilon coffee clones. Coffee Sci. 7(2):177-186. [in portuguese]

Seemann, JR, Sharkey TD, Wang JL, Osmond CB (1987). Environmental effects on photosynthesis, nitrogen use efficiency, and metabolic pools in leaves of sun and shade plants. Plant Physiol. 84:796-802.

Taiz L, Zeiger E (2013). Fisiologia vegetal. 5 ed. Porto Alegre, Artmed. 918 p.

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Pod quality of snap bean as affected by Nitrogen fixation, cultivar and climate zone under dryland agriculture

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Snap bean (*Phaseolus vulgaris* L.) is one of the major vegetable crops in Ethiopia grown for export and local markets. The crop is mainly produced during the dry season under irrigation. Snap bean has higher price than other vegetables in local markets in Ethiopia; however, the high cost of production under irrigation restricts the majority of local farmers from taking this opportunity. The main objective of this research was to investigate the influence of nitrogen (N) treatment, cultivar and contrasting environments on pod quality of snap bean under rain fed conditions. Three N treatments (0 and 100 kg N ha^{-1}, and *Rhizobium etli*[HB 429]) and eight snap bean cultivars were evaluated in a factorial experiment arranged as a randomized block design with three replications. The experiment was conducted at three locations (DebreZeit, Hawassa and Ziway) in 2011 and 2012. Applied N and rhizobium inoculant increased marketable pod yield by 43 and 18%, respectively. Cultivar Melkassa 1 had the greatest marketable yield, but had lower pod physical qualities than other cultivars. The highest zinc concentration in pods was obtained at Hawassa location. In conclusion, viable option for the production of high quality snap bean can be realised under rain fed condition using rhizobial inoculant as N source. These results open new opportunity for resource limited farmers in Ethiopia to produce snap bean with acceptable quality using rhizobial inoculation as N source under rain fed condition.

Key words: Snap bean, cultivars, quality, rhizobium, nutrient concentrations.

INTRODUCTION

Snap bean cultivars are specific cultivars of common bean (*Phaseolus vulgaris* L.) grown for their green pods used as vegetable and serve as an important source of protein. The pod physical quality of snap bean is a combination of appearance and physical condition. Acceptable snap bean quality includes well-formed and straight pods, and pods should be bright in color with a fresh appearance, free of defects, and tender, not tough or stringy and firm (Cantwell and Suslow, 1998). The quality of snap bean pods can also be expressed in terms

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of nutrient concentrations because of their importance in human nutrition. Over two billion people are affected by micronutrient malnutrition in the developing world (Cakmak et al., 2010). Iron (Fe) and zinc (Zn) deficiencies are leading micronutrient deficiencies affecting preschool children that impaired physical growth and mental development (Fe), hampered growth and development (Zn), and weaken the immune system (Zn) (Cakmak et al., 2010). Consumption of common bean which its pods are rich in quality protein, fibre, micronutrients such as iron, zinc and vitamin A may help to alleviate micronutrient malnutrition (Ugen et al., 2012). Common bean also contains high protein that contains the essential amino acid lysine (Baudoin and Maquet, 1999). Although information is lacking for direct comparison, independent research results showed that snap bean immature pods on a dry weight basis contain a similar range of protein concentration as dry seeds of common bean (Abubaker, 2008; Pereira et al., 2009). Report also showed that snap bean possesses relatively high bio available calcium when compared to other vegetables (Quintana et al., 1999a).

Protein and mineral concentrations of snap bean pods can be affected by cultural practices including N fertilizer (Abubaker, 2008; Ahmed et al., 2010). Further, yield and quality of snap bean plant were significantly improved by organic fertilizers (Salinas-Ramírez et al., 2011) and by both macro and micro nutrient applications (Tantawy et al., 2009; Abdel-Mawgoud et al., 2011).

The use of synthetic N for improvement of snap bean pod quality has been well documented and extensively studied. However, dependency on synthetic fertilizers needs to be minimized due to some reasons including high fertilizer cost. Synthetic fertilizers may also release greenhouse gases and may loss in the field resulting from less effective application strategy (Reid et al., 2011; Ferguson et al., 2010; Ferguson, 2013). Extensive reports are available on the use of rhizobium inoculation for increasing yield of chickpea (Bhuiyan et al., 2008), soybean (Sall and Sinclair, 1991), field bean (Bildirici and Yilmaz, 2005; Otieno et al., 2009), and many other legumes. However, the use of rhizobium inoculant for improving the quality of snap bean is lacking. Some reports also argued the effectiveness of using rhizobium inoculation for vegetable legume production including snap bean because nitrogen fixation may not produce adequate N early in the season to support pod production (Rubatzky and Yamaguchi, 1997).

The productivity and quality of a given crop species or cultivarare determined by crop management and agro-meteorological variables such as soil properties, rainfall and temperature (Dapaah, 1997; Hoogenboom, 2000). Individual legume species or cultivars often require specific ecological niches for maximum production (Masaya and White, 1991), which should be considered when selecting the production site suitability whether at local, national or international levels (Valentine and Matthew, 1999). Knowledge of the developmental and environmental factors contributing to yield and quality variation is, therefore, required to maximize yield and quality of agricultural crops. Yield variation was observed among different *P. vulgaris* genotypes along different locations in Tanzania (Giller et al., 1998). Nutrient concentration in seeds of common bean was also influenced by genotype (Beebe et al., 2000; Gregorio, 2002; Nchimbi-Msolla and Tryphone, 2010; Prolla et al., 2010) and environment (Quintana et al., 1999b; Nchimbi-Msolla and Tryphone, 2010).

However, studies are lacking on the influence of climatic zones (environment) on the physical properties and nutrient concentrations of snap bean pods. The interactive effects of environments and cultivars on pod quality of snap bean also need investigation. We hypothesized that rhizobium inoculation can be the main N source to produce quality snap bean under low input production system. Further, rhizobium inoculation, selection of suitable climatic zone (location) and cultivar can improve pod physical qualities and nutrient concentrations in the pods of snap bean.

The objectives of the study were first to assess the possibility of using rhizobium inoculation as the main source of N to produce quality snap bean pods relative to the use of synthetic N fertilizer, and second to evaluate the cultivars of snap bean for their marketable yield, physical pod qualities and nutrient concentrations in the pods under different N treatments across different agro-ecologies in the Rift Valley of Ethiopia.

MATERIALS AND METHODS

Site characteristics

The study was conducted at three sites across different agro-ecologies in the Rift Valley of Ethiopia. The three sites were DebreZeit, Hawassa and Ziway. DebreZeitis found at 8°44’52’’N, 38°05’53’’E, in a tepid to cool sub-moist climate zone characterized by moderate temperature and a definitive rainfall patterned between July to September (Table 1). It is situated at higher altitude in the transitional region of the Rift Valley and associated mountain ranges. The area is dominated by clay soils with higher copper and cation exchange capacity, and a neutral pH (Table 2).

Hawassa is situated at 7°4’ N, 38°31’ E and it is in a hot to warm sub-moist humid climate zone with warmer temperature especially during the dry season (February to April) (Table 1). It has a longer growing season and a less definitive pattern of rain fall during the growing season (Table 1). It is a mid-highland area in the Rift Valley zone. The soil is loam characterized by slightly acidic pH and higher concentrations of micronutrients such as manganese, iron and zinc (Table 2).

Ziway is found at 8°00’ N, 38°45’E in a tepid to cool semi-arid climate zone with erratic rainfall and unpredictable climate (Table 1). The area is in the Rift Valley zone with a mid-altitude. It has warmer temperature particularly during the dry season. The soil is sandy loam with a very high pH and relatively higher exchangeable sodium (Table 2). Ziway is located at a distance of around100 km equidistant between DebreZeit and Hawassa.

Experimental design and crop management

The field experiments were conducted under rain fed conditions in

Table 1. Average rainfall, maximum and minimum temperature during 2011 and 2012 growing seasons atDebreZeit, Hawassa and Ziway, Ethiopia. Ten year normal climate, altitude and climate zone of each location are presented.

Year		DebreZeit			Hawassa			Ziway		
		Rainfall	Max. T‡	Min. T§	Rainfall	Max. T‡	Min. T§	Rainfall	Max. T‡	Min. T§
		mm	°C	°C	mm	°C	°C	mm	°C	°C
	July	134.6	26.9	13.5	129.6	25.7	12.8	133.7	25.8	14.8
2011	August	241.7	25.0	14.9	157.3	25.3	13.0	114.8	24.6	15.1
	September	82.6	25.0	14.9	113.3	25.7	13.3	56.2	25.5	13.3
	Annual	724.1	26.4	11.3	776.1	28.0	12.1	598.3	29.1	13.0
	July	197.4	25.0	13.5	232.5	24.9	14.7	326.3	23.2	15.0
2012	August	256.5	24.5	12.6	72.7	24.4	14.5	171.4	24.3	14.7
	September	103.0	25.6	12.5	139.8	27.0	15.3	136.6	27.8	9.7
	Annual	726.3	26.7	10.4	884.8	28.1	12.7	856.8	28.6	12.4
10 years	Normal	747.0	26.4	10.7	786.5	27.9	12.3	763.9	27.5	13.9
Altitude (m above sea level)			1950			1700			1645	
Climate Zone †		Tepid to cool sub-moist			Hot to warm sub-moist Humid			Tepid to cool Semi arid		

Data collected by DebreZeit Agricultural Research Center (DebreZeit), South Agricultural research Center (Hawassa), and Adame Tulu Agricultural Research Center (Ziway). †According Ethiopian Ministry of Agriculture (2000); ‡Maximum temperature; § Minimum temperature.

2011 and 2012 during the main rainy season from June to September (normal planting season in the region). In 2011 seeding occurred on June 27, July 6 and 19 at Ziway, DebreZeit and Hawassa, respectively. In the second year, crops were seeded on July 1, 2 and 4, 2012, at Hawassa, Ziway and DebreZeit, respectively. At each of the three sites, eight snap bean cultivars (six commercial: Andante, Boston, Contender Blue, Lomami, Paulista and Volta; and two locally recommended cultivars from Melkassa Agricultural Research Center: Melkassa 1 and Melkassa 3) were tested against three N treatments (0kg N ha^{-1}, *Rhizobium etli* [strain HB 429] and 100 kg N ha^{-1}). The rhizobium strain used in the experiment was developed by the National Soil Testing Center at Addis Ababa, Ethiopia. The strain is being used by local farmers for dry bean production. Seeds of snap bean cultivars for rhizobium inoculation treatment were coated with charcoal based rhizobium inoculum (*R. etli* [HB 429]) at a concentration of 1×10^9 cells g^{-1} material. Fresh inoculum impregnated in charcoal was taken from National Soil Testing Centre, Addis Ababa, Ethiopia, one week before seeding date. On the date of seeding, the snap bean seeds for inoculation were wetted with water with a spoon of sugar in it as a sticker solution. The charcoal base rhizobium inoculum was mixed thoroughly with seeds with sticker for proper coating. Then the coated seeds were put under shade for approximately 20 to 30 min and then seeded immediately. The detailed procedure is summarized in N2Africasite (http://www.n2africa.org/). The 100 kg N $ha^{-1}$is the average rate of commonly used N fertilizer by commercial snap bean producers in Ethiopia.

The treatments were applied as factorial combinations in a Completely Randomized Block Design with three replications at each location and year. The size of the plot was 2.5 m × 2.0 m. Each plot had five rows. Row length was 2.0 m with 0.1 m between plants within each row and 0.5 m between rows. The two outer rows were considered as border rows. Plant population was maintained by planting two seeds per hill and thinned to one upon appearance of trifoliate leaves.

The recommended rate of phosphorus fertilizer (21 kg P ha^{-1}) was applied at the time of seeding in the form of triple super phosphate for all locations. Weeds were controlled by hand weeding and hoeing. Fungicide (Mancozeb) was also sprayed to protect from fungal diseases at three week intervals until the pod setting stage.

Measurements

Marketable yield and other physical qualities

Pods at optimum maturity (firm, bright green, and tender fleshy pods with small green immature seeds at60 to 70 days after planting) were harvested. Three to four rounds or passes of harvesting were made depending on the cultivar. The weights of marketable pods from the total harvest were calculated as tonnes per hectare. The length and diameter of pods from four randomly selected sample plants per plot were measured with a tape measure and sieve, respectively. Pod texture (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/rough, 5 = very coarse/rough) and pod appearance (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected) were rated visually using the scale modified from Proulx et al. (2010) and Martinez et al. (1995). Pod texture and appearance were rated by five experts who grade and pack snap bean for export markets. For titrate be acidity, aliquots (10.0 g) of juice were diluted with 50 mL distilled water and acidity was determined by titration with 0.1 N NaOH end point (pink color). The results were converted into percentage malic acid, which is the main organic acid in snap bean (Martinez et al., 1995) using the formula (1) of Proulx et al. (2010).

$$TA = \frac{mL\ NaOH * 0.1\ N * 0.067\ meq}{10.0\ g} \times 100 \quad (1)$$

Where TA = Titratable acidity, mL = milliliter, NaOH = Sodium hydroxide, N = Normal (normality of NaOH), meq = milli-equivalent (molecular weight of malic acid = 67), and g = gram (juice).

The total soluble solids (TSS) of pods were measured using a hand-held refractometer for Brix (TBT, RHB0-80, Jiangsu, China).

Nutrient concentration

Total N and phosphorus in green pods of snap bean were measured by a sulfuric acid-hydrogen peroxide digestion using a temperature-controlled digestion block (Thomas et al., 1967), followed by determination of total N and phosphate concentration in

Table 2. Soil physicochemical characteristics at DebreZeit, Hawassa and Ziway, Ethiopia during the 2011 and 2012 growing seasons.

Profile code	DebreZeit 2011	Hawassa 2011	Ziway 2011	DebreZeit 2012	Hawassa 2012	Ziway 2012
Sand (%)	13.59	47.03	83.62	15.57	51.69	74.17
Silt (%)	14.75	29.66	14.33	10.30	30.20	17.22
Clay (%)	71.65	23.31	2.05	74.14	18.12	8.61
Texture class†	Clay	Loam	Sandy loam	Clay	Loam	Sandy loam
pH-H_2O (1:2.5) ‡	6.98	6.10	8.38	6.98	6.10	8.20
pH-KCl (1:2.5) ‡	5.96	5.31	7.61	6.02	5.22	7.58
EC (ms cm^{-1}) (1:2.5)	0.16	0.17	0.15	0.26	0.17	0.26
Exch.Na (cmolc kg^{-1} soil) §	0.44	0.65	1.19	0.70	0.60	1.35
Exch.K (cmolc kg^{-1} soil) §	0.36	1.50	1.84	0.32	2.41	2.20
Exch.Ca (cmolc kg^{-1} soil) §	32.32	12.93	18.58	28.28	12.93	21.82
Exch.Mg (cmolc kg^{-1} soil)§	15.35	11.31	6.87	12.12	8.08	0.81
sum of cations (cmolc kg^{-1} soil)	52.70	36.01	34.69	44.35	36.01	37.77
CEC (cmolc kg^{-1} soil)	48.47	26.39	28.48	41.42	24.01	26.18
Organic Carbon (%) ¶	1.5	1.59	0.96	1.47	1.55	1.15
Nitrogen (%) ††	0.11	0.11	0.10	0.08	0.10	0.07
Available P (mg P_2O_5 kg^{-1} soil) #	43.66	49.32	43.81	41.89	91.68	83.46
Available K (mg K_2O kg^{-1} soil) §	158.22	620.7	778.91	141.18	973.64	864.11
$CaCO_3$ (%)						
Exchangeable sodium % (ESP) §	0.83	1.80	3.42	1.58	1.66	3.58
Micronutrients ‡‡						
Cu (mg kg^{-1} soil)	2.04	0.30	0.33	1.47	0.39	0.32
Fe (mg kg^{-1} soil)	12.46	28.96	3.13	10.64	25.93	4.58
Mn (mg kg^{-1} soil)	9.27	20.76	2.70	7.82	27.03	4.63
Zn (mg kg^{-1} soil)	0.86	3.61	1.08	0.86	3.78	1.50

Methods: †Hydrometer; ‡Acid neutralization; §Ammonium acetate; #Olsen; ¶Walklay and Black; ††Kjeldahl; ‡‡ Instrumental.

the digest (Wall et al., 1975; Watanabe and Olsen, 1965), using automated colorimetry (Technicon Instruments Corporation, New York, USA). Protein was estimated by multiplying total N by 6.25 (Imran et al., 2008). The zinc and iron concentrations were analyzed on a novAA®330 Atomic Absorption Spectrometer (Analytikjena, Jena, Germany) using an air/acetylene flame. The calcium and potassium concentrations were analyzed using the same NovAA330 Atomic Absorption Spectrophotometer using nitrous oxide as the oxidant for the acetylene.

Statistical analysis

Data analysis was done using the PROC MIXED procedure of the SAS software version 9.3 (SAS Institute Inc., 2012). The assumptions of ANOVA for normality of distribution and homogeneity of variance were checked. The two years data were combined for analysis. The covariance parameter estimate showed there was year by location by cultivar interactions for protein and calcium concentrations. Therefore, a separate analysis was done for each year to identify the effect of location by cultivar interaction on these response variables. The TSS and acidity data were available only in 2012. Nitrogen treatments, cultivar and locations (agro-ecology) were considered as fixed effects. Year, block nested in year, the interaction of each of main plot factors (N treatment, cultivar and location) with year and the two-way and three-way interactions of main plot factors with year were considered as random. The non-significant covariance parameters were eliminated starting from the higher level of interaction from the model according to AIC values to simplify the model for better model fit (Littell et al., 2005). Interaction effects between main effects (cultivar by location, cultivar by N treatment, N treatment by location, and three way interaction cultivar by location by N treatment) were presented only when statistically significant. The absence of significant interaction shows no particular reaction of one main effect (for example cultivar)at another specific main effect (for example location). Means were separated according Fisher's protected LSD at $P < 0.05$.

RESULTS

Pod marketable yield and other physical qualities

The combined analysis showed that N treatment significantly affected marketable yield, pod appearance and titratable acidity (Table 3). Cultivar significantly affected marketable yield and all other pod physical quality parameters (Table 3).Location significantly affected marketable yield and titratable acidity (Table 3). Cultivar by location interaction significantly ($P < 0.05$) affected TSS of snap bean pods but did not significantly

Table 3. *P*-values from mixed model ANOVA F-test for marketable yield, pod length, pod diameter, pod texture, pod appearance, titratable acidity (TA) and total soluble solids (TSS) of snap bean affected by nitrogen treatment, cultivar and location in 2011 and 2012 under rain fed conditions.

Source	Marketable yield	Pod length	Pod diameter	Texture	Appearance	TA	TSS
	t ha^{-1}	mm	mm	1-5	1-5	%	^{o}Brix
Nitrogen treatment (N)	0.0001***	0.3794	0.1192	0.0986	0.0054**	0.002**	0.0727
Cultivar (V)	<.0001***	<.0001***	<.0001***	0.0316*	0.0046**	0.0072**	0.0225*
Location (L)	0.0173*	0.0868	0.3865	0.6253	0.6626	0.0016**	0.5567
L*V	0.473	0.3774	0.292	0.272	0.5378	0.2464	0.0001***
L*N	0.1543	0.2169	0.9079	0.0078**	0.9636	0.1993	0.8423
V*N	0.6966	0.2525	0.8376	0.0299*	0.0226*	0.6749	0.4801
L*V*N	0.7419	0.4971	0.8746	0.6752	0.6962	0.0567	0.8689

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

Table 4. Pod marketable yield, length, diameter, texture, appearance, titratable acidity and total soluble solids (TSS) of snap bean affected by nitrogen treatment, cultivar and location (combined 2011 and 2012).

Nitrogen treatment	Marketable yield (t ha^{-1})	Pod length (mm)	Pod diameter (mm)	Texture (1-5)†	Appearance (1-5)‡	Titratable acidity (%)§	TSS (^{o}Brix)§
100 kg N ha^{-1}	20.54^{a}	125.0	7.56	1.21	1.21^{c}	0.0769^{a}	5.54
Rhizobium etli (HB 429)	16.92^{b}	122.0	7.49	1.91	1.81^{b}	0.0747^{a}	5.50
Zero N	14.39^{c}	120.2	7.38	1.98	2.13^{a}	0.0701^{b}	5.46
Cultivar							
Andante	11.70^{c}	106.4^{e}	6.01^{e}	1.42^{b}	1.57^{b}	0.0765^{a}	5.44^{b}
Boston	17.94^{b}	123.1^{bc}	7.11^{d}	1.44^{b}	1.54^{b}	0.0768^{a}	5.41^{b}
Contender Blue	16.94^{b}	112.8^{d}	7.38^{cd}	1.55^{b}	1.56^{b}	0.0747^{ab}	5.47^{ab}
Lomami	18.14^{ab}	122.7^{c}	7.44^{cd}	1.59^{b}	1.63^{b}	0.0775^{a}	5.51^{ab}
Melkassa 1	20.60^{a}	125.8^{bc}	8.68^{a}	2.26^{a}	2.26^{a}	0.0668^{c}	5.49^{ab}
Melkassa 3	16.95^{b}	133.8^{a}	8.32^{b}	2.15^{a}	2.15^{a}	0.0726^{abc}	5.56^{a}
Paulista	17.98^{b}	126.5^{bc}	7.36^{cd}	1.57^{b}	1.5^{b}	0.0700^{bc}	5.57^{a}
Volta	18.00^{b}	128.1^{b}	7.48^{c}	1.61^{b}	1.54^{b}	0.0763^{a}	5.56^{a}
Location							
DebreZeit	18.45^{a}	122.2	7.22	1.77	1.70	0.0789^{a}	5.54
Hawassa	21.23^{a}	129.2	7.81	1.69	1.71	0.0782^{a}	5.50
Ziway	12.17^{b}	115.8	7.39	1.65	1.74	0.0647^{b}	5.47

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, *P*<0.05. Absence of letter in a grouping column denotes non significance. % determined on the basis of g 100 g^{-1} of pod dry weight. †Score (1 = very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough). ‡Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected). § Data only 2012.

affect marketable yield, pod length, pod diameter, texture, and pod appearance. Location by N treatment significantly (P < 0.05) affected pod texture but had no effect on other pod physical quality parameters considered in this section (Table 3). Cultivar by N treatment interaction significantly affected pod texture and appearance but had no significant effect on other physical quality parameters (Table 3). The three ways interaction (N treatment by cultivar by location) had no effect on all of the parameters considered (Table 3).

Nitrogen treatments, cultivars and locations significantly affected marketable pod yield (Table 3). The highest marketable pod yield was produced using 100 kg N ha^{-1} (Table 4). Rhizobium inoculation resulted in significantly higher marketable pod yield than the no N application (control). The greatest and the least marketable yield were obtained from Melkassa 1 and Andante, respectively (Table 4). Hawassa and DebreZeit were found to be suitable areas to produce snap bean and both had significantly greater marketable yield than Ziway

Table 5. Nitrogen treatment by cultivar interaction for snap bean pod texture and pod appearance. Nitrogen treatment by location interaction for snap bean pod texture. Location by variety interaction for total soluble solids (TSS). (Combined2011 and 2012).

Cultivar	Texture (1-5)†			Appearance (1-5)‡			TSS (°Brix)§		
	Nitrogen treatment			Nitrogen treatment			Location		
	0 kg N ha^{-1}	*Rhizobium Etli* (HB 429)	100 kg N ha^{-1}	0 kg N ha^{-1}	*Rhizobium etli* (HB 429)	100 kg N ha^{-1}	DebreZeit	Hawassa	Ziway
Andante	1.61^{cde}	1.67^{de}	1.00^{e}	1.83^{bcd}	1.78^{bcd}	1.11^{e}	5.6^{ab}	5.57^{a_d}	5.16^{f}
Boston	1.61^{cde}	1.67^{de}	1.06^{e}	1.83^{bcd}	1.78^{bcd}	1.00^{e}	5.6^{ab}	5.2^{ef}	5.42^{bcd}
Contender Blue	1.83^{bcd}	1.83^{bcd}	1.00^{e}	1.94^{bcd}	1.67^{cd}	1.06^{e}	5.52^{a_d}	5.53^{a_d}	5.35^{de}
Lomami	1.83^{bcd}	1.94^{abcd}	1.00^{e}	1.94^{bcd}	1.89^{bcd}	1.06^{e}	5.42^{bcd}	5.5^{a_d}	5.61^{ab}
Melkassa 1	2.61^{a}	2.33^{abc}	1.83^{bcd}	2.83^{a}	2.06^{b}	1.89^{bcd}	5.51^{a_d}	5.38^{cde}	5.59^{abc}
Melkassa 3	2.5^{ab}	2.17^{abcd}	1.78^{bcd}	2.78^{a}	2.11^{b}	1.56^{cd}	5.53^{a_d}	5.6^{ab}	5.53^{a_d}
Paulista	1.89^{bcd}	1.83^{bcd}	1.00^{e}	1.94^{bcd}	1.56^{d}	1.00^{e}	5.57^{a_d}	5.62^{ab}	5.53^{a_d}
Volta	2^{bcd}	1.83^{bcd}	1.00^{e}	1.94^{bcd}	1.67^{cd}	1.00^{e}	5.54^{a_d}	5.58^{abc}	5.57^{a_d}
Location									
DebreZeit	2.15^{a}	1.92^{abc}	1.25^{bc}						
Hawassa	1.85^{abc}	1.85^{abc}	1.21^{bc}						
Ziway	1.81a^{bc}	1.96a^{bc}	1.17^{c}						

Means followed by different letters in the same interaction groups (nitrogen treatment x cultivar; nitrogen treatment x location; location x cultivar) in the same parameter differ significantly based on LSD, $P< 0.05$. †Score (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough). ‡Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected). § data only 2012.

(Table 4).

Cultivar had significant effects on pod length and pod diameter of snap bean. However, N treatments and locations had no effect on pod length and diameter (Table 3). Melkassa 3 produced the longest pods of all cultivars (Table 4). Among the commercial cultivars, Volta produced longer pods than Andante, Contender Blue and Lomami. In contrast, Andante produced the shortest pods of all cultivars followed by Contender Blue (Table 4). Melkassa 1 produced the largest pod diameter followed by Melkassa 3 (Table 4).Volta produced largest pod diameters among the commercial cultivars (Table 4). Among commercial cultivars, pods of cultivar Volta were similar in diameter to pods from Contender Blue, Lomami and Paulista (Table 4). On the other hand, Andante produced the smallest pod diameters of all cultivars (Table 4).

Cultivar significantly affected the texture of snap bean pods (Table 3). The interactions of N treatment by cultivar, and N treatment by location significantly affected pod texture (Table 3). Commercial cultivars generally had better pod texture than Melkassa cultivars (Table4). Commercial cultivars had smooth and uniform pod texture in contrast to pods from Melkassa cultivars which were rough and lacked uniformity. The cultivar differences in pod texture were enhanced by N as seen in the interaction of N treatment by cultivar (Table 5). The best textures seen in Contender Blue, Lomami, Paulista and Volta were all obtained under 100 kg N ha^{-1} application apart from Andante and Boston which were already at their best regardless of N treatment (Table 5). Nitrogen application also improved the texture of Melkassa 1 (Table 5). The results from the N treatment by location interaction showed that N application at Ziway resulted in better pod texture than the control at DebreZeit (Table 5). Generally, N application improved the texture of snap bean pods at all location (Table 5).

Nitrogen treatment and cultivar had significant effect on the appearance of snap bean pods, while the effect of location was not significant

Table 6. *P*-values from mixed model ANOVA F-test for protein, phosphorus (P), zinc Zn), calcium (Ca) and potassium (K) concentration of snap bean pods affected by nitrogen treatment, cultivar and location in 2011 and 2012 under rain fed conditions.

Source	Protein (%)	P (%)	Zn (ppm)	Ca (%)	K (%)
Nitrogen treatment (N)	0.3093	0.0232*	0.3792	0.4652	0.1106
Cultivar (V)	0.050	0.0131*	0.0132*	0.0178*	0.069
Location (L)	0.9413	0.8254	0.001**	0.0753	0.1482
L*V	0.8561	0.6891	0.1245	0.9281	0.0051**
L*N	0.8768	0.6424	0.2873	0.1451	0.0197*
V*N	0.641	0.162	0.5638	0.7692	0.8836
L*V*N	0.6985	0.6057	0.5623	0.3658	0.693
Year*L*V	0.0469*	-	-	0.0136*	-

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

(Table 3). The interaction of N treatment by cultivar also significantly affected snap bean pod appearance (Table 3). Excellent pod appearance was obtained from 100 kg N ha^{-1} (Table 4). Rhizobium inoculation also improved the appearance of snap bean pods as compared to the control (Table 4). Commercial cultivars produced the best pod appearance (Table 4). Pod appearance of Melkassa cultivars were in an acceptable range but not at the level of commercial cultivars (Table 4).

Nitrogen by cultivar interaction significantly affected pod appearance. Pods from commercial cultivars was at their best appearance when they were treated by 100 kg N ha^{-1} (Table 5). Applied N fertilizer resulted in better appearance than rhizobium inoculation for all cultivars except Melkassa 1 (Table 5). Pod appearance of Melkassa cultivars was better under rhizobium inoculation than undercontrol treatment (Table 5). There was no difference between rhizobium inoculation and the zero N control for pod appearance of commercial cultivars (Table 5).

Nitrogen treatment, cultivar and locations significantly affected titratableacidity of snap bean pods (Table 3). N application and rhizobium inoculation in particular increased titratableacidity of snap bean pods (Table 4). For the cultivar response, Lomami hadgreaterpod titratableacidity, though it was not significantly different than Andante, Boston, Contender Blue, Melkassa 3 and Volta (Table 4). Growing snap bean at Hawassa and DebreZeit resulted in higher percentage of titratable acidity in the pods than that grown at Ziway (Table 4).

Cultivar significantly affected the TSS of snap bean pods. Nitrogen treatment and location had no effect on TSS of pods (Table 3). The location by cultivar interaction had also a significant effect on the TSS of snap bean pods. Within cultivars, there was only a slight range of TSS. Cultivars Melkassa 3, Paulista and Volta had greater TSS than Andante and Boston (Table 4). TSS was also affected by the interaction of cultivar and location (Table 5).

Nutrient concentrations

All main factors and their interactions had no significant effect on protein concentrations (Table 6). However, year by cultivar by location interactions significantly affected protein concentration as shown on covariate parameter estimate (Table 6). Nitrogen treatment significantly affected only phosphorus concentrations, and cultivar significantly affected phosphorus, zinc and calcium concentrations (Table 6). Location had significant effect only on zinc concentration. For the interaction effects, both location by cultivar and location by N treatment interactions significantly affected potassium concentration. All other interactions had no significant effect on nutrient concentration of snap bean pods, except calcium concentration and protein, which were affected by year by location by cultivar interaction (Table 6).

Analysis from combined data showed that N treatment, cultivar and location had no significant effect on the protein concentrations of snap bean pods (Table 6). However, year by cultivar by location interaction was significant. This indicates that year had significant influence on the cultivar by location interaction. From separate analyses for each year, 2011 (P = 0.018) and 2012 (P = 0.0001), significant interactions for cultivar by location interactions were seen. Paulista at DebreZeit and Volta at Hawassa produced the highest protein in 2011 and 2012, respectively (Table 6). Melkassa 3 at DebreZeit in 2011 and at Ziway in 2012 produced the lowest protein (Table 8). Protein levels in other cultivars were inconsistent from location to location and from year to year. Generally, most cultivars produced high protein concentration at Ziway in 2011 and at Hawassa in 2012 (Table 8).

The effects of N treatment and cultivar were significant on the phosphorus concentration of snap bean pods (Table 6). Location had no effect on phosphorus concentrations in the pods. Applied N improved

Table 7. Protein, phosphorus, zinc, calcium and potassium concentrations of snap bean pods affected by nitrogen treatment, cultivar and location (combined 2011 and 2012).

Parameter	Protein (%)	Phosphorus (%)	Zinc (ppm)	Calcium (%)	Potassium (%)
Nitrogen treatment					
0 kg N ha^{-1}	17.9	0.399^{b}	28.96	0.68	3.2
Rhizobium etli (HB 429)	18.1	0.406^{ab}	29.42	0.67	3.1
100 kg N ha^{-1}	18.4	0.413^{a}	29.93	0.69	3.2
Cultivar					
Andante	18.6	0.413^{ab}	31.21^{a}	0.76^{a}	3.2
Boston	18.1	0.401^{bc}	30.70^{ab}	0.70^{ab}	3.2
Contender Blue	18.3	0.406^{bc}	30.88^{a}	0.65^{bc}	3.3
Lomami	18.9	0.423^{a}	29.69^{abc}	0.69^{ab}	3.3
Melkassa 1	17.7	0.397^{bc}	28.36^{bc}	0.64^{bc}	3.0
Melkassa 3	16.9	0.400^{bc}	28.13^{c}	0.59^{c}	3.1
Paulista	18.0	0.411^{abc}	29.06^{abc}	0.72^{ab}	3.3
Volta	18.2	0.394^{c}	27.45^{c}	0.68^{ab}	3.2
Location					
DebreZeit	18.3	0.404	27.87^{b}	0.66	2.9
Hawassa	18.3	0.415	36.22^{a}	0.61	3.2
Ziway	17.7	0.399	24.20^{c}	0.77	3.4

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, $P < 0.05$. Absence of letter in a grouping column denotes non significance. % determined on the basis of g 100 g^{-1} of pod dry weight.

phosphorus concentration, but no different in phosphorus concentration from rhizobium inoculation and zero N application (Table 7). Lomami produced the highest phosphorus concentration, significantly more than Boston, Contender Blue, Melkassa 1, Melkassa 3 and Volta (Table 7).

Snap bean cultivars and locations significantly affected zinc concentration inthe pods (Table 6). The N treatment did not significantly change zinc concentration in pods. Numerically, the highest pod zinc concentration was recorded from Andante. Pod zinc concentration among Andante, Boston, Contender Blue and Paulista was similar (Table 7). Snap bean produced the highest zinc concentration when grown at Hawassa followed by DebreZeit and Ziway (Table 7).

The combined data analysis of the two year experiment showed that cultivar had a significant effect on calcium concentration in snap bean pods. Calcium concentration was not affected by N treatment and location (Table 6). Year by cultivar by location interaction was also significant (Table 6). The separate analysis for 2012 indicated that the cultivar by location interaction significantly ($P = 0.0008$) affected calcium concentrations of snap bean pods. In 2011, the cultivar by location ($P = 0.375$) interaction was not significant. Combined analysis across two years showed that Andante produced higher calcium concentration than Contender Blue and Melkassa cultivars (Table 7). The cultivar by location interaction in 2012 showed that Andante produced the highest calcium when grown at Ziway (Table 8). Melkassa 3 pods had the lowest calcium concentration at Ziway (Table 6). Cultivars had similar pod calcium concentrations within DebreZeit, except Melkassa 1 at Hawassa (Table 8).

Pod potassium concentration was not affected by N treatment, cultivar or location. But the cultivar by location interaction significantly affected potassium concentration of snap bean pods (Table 6). Numerically, pods from Lomami at Ziway had the highest potassium concentration (Table 9). Overall, Lomami was the most consistent cultivar forpod potassium concentration across all locations (Table 9). Melkassa cultivars had pods with lower potassium at Hawassa than other cultivars group in the same location (Table 9).

The interaction of N treatment by location significantly affected potassium concentration in the pods. Applied N at Ziway resulted in higher pod potassium concentration than rhizobium inoculation and Zero N at DebreZeit (Table 9). Generally, cultivars produced lower potassium concentration at DebreZeit than at Hawassa and Ziway (Table 9).

DISCUSSION

The results demonstrated that applied N and rhizobium

Table 8. Protein (%) and calcium (%) concentrations of snap bean pods affected by cultivar by location interaction in 2011 and 2012.

Cultivar	2011			2012			2012		
	Protein (%)			Protein (%)			Calcium (%)		
	Location			Location			Location		
	DebreZeit	Hawassa	Ziway	DebreZeit	Hawassa	Ziway	DebreZeit	Hawassa	Ziway
Andante	17.54^{a-g}	16.19^{g-j}	18.16^{a-e}	19.93^{a-f}	20.49^{abc}	19.26^{b-g}	0.757^{b-e}	0.632^{f-k}	1.016^{a}
Boston	17.31^{a-g}	16.32^{f-i}	17.13^{b-h}	18.83^{d-j}	20.84^{a}	17.91^{h-l}	0.763^{b-e}	0.616^{g-k}	0.793^{bc}
Contender Blue	17.69^{a-g}	15.18^{ij}	18.90^{abc}	19.81^{a-g}	20.80^{a}	17.41^{j-m}	0.667^{d-i}	0.550^{jl}	0.769^{bcd}
Lomami	18.34^{a-d}	17.05^{d-h}	18.42^{a-d}	20.12^{ab}	21.07^{a}	18.48^{f-i}	0.658^{d-j}	0.551^{ijl}	0.819^{b}
Melkassa 1	16.32^{f-i}	14.88^{ij}	17.19^{b-h}	19.69^{a-g}	20.87^{a}	17.13^{lm}	0.743^{b-f}	0.659^{d-g}	0.697^{e-f}
Melkassa 3	14.38^{j}	15.45^{hij}	18.05^{a-f}	18.97^{c-h}	19.74^{b-g}	15.03^{n}	0.691^{c-h}	0.550^{jl}	0.530^{kl}
Paulista	18.91^{ab}	16.37^{e-i}	17.42^{a-g}	18.87^{d-j}	20.12^{a-e}	16.47^{m}	0.713^{b-g}	0.615^{g-k}	0.804^{bc}
Volta	17.21^{c-h}	15.91^{g-j}	18.17^{a-e}	18.67^{e-k}	21.10^{a}	18.32^{g-k}	0.664^{d-j}	0.562^{ijl}	0.793^{bc}

Means followed by the different letters in the same interaction group (cultivar x location) differ significantly based on LSD, $P< 0.05$. Letters a-g indicate all alphabetical letters included in the range from a to g. % determined on the basis of g 100 g^{-1} of pod dry weight.

Table 9. Potassium (%) of snap bean pods as affected by cultivar by location interaction; and Nitrogen treatment by location interaction in 2011 and 2012.

Parameter	Combined 2011 and 2012		
	Potassium (%)		
	Location		
	DebreZeit	Hawassa	Ziway
Cultivar			
Andante	2.86^{f-j}	3.40^{a-d}	3.20^{d-h}
Boston	2.78^{g-j}	3.26^{a-g}	3.42^{a-e}
Contender Blue	3.02^{b-g}	3.38^{a-f}	3.50^{abc}
Lomami	3.08^{a-f}	3.25^{a-g}	3.58^{a}
Melkassa 1	2.63^{j}	2.95^{e-j}	3.36^{a-f}
Melkassa 3	2.90^{d-h}	3.02^{c-i}	3.32^{b-f}
Paulista	3.02^{b-g}	3.33^{a-f}	3.38^{a-f}
Volta	2.87^{f-j}	3.32^{a-f}	3.25^{b-g}
Nitrogen treatment			
0 kg N ha^{-1}	2.83^{c}	3.29^{abc}	3.39^{ab}
Rhizobium etli (HB 429)	2.87^{bc}	3.24^{abc}	3.28^{abc}
100 kg N ha^{-1}	2.98^{abc}	3.18^{abc}	3.47^{a}

Means followed by different letters in the same interaction group (cultivar x location; nitrogen treatment x location) differ significantly based on LSD, $P < 0.05$. Letters a-g indicate all alphabetical letters included in the range from a to g. % determined on the basis of g 100 g^{-1} of pod dry weight.

inoculation were effective in improving the marketable yield of snap bean pods by 43 and 18%, respectively (Table 3). The result agreed with Mahmoud et al. (2010); El-Awadi et al. (2011) and Salinas-Ramírez et al. (2011) all of whom reported that applied N improved yield and yield components of dry bean. Results also confirmed Bildirici and Yilmaz (2005) who reported significant yield improvement in dry bean by rhizobium inoculation. The current result, however, was in contrast to Otieno et al. (2009) who found no yield response to rhizobium inoculation on dry bean. Most of these reports were focused on grain yield of dry bean. The current finding demonstrated that the benefit of rhizobium inoculation can be realized at earlier crop growth stage at immature pod stage. Our investigation showed the possibility of producing export quality snap bean under reduced inputs that minimizes the reliance of vegetable production on heavy N fertilizer especially for resource limited farmers.

The significant differences among cultivars for marketable yield may be due to size of the plant that attributed to increased photosynthetic area (leaf area index) and relatively more pod sites. Melkassa 1, the best cultivar for marketable yield was characterized by tall plants and a larger leaf area index (data not presented) that determined its high yield capacity. In addition, Melkassa 1 was a well-adapted cultivar to a reduced input production system, especially dry land agriculture as it was developed under Ethiopian conditions. The yield potential of commercial cultivars may be limited by environmental variables; potentially moisture shortage because this experiment was conducted under natural rain fed conditions. The marketable yield of snap bean cultivars was similar at DebreZeit and Hawassa, but it was lower at Ziway. Ziway is characterized by a high soil pH, semi-arid environment with erratic and unpredictable rain fall (Tables 1 and 2). This may limit the productivity and quality of snap bean. The high marketable yield at DebreZeit and Hawassa may be due to suitability of the agro-ecology at these locations for enabling better utilization of soil fertility (Tables 1 and 2).

The length and diameter of snap bean pods were not affected by N treatment and location under rain fed conditions (Table 3). Pod size is therefore highly controlled by genetic factors (additive gene effect) and less affected by environmental factors (Arunga et al., 2010). From our study, cultivars could be grouped into three categories based on pod diameter. Andante is an extra fine cultivar with very small pod diameter ranging from 5.0 mm to 6.2 mm and Melkassa cultivars (Melkassa 1 and Melkassa 3) were at the other extreme, being bobby cultivars with pod diameters ranging from 8.0 mm to 8.7 mm (Tables 3). The remaining cultivars were fine cultivars with pod diameter of 7.0 mm to 7.6 mm (Wahome et al., 2013).

Texture and appearance of snap bean pods are the two most critical parameters that influence their marketability. Snap bean pods are graded into marketable and unmarketable pods depending on texture and appearance. Texture and appearance of pods depend on smoothness, uniformity and overall look of the pods in the absence of disease, insect damage and other defects. The appearance of pods was improved by the application of N fertilizer (Tables 4 and 5).The texture and appearance of all the commercial cultivars were improved by N fertilizer application. Melkassa cultivars responded well for rhizobium inoculations especially pod appearance (Table 5). The result is in agreement with previous studies stating N application increased the quality of green bean (Mahmoud et al., 2010; Kamanu et al., 2012).Our findings demonstrated that rhizobium inoculant can provide sufficient N to improve the appearance of snap bean pods at least for some cultivars. Improved N nutrition turned green pods into well-formed and straight, bright in color and acceptable quality.

Commercial cultivars produced the highest quality pods due to their fine texture, and well-rounded straight pods. Melkassa cultivars lacked some quality characteristics including smoothness and uniformity of pods, particularly for Melkassa 1 which had a high marketable yield. Therefore, breeding work is needed to improve the pod appearance for Melkassa 1 to bring this cultivar to the premium level. Generally, all cultivars had fine texture and acceptable appearance at all locations. This indicates that it is possible to produce snap bean with acceptable texture and appearance for export markets even without N application and inoculation at any of the three sites.

Nitrogen application and rhizobium inoculation increased the titratable acidity of snap bean pods. Studies on tomato (Wright and Harris, 1985; Erdal et al., 2007) and grape (Baiano et al., 2011) fruits indicated that increasing N fertilizer increased titratable acidity of the fruit. As titratable acidity is the prime taste quality determinant in fruit juice (Zagory and Kader, 1989), the authors assumed applied N and rhizobium inoculation would improve the taste quality of pods by increasing titratable acidity. The titratable acidity of the cultivars was in the range for snap bean determined by Proulx et al. (2010). The higher titratable acidity at DebreZeit and Hawassa may be due to favorable growing conditions for snap bean production as reflected in other parameters such as marketable yield. Nitrogen nutrition, cultivar and growing location may additionally affect the taste quality of snap bean in terms of titratable acidity.

Some cultivars had consistent pod TSS from location to location but others did not. Melkassa 3, Paulista and Volta were numerically the most stable cultivars found in the top group of pod TSS at all locations. This may be due to environmental variables of a specific location determining the TSS of a particular cultivar (Hoogenboom, 2000). In soybean, TSS of pods is directly associated with the photo-assimilate manufactured by the plant (Liu et al., 2011), and affects the relative concentrations of soluble sugars in the pod. Generally, factors that affect soluble sugars also influence TSS (Caliman et al., 2010). TSS is another taste quality determinant (Champa et al., 2008), and cultivars with higher TSS have higher taste quality particularly when in combination with high titratable acidity (Al-Jamali and Hani, 2009).

In 2012, a cultivar by location interaction resulted in generally higher pod protein at Hawassa but the reverse was observed in 2011 with numerically better protein at Ziway. The low pod protein concentrations for most of the cultivars at Ziway may be due to unfavorable weather conditions especially erratic and excess rain fall during the early growth periods of these snap bean in 2012 (Table 1). Nitrogen from agricultural fields may be lost by moderate to high rain fall (Scharf and Lory, 2006), an effect which would be magnified by the sandy nature of the soil at Ziway (Table 2). Pod protein concentrations of cultivars were inconsistent from year to year and location

to location.

Applied N increased the phosphorus concentration in snap bean pods. This result was supported by Apthorp et al. (1987) who reported that N fertilizer application increased phosphorus uptake by plants. Rhizobium inoculation and applied N were similar in increasing pod phosphorus concentration. However, only the latter was significantly different from the control. The concentrations of phosphorus in green pods of snap bean showed variation among cultivars. Phosphorus shoot tissue concentration and its uptake by the plant were affected by varietal differences in common bean (Mourice and Tryphone, 2012).

Applied N and rhizobium showed a trend of enhanced zinc pod concentrations of snap bean pods numerically (Table 7). The variations among cultivars for zinc are supported by the report from Beebe et al. (2000) and Gregorio (2002) who reported the presence of sufficient variability in zinc concentration among bean cultivars. The authors found that the tested cultivars had pod zinc concentrations close to mean values reported by Beebe et al. (2000). Zinc concentration of pods was highest at Hawassa followed by DebreZeit. The soil analysis from each locations showed that high zinc content in the soil was found at Hawassa followed by Ziway, and DebreZeit had the least (Table 2). This may suggest that a high zinc concentration in pods at Hawassa was due to high zinc content in the soil. However, the pod zinc concentration at DebreZeit was higher than at Ziway. This may indicate that environmental variables other than zinc concentration in the soil may also contribute to zinc concentration in snap bean pods. Studies indicated that higher pH reduced the availability and plant uptake of zinc in the soil solution (Jeffery and Uren, 1983), which may explain the lower zinc concentration in pods at Ziway where high soil pH occurred (Table 2). Both zinc content and pH of the soil affected zinc concentration in snap bean pods. Locations with higher zinc content or slightly acidic soils resulted in pods with higher zinc concentration compared to locations with low zinc content or alkaline soils.

The result from 2012 showed that Andante was the top cultivar in producing calcium in its pod when grown at Ziway. This result is in agreement with reports of cultivars having small pod diameters have higher calcium concentration (Grusak and Pomper, 1999). Generally, snap bean cultivars had numerically, greater pod calcium when grown at Ziway and DebreZeit. Low calcium concentration in pods at Hawassa may be due to lower calcium concentration in the soil (Table 2). Calcium concentration in snap bean pods is influenced by cultivar and environmental conditions such as heat units (temperature), rainfall and water availability for crop uptake, and soil calcium concentration (Quintana et al., 1999b).

Applied N at Ziway improved the potassium concentration of snap bean pods when compared to rhizobium inoculation and no N at DebreZeit. Hirzel and Walter (2008) also reported that NPK fertilizer application increased soil and tissue concentrations of potassium in sweet corn.

Conclusion

Nitrogen application and rhizobium inoculation increased marketable yield and titratable acidity of snap bean compared to no N application. Nitrogen treatments interacted with cultivars to affect pod texture and pod appearance. Nitrogen application was almost always better than rhizobium inoculation for improving pod appearance, and consistently resulted in improved pod appearance compared to a zero N control. However, rhizobium inoculation also improved the appearance, particularly of the two Melkassa cultivars. Melkassa 1 was well-adapted to rain-fed conditions in that it gave numerically the highest overall marketable yield across all locations. Melkassa 1 had the largest pod diameter of any tested cultivar, and it is frequently ranked below commercial cultivars for pod texture and pod appearance. Locations interacted with cultivars and affected the pod traits TSS and concentrations of protein, calcium, and potassium. Snap bean pods produced at DebreZeit and Hawassa were similar in marketable yield and several other traits. Pod zinc concentration was particularly highest at Hawassa. Ziway, with a more arid climate and soil pH above 8.0, was the least favorable location for production of export-quality snap bean as compared to other locations tested. Generally, production of marketable quality snap bean pods can be achieved by using rhizobial inoculation as N source particularly for resource limited farmers. Hawassa, which is characterized by higher soil zinc content, slightly acidic soil and hot to warm sub-moist humid climate, is most suitable to produce snap bean pods with high zinc concentration. Further breeding works are required to improve the pod quality of cultivar Melkassa 1 to gain maximum benefits from its high yielding potential.

Conflict of Interest

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

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REFERENCES

Abdel-Mawgoud AMR, El-Bassiouny AM, Ghoname A, Abou-Hussein SD (2011). Foliar application of amino acids and micronutrients enhance performance of green bean crop under newly reclaimed land conditions. Aust. J. Basic Appl. Sci. 5:87-95.

Abubaker S (2008). Effect of plant density on flowering date, yield and quality attributes of bush bean (*Phaseolus vulgaris* L.) under center pivot irrigation system. Am. J. Agric. Biol. Sci. 3:666-668.

Ahmed H, Nesiem MR, Hewedy AM, Sallam HEl-S (2010). Effect of some simulative compounds on growth, yield and chemical composition of snap bean plants grown under calcareous soil conditions. J. Am. Sci. 6:552-569.

Al-Jamali AF, Hani MTB (2009). Croplife-B with nitrous oxide or carbon dioxide extends shelf life of green bean (*Phaseolus vulgaris* L.) at ambient temperatures. J. Food Agric. Environ. 7:38-43.

Apthorp JN, Hedley MJ, Tillman RW (1987). The effects of nitrogen fertilizer form on the plant availability of phosphate from soil, phosphate rock and mono-calcium phosphate. Fert. Res. 12:269-284.

Arunga EE, Van Rheenen HA, Owuoche JO (2010).Diallel analysis of Snap bean (*Phaseolus vulgaris* L.) cultivars for important traits. Afr. J. Agric. Res. 5(15):1951-1957.

Baiano A, La Notte E, Coletta A, Terracone C, Antonacci D (2011). Effects of irrigation volume and nitrogen fertilization on Redglobe and Michele Palieri table-grape cultivars. Am. J. Enol. Vitic. 62:57-65.

Baudoin J, Maquet A (1999). Improvement of protein and amino acid contents in seeds of food legumes. A case study in *Phaseolus*. Biotechnol. Agron. Soc. Environ. 3:220–224.

Beebe S, Gonzalez A, Rengifo J (2000). Research on trace minerals in the common bean. Food Nutr. Bull. 21:387-391.

Bhuiyan MAH, Khanam D, Hossain MF, Ahmed MS (2008). Effect of rhizobium inoculation on nodulation and yield of chickpea in calcareous soil. Bang. J. Agric. Res. 33:549-554.

Bildirici N, Yilmaz N (2005). The effect of different nitrogen and phosphorus doses and bacteria inoculation (Rhizobium phaseoli) on the yield and yield components of field bean (*Phaseolus vulgaris* L.). J. Agron. 4:207-215.

Cakmak I, Pfeiffer HW, McClafferty B (2010). Bioforification of durum wheat with zinc and iron: Review. Cereal Chem. 87:10–20.

Caliman FRB, Da Silva DJH, Stringheta PC, Fontes PCR, Moreira GR, Mantovani EC (2010). Quality of tomatoes grown under a protected environment and field conditions. IDESIA 28:75-82.

Cantwell M, Suslow T (1998). Bean, Snap: Recommendations for maintaining postharvest quality. Cited 2014 Oct 3. Available from: Davis. http://postharvest.ucdavis.edu/pfvegetable/BeansSnap/

Champa WAH, Weerakkody WAP, Palipane KB (2008). Maturity indices for harvesting of beans (*Phaseolus vulgaris* L.) variety Kentuckey Wonder Green in dry zone cultivation in Sri Lanka. Trop. Agric. Res. 20:123-133.

Dapaah HK (1997). Environmental influences on the growth, development and yield of pinto beans (*Phaseolus vulgaris* L.). Ph.D. Thesis, Lincoln University, Christchurch, New Zealand.

El-Awadi ME, El-Bassiony AM, Fawzy ZF, El-Nemr MA (2011). Response of snap bean (*Phaseolus vulgaris* L.) plants to nitrogen fertilizer and foliar application with methionine and tryptophan. Nature Sci. 9:87-94.

Erdal I, ErtekA,Senyigit U, Koyuncu MA (2007). Combined effects of irrigation and nitrogen on some quality parameters of processing tomato. World J. Agric. Sci. 3:57-65.

Ferguson BJ (2013). The development and regulation of soybean nodules. In: Board JE, editor. A comprehensive survey of international soybean research-genetics, physiology, agronomy and nitrogen relationships. Rijeka: InTech. pp. 31-47.

Ferguson BJ, Indrasumunar A, Hayashi S, Lin MH, Lin YH, Reid DE, Gresshoff PM (2010). Molecular analysis of legume nodule development and auto-regulation. J. Integr. Plant Biol. 52:61-76.

Giller KE, Amijee F, Brodrick SJ, Edje OT (1998). Environmental constraints to nodulation and nitrogen fixation of *Phaseolus vulgaris*L.in Tansania. II. Response to N and P fertilizers and inoculation with Rhizobium. Afr. Crop Sci. J. 6:171-178.

Gregorio GB (2002). Progress in breeding for trace minerals in staple crops. J. Nutr. 132:500S-502S.

Grusak MA, Pomper KW (1999). Influence of pod stomatal density and pod transpiration on the calcium concentration of snap bean pods. J. Am. Soc. Hortic. Sci. 124:194-198.

Hirzel J, Walter I (2008). Availability of nitrogen, phosphorus and potassium from poultry litter and conventional fertilizers in a volcanic soil cultivated with silage corn. Chilean J. Agric. Res. 68:264-273.

Hoogenboom G (2000). Contribution of agrometeorology to the simulation of Crop production and its application. Agric. For. Meteorol. 103:137-157.

Imran M, Khan H, Hassan SS, Khan R (2008). Physicochemical characteristics of various milk samples available in Pakistan. J. Zhejiang Univ. Sci. B. 9:546-551.

Jeffery JJ, Uren NC (1983). Copper and zinc species in the soil solution and the effects of soil pH. Aust. J. Soil Res. 21:479-88.

Kamanu JK, Chemining'wa GN, Nderitu JH, Ambuko J (2012). Growth, yield and quality response of snap bean (*Phaseolus vulgaris* L.) plants to different inorganic fertilizers applications in central Kenya. J. Appl. Biosci. 55:3944– 3952.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2005). SAS System for Mixed models (second ed.) SAS Institute Inc., Cary, NC: USA.

Liu B, Li YH, Liu XB, Wang C, Jin J, Herbert SJ (2011). Lower total soluble sugars in vegetative parts of soybean plants are responsible for reduced pod number under shading conditions. Aust. J. Crop Sci. 5:1852-1857.

Mahmoud AR, El-Desuki M. Abdel-Mouty MM (2010). Response of snap bean plants to bio-fertilizer and nitrogen level application. Int. J. Acad. Res. 2:179-183.

Martinez C, Ros G,Periago MJ, Lopez G, Ortuno J, Rincon F (1995). Physicochemical andsensory quality criteria of green beans (*Phaseolus vulgaris* L.). Lebensm. Wiss. u.Technol. 28:515-520.

Masaya P, White JW (1991). Adaptation to photoperiod and temperature. In:Van Schoonhoven A, VoysestO (eds.) Common beans: research for crop improvement. Cali: CAB International and CIAT, pp. 455-507.

Ministry of Agriculture (2000). Agro-ecological zones of Ethiopia. Addis Ababa:Natural Resources Management and Regulatory Department (Ethiopia).

Mourice SK, Tryphone GM (2012). Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for adaptation to low phosphorus. ISRN Agron. doi:10.5402/309614

N2Africa (2014). Better soybean through good agricultural practice. Africa Soil Health Consortium. http://africasoilhealth.cabi.org/wpcms/wp-content/uploads/2014/09/292-N2Africa-Ethiopia-common-beans-booklet.pdf (Accessed 20 April 2015).

Nchimbi-Msolla S, Tryphone GM (2010). The effect of the environment on iron and zinc concentrations and performance of common bean (*Phaseolus vulgaris* L.) genotypes. Asian J. Plant Sci. 9:455-462.

Otieno PE, Muthomi JW, Chemining'wa GN, Nderitu JH (2009). Effect of Rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected food grain legumes. J. Biol. Sci. 9:326-332.

Pereira T, Coelho CMM, Bogo A, Guidolin AF, Miquelluti DJ (2009). Diversity in common bean landraces from south Brazil. Acta Bot. Croat. 68(1):79-92.

Proll IRD, Barbosa RG, Veeck APL, Augusti PR, Da Silva LP, Ribeiro ND, Emanuelli T(2010). Cultivar, harvest year, and storage conditions affecting nutritional quality of common beans (*Phaseolus vulgaris* L.). Ciênc. Tecnol. Aliment. Campinas 30(1):96-102.

Proulx E, Yagiz Y, Nunes MCN, Emond J (2010). Quality attributes limiting snap bean (*Phaseolus vulgaris* L.) postharvest life at chilling and non-chilling temperatures. Hortic. Sci. 45:1238-1249.

Quintana J M, Harrison HC, Nienhuis J, Palta JP, Kmiecik K (1999a).

Comparison of pod calcium concentration between two snap bean populations. J. Am. Soc. Hortic. Sci. 124:273-276.
Quintana JM, Harrison HC, Nienhuis J, Palta JP, Kmiecik K (1999b). Differences in pod calcium concentration for eight snap bean and dry bean cultivars. Hortic. Sci. 34:932-934.
Reid DE, Ferguson BJ, Hayashi S, Lin YH, Gresshoff PM (2011). Molecular mechanisms controlling legume autoregulation of nodulation. Ann. Bot. 108:789-795.
Rubatzky VE, Yamaguchi M (1997). World vegetables: principles, productions and nutritive values.2nded. New York: Chapman and Hall (US).
Salinas-Ramírez N, Escalante-Estrada JA, Rodríguez-González MT, Sosa-Montes E (2011). Yield and nutritional quality of snap bean in terms of biofertilization. Tropical and Subtropical Agroecosystems 13:347-355.
Sall K, Sinclair TR (1991). Soybean genotypic differences insensitivity ofsymbiotic nitrogen fixation to soil dehydration. Plant Soil 133:31-37.
SAS Institute (2012). The SAS system for Windows. Release 9.3. Cary:SAS Institute, Inc. (US).
Scharf P, Lory J (2006). Integrated pest management: best management practices for nitrogen fertilizer in Missouri. Missouri:MU Extension, University of Missouri (Columbia).
Tantawy AS, Abdel-Mawgoud AMR, Habib HAM, Hafez MM (2009). Growth, productivity and pod quality responses of green bean plants *Phaseolus vulgaris* to foliar application of nutrients and pollen extracts. Res. J. Agric. Biol. Sci. 5:1032-1038.
Thomas R, Sheard RW, Moyer IP (1967). Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digest. Agron. J. 99:240-243.
Ugen M, Musoni A, Chemining'wa G, Kimani P, Mucharo M, Katafire M (2012). Utilization of common bean for improved health and nutrition in eastern and central Africa. Entebbe (Uganda):Association for Strengthening Agricultural Research in Eastern and Central Africa.
Wahome SW, Kimani PM, Muthomi JW, Narla RD (2013). Quality and yield of snap bean lines locally developed in Kenya. IJAAR 3:1-10.
Wall LL, Gehrke CW, Neuner TE, Cathey RD, Rexnord PR (1975). Cereal protein nitrogen: Evaluation and comparison of four different methods. J. Assoc. Off. Anal. Chem. 58:811-817.
Watanabe FS, Olsen SR (1965). Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc. 29:677-678.
Wright DH, Harris D (1985). Effect of nitrogen and potassium fertilization on tomato flavor. J. Agric. Food Chem. 33:355-358.
Zagory D, Kader AA (1989). Quality maintenance in fresh fruits and vegetables by controlled atmospheres. In: Jen JJeditor. Quality factors of fruits and vegetables-chemistry and technology. Washington DC: American Chemical Society (US).

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Genetic variability studies of fruit yield and its traits among indeterminate tomato genotypes under open field condition

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The present study was aimed to investigate the yield and its contributing traits among indeterminate tomato genotypes in order to generate information regarding the extent of genetic variability, heritability and genetic advance. The experiment was conducted using a randomized complete block design with three replications at Vegetable Research Farm, Department of Horticulture, SHIATS, Allahabad during 2012-2013 cropping season. The analysis of variance revealed highly significant differences among all genotypes for the characters. Analysis of coefficient of variation revealed that the magnitude of phenotypic coefficient of variation was higher than genotypic coefficient of variation for all traits under study. The leaf curl incidence (39.73 and 39.74) and ascorbic acid (27.62 and 27.67) recorded high genotypic and phenotypic coefficients of variation, indicating higher magnitude of variability for these characters, thus the scope for improvement of these characters through simple selection would be better. The estimates of heritability were high for all the traits and ranged from 95 to 100 percent, suggested that selection based on phenotypic expression could be relied upon as there is major role of genetic constitution in the expression of these characters. High heritability accompanied with high genetic advance were noted for fruit yield per plant (1129.78), plant height (43.37), number of flowers per plant (40.35), number of leaves per plant (25.48) and ascorbic acid (21.68) indicating that these characters are under additive gene effects and that these traits could be considered as reliable indices for selection and higher responses of this trait could be expected from selection.

Key words: Genetic variability, heritability, genetic advance, *Solanum lycopersicum* L., yield, yield traits.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) occupies the prime position among different vegetables and is an important vegetable cultivated in India (Shankarappa et al., 2008; Narolia et al., 2012). It is a very versatile vegetable for

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culinary purposes (Kumar et al., 2014). Ripe fresh fruits are consumed as salads, in cooked form as stew and utilized in the preparation of various forms of processed products such as juice, paste, powder, ketchup, sauce and canned whole fruits (Grandillo et al., 1999). Unripe green fruits are used for preparation of pickles and chutney (Adebooye et al., 2006; Osekita and Ademiluyi, 2014). All the species of tomato are native to Western South America (Rick, 1976). Tomatoes are the main source of lycopene (an antioxidant), ascorbic acid and ß-carotene and also are valued for its colour and flavor (Krumbein et al., 2006). Lycopene is the principle carotenoid, causing the characteristic red hue of tomatoes (Shi and Le-Maguer, 2000) used in treating various chronic human diseases like cancer, cardiovascular diseases, osteoporosis and diabetes (Bai and Lindhot, 2007). Tomato is an important cash-generating crop for small scale farmers and also provides employment opportunities in production and processing industries. Considering the importance of tomato as one of the potential vegetable crop for domestic consumption as well as export markets, it is important to increase its productivity along with desirable attributes through genetic manipulation.

For improving yield potential of tomato, there is a need of systemic breeding approach. Systematic study and evaluation of tomato genotypes is of great importance for current and future agronomic and genetic improvement of this crop. Furthermore, if an improvement program is to be carried out, evaluation of genotypes is imperative, in order to understand the genetic background and the breeding value of the available genotypes (Agong et al., 2000). In any crop-improvement programme the success of selection as a breeding method is determined by the magnitude of genetic variability for yield and yield components (Dudley and Moll, 1969). The genetic variance of any quantitative trait is composed of additive variance (heritable) and non-additive variance and include dominance and epitasis (non-allelic interaction).

Therefore, it becomes necessary to partition the observed phenotypic variability into its heritable and non-heritable components with suitable parameters such as phenotypic and genotypic coefficient of variation, heritability and genetic advance. In genetic studies, characters with high genotypic coefficient of variation indicate the potential for an effective selection (Sadiq et al., 1986). Determining the components of variability in yield and its components enable us to know the extent of environmental influence on yield, taking into consideration of the fact that yield and its component are quantitative characters that are affected by the environment (Ahmed et al., 2007). Heritability provides an idea of the extent of genetic control for expression of a particular character and the reliability of phenotype in predicting its breeding value and the extent of which a particular genetic character can be transmitted to the successive generations (Mangi et al., 2010). High heritability indicates less environmental influence in the observed variation (Songsri et al., 2008). Heritability value alone cannot provide information on amount of genetic progress that would result from selection of best individuals.

Johnson et al. (1955) reported that heritability estimates along with genetic advance would be more successful in predicting the effectiveness of selecting the best individuals. Genetic advance which estimates the degree of gain in a trait obtained under a given selection pressure is an important parameter that guides the breeder in choosing a selection programme (Hamdi et al., 2003). High heritability and high genetic advance for a given trait indicates that it is governed by additive gene action and, therefore, provides the most effective condition for selection (Nwosu et al., 2014). So, proper evaluation of genetic resources is essential to understand and estimate the genetic variability and heritability. Studies on genetic parameters provide information about the expected response of various characters to selection and it will help in developing optimum breeding procedure. Keeping in view of this, an attempt was made to know the nature and magnitude of genetic variability existing for yield and its contributing traits in the available genotypes of indeterminate tomato.

MATERIALS AND METHODS

Genotype collection and seedling establishment

The experimental materials comprised of nineteen indigenous genotypes of indeterminate growth tomato collected from Indian Institute of Vegetable Research (IIVR), Varanasi and Vegetable Research Station (VRS), Junagadh Agricultural University, Junagadh, Gujarat, India (Table 1). For raising good and healthy seedlings, the seeds were treated with carbendazim using 2.0 g/kg of seed. Afterwards, the seeds of nineteen genotypes of tomato were sown in lines 10 cm apart on the nursery beds.

Establishment of tomato genotypes in field

The present investigation was conducted at Vegetable Research Farm, Department of Horticulture, SHIATS, Allahabad, India during 2012-2013 cropping season. Allahabad is situated at an elevation of 98 m above sea level at 25° 28' N latitude and 81° 54' E longitude. This region has a sub-tropical climate prevailing in the south-eastern part of the state Uttar Pradesh with extremes temperatures, the winter and the summer. During winter, frosts and during summer, hot scorching winds are also not uncommon. The average rainfall is around 1027 mm (40.4 inches) with maximum concentration from July to September. The mean monthly agro-meteorological observations were recorded during the crop season (Figure 1).

The experiment was laid out in a randomized complete block design (RCBD) with three replications. Thirty-days-old seedlings of all genotypes were transplanted in small plots (2.0 m × 2.0 m) in open-field where row-to-row and plant-to-plant spacing was 60 cm × 60 cm that contained 9 plants. All the recommended agronomic package of practices were followed (like staking, earthing up, pruning and training, irrigation, weeding, fertilizers applications), as recommended for commercial tomato production.

Table 1. Lists of genotypes used for the study.

S/N	Name of genotype	Source	S/N	Name of genotype	Source
1.	2011/TOINDVAR-1	IIVR, Varanasi	11.	EC 620430	IIVR, Varanasi
2.	2011/TOINDVAR-2	IIVR, Varanasi	12.	EC 620432	IIVR, Varanasi
3.	2011/TOINDVAR-3	IIVR, Varanasi	13.	EC 620434	IIVR, Varanasi
4.	2011/TOINDVAR-4	IIVR, Varanasi	14.	EC 620437	IIVR, Varanasi
5.	2011/TOINDVAR-5	IIVR, Varanasi	15.	EC 620449	IIVR, Varanasi
6.	2012/TOINDVAR-1	IIVR, Varanasi	16.	AJETA-32	IIVR, Varanasi
7.	2012/TOINDVAR-2	IIVR, Varanasi	17.	ARKA VIKAS	IIVR, Varanasi
8.	2012/TOINDVAR-3	IIVR, Varanasi	18.	ANGOOR LATA	IIVR, Varanasi
9.	2012/TOINDVAR-4	IIVR, Varanasi	19.	2012/GT-1	VRS, JAU, Junagadh
10.	EC 620421	IIVR, Varanasi			

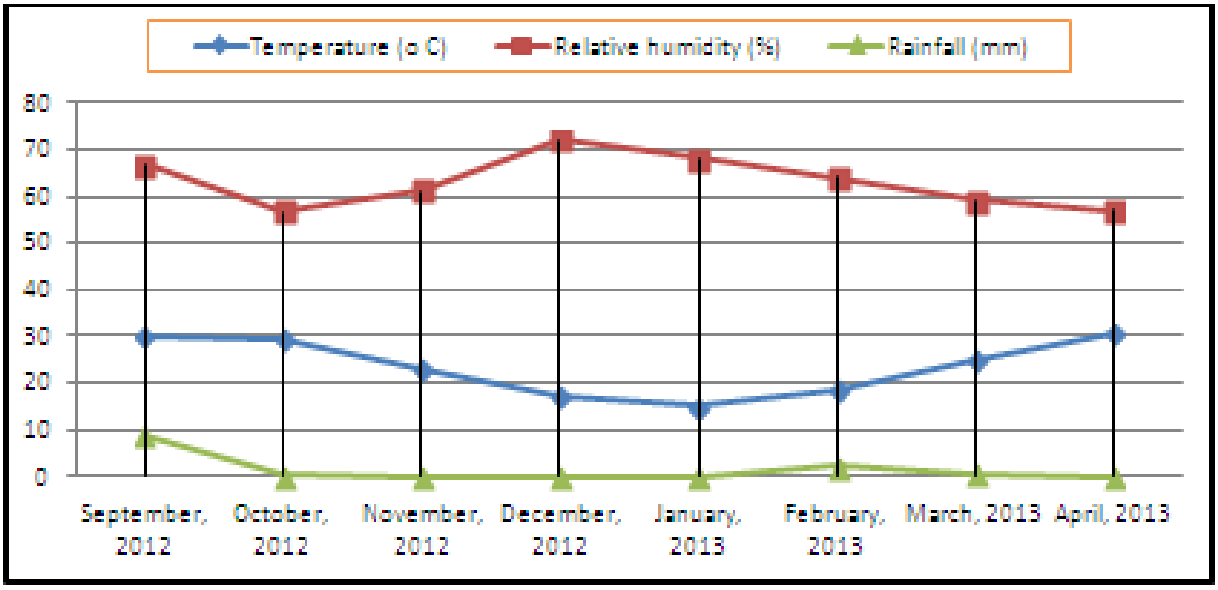

Figure 1. Mean monthly agro-meteorological observations recorded during crop season 2012-2013.

Recording of observations and biochemical analysis

The observations were recorded on a randomly selected five plants from each replication for morphological and biochemical characters viz., (1) plant height (cm), (2) number of branches per plant, (3) number of leaves per plant, (4) days to flowering, (5) number of flower clusters per plant, (6) number of flowers per plant, (7) number of fruits per plant, (8) fruit set percentage, (9) fruit weight (g), (10) radial diameter of fruit (mm), (11) polar diameter of fruit (mm), (12) fruit yield per plant (g), (13) leaf curl incidence percentage (based on the scale given by Joshi and Choudhary (1981), (14) TSS °Brix and (15) ascorbic acid (mg/100 g).

Total soluble solids (TSS) (◦brix)

The total soluble solids of the selected samples were determined with a hand refractometer, Model ATAGO, Tokyo, Japan (0-32° Brix range). The refractometer was washed with distilled water each time after use and dried with blotting paper to avoid contamination.

Ascorbic acid (mg/100 g)

Ascorbic acid was estimated by 2,6-dichlorophenol indophenol method (AOAC, 1975). A two milliliter juice sample was added to an equal volume of 6% metaphosphoric acid in a conical flask and titrated with standard dye solution. The end point was indicated by the appearance of pink colour, which persisted for about 15 s. The dye was standardized with standard stock solution (1 mg/1 ml) of ascorbic acid. The results were expressed as milligrams ascorbic acid/100 g of tomato juice and calculated as follows:

$$\text{Ascorbic acid} = \left[\frac{Y}{X}\right] \times 100$$

Where *Y* is the volume of dye used (ml) in titrating 2 ml juice and *X* the volume of dye used (ml) in titrating 2 ml standard stock solution.

Table 2. Analysis of variance for 15 characters of indeterminate tomato genotypes.

Source of variance	df	Plant height	No. of branches / plant	No. of leaves/ plant	Days to flowering	Flower clusters/ plant	No. of flowers/ plant	No. of fruits/ plant	Fruit set (%)	Average fruit weight	Radial diameter of fruit	Polar diameter of fruit	Fruit yield/ plant	Leaf curl incidence (%)	TSS ° brix	Ascorbic acid
Replication	2	1.69	0.16	0.34	0.006	0.04	0.37	0.08	0.07	1.43	0.42	0.23	5206.96	0.004	0.01	0.17
Treatment	18	1331.66**	11.65**	461.38**	153.96**	24.02**	1159.45**	228.04**	129.30**	225.66**	172.18**	70.06**	912915.31**	257.55**	1.33**	333.75**
Error	36	0.46	0.20	0.60	0.31	0.06	2.06	0.28	0.56	0.36	0.12	0.39	2657.22	0.02	0.01	0.39

** Significant at 0.01%.

Statistical analysis

Analysis of variance was carried out by the method suggested by Panse and Sukhatme (1985). The genotypic and phenotypic coefficients of variation were calculated using the formula of Burton and De Vane (1953). Heritability and genetic advance were calculated according to Allard (1960) and genetic advance as percent of mean was estimated using the method of Johnson et al. (1955).

RESULTS AND DISCUSSION

Analysis of variance

The result on analysis of variances (ANOVA) using randomized complete block design revealed that the genotypes exhibited highly significant differences for all the characters studied (Table 2). Fruit yield per plant (912915.31), plant height (1331.66), number of flowers per plant (1159.45), number of leaves per plant (461.38) and ascorbic acid (333.75) were some of the traits which showed highly significant variation. The significant variation among the genotypes revealed that presence of adequate variability which can be exploited through selection. This is in agreement with the findings of Singh et al. (2006), Dar et al. (2012), Singh et al. (2014), Pandey et al. (2015) and Senapati and Kumar (2015).

Estimation of range and mean

There were high differences observed between the least and highest mean values for all characters studied (Table 3). A wide range of variation was observed for fruit yield per plant (2186.71-4356.49), followed by plant height (120.82-207.27), number of flowers per plant (87.67-153.60), number of leaves per plant (186.83-234.90), radial diameter of fruit (34.72-72.37), and leaf curl incidence percentage (11.39-46.76) indicating their maximum contribution to the total variability observed among the tomato genotypes. This showed the possibility to improve the various desirable traits through direct selection as short term strategy. The wide range of variation obtained may be due to divergent genotypes included in the study. Similar finding were also reported by Haydar et al. (2007), Mehta and Asati (2008) and Kaushik et al. (2011) for fruit yield per plant; Patil et al. (2013) for plant height, yield per plant and fruit diameter.

Estimation of phenotypic and genotypic variability

In the present study, maximum genotypic and phenotypic variance (σ^2_g and σ^2_p), respectively were recorded for fruit yield per plant (303419.38 and 306076.59), plant height (443.74 and 444.20), number of flowers per plant (385.79 and 387.86), number of leaves per plant (153.60 and 154.20), ascorbic acid (111.12 and 111.51), leaf curl incidence (85.84 and 85.87), number of fruits per plant (75.92 and 76.20), fruit weight (75.10 and 75.47) whereas the minimum for TSS (0.44 and 0.46). High genotypic variance indicating more contribution of genetic component for the total variation. Therefore, these characters could be considered and exploited for selection purpose, whereas, high phenotypic variance indicating the strong influence of environmental factors during the growth period for their expression. These results are in accordance of the results obtained by Haydar et al. (2007), Shashikanth et al. (2010) and Mohamed et al. (2012) in tomato.

The nature and extent of genetic variability is one of the most important criteria in formulating an efficient breeding programme and knowledge of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) is much helpful in predicting the amount of variation present in a given genetic stock. In general, the phenotypic coefficient of variations were slightly higher than the corresponding genotypic coefficient of variations for all the traits studied (Table 3 and Figure 2), which indicated that the apparent variation is not only due to genotypes

Table 3. Range, mean, variance, coefficient of variations, heritability, genetic advance and genetic advance as percent of mean for 15 characters of indeterminate tomato genotypes.

Characters	Range		Mean	GV ($\sigma^2 g$)	PV ($\sigma^2 p$)	CV		h^2 (bs) (%)	GA	GA as % of mean
	Min.	Max.				GCV (%)	PCV (%)		5%	5%
Plant height	120.82	207.27	146.30	443.74	444.20	14.40	14.41	100	43.37	29.64
No. of branches/plant	15.20	22.93	18.37	3.82	4.02	10.64	10.91	95	3.92	21.35
No. of leaves/plant	186.83	234.90	199.16	153.60	154.20	6.22	6.23	100	25.48	12.79
Days to flowering	47.27	73.00	58.53	51.22	51.53	12.23	12.27	99	14.70	25.11
No. of flower clusters/plant	17.53	27.20	22.08	7.99	8.05	12.80	12.85	99	5.80	26.26
No. of flowers/plant	87.67	153.60	125.34	385.79	387.86	15.67	15.71	99	40.35	32.20
Average no. of fruits/plant	41.13	76.23	56.58	75.92	76.20	15.40	15.43	100	17.92	31.67
Fruit set (%)	37.17	61.71	45.53	42.91	43.48	14.39	14.48	99	13.41	29.44
Average fruit weight	34.60	69.67	53.61	75.10	75.47	16.17	16.21	100	17.81	33.22
Radial diameter of fruit	34.72	72.37	53.91	57.35	57.48	14.05	14.06	100	15.58	28.91
Polar diameter of fruit	38.97	54.25	47.75	23.22	23.62	10.09	10.18	98	9.84	20.62
Fruit yield/plant	2186.71	4356.49	3000.71	303419.38	306076.59	18.36	18.44	99	1129.78	37.65
Leaf curl incidence (%)	11.39	46.76	23.32	85.84	85.87	39.73	39.74	100	19.08	81.83
TSS ° brix	2.87	5.60	4.52	0.44	0.46	14.69	14.93	97	1.35	29.77
Ascorbic acid	19.25	51.79	38.16	111.12	111.51	27.62	27.67	100	21.68	56.80

but also due to the influence of environment in the expression of the traits. Similar finding were also reported by Kaushik et al. (2011), Islam et al. (2012), Patil et al. (2013), Saleem et al. (2013) and Senapati and Kumar (2015). In present study, the difference between values of PCV and GCV were less for all traits except number of branches and total soluble solid (TSS). It means that these traits were less influenced by environment and hence, they could be improved by following different phenotypic selections like directional, disruptive and stabilized selections. The leaf curl incidence (39.73 and 39.74) and ascorbic acid (27.62 and 27.67) recorded high genotypic and phenotypic coefficients of variation, indicating higher magnitude of variability for these characters. Similar findings were also reported by Narolia et al. (2012) and for ascorbic acid. The moderate amount of GCV and PCV, respectively were recorded for fruit yield per plant (18.36 and 18.44), fruit weight (16.17 and 16.21), number of flowers per plant (15.67 and 15.71), number of fruits per plant (15.40 and 15.43), TSS (14.69 and 14.93), plant height (14.40 and 14.41), fruit set percentage (14.39 and 14.48), radial diameter of fruit (14.05 and 14.06), number of flower clusters per plant (12.80 and 12.85), days to flowering (12.23 and 12.27), number of branches per plant (10.64 and 10.91) and polar diameter of fruit (10.09 and 10.18). Moderate rate of GCV and PCV are indication of ample scope for improvement through selection. These results corroborate with the findings of earlier researchers for average fruit weight, TSS, plant height (Ara et al., 2009); fruit diameter (Singh, 2009; Kumar et al., 2013); plant height, fruits per plant, fruit weight (Kumar, 2010); fruit yield per plant, fruit diameter (Tasisa et al., 2011).

Estimates of broad sense heritability and genetic advance

Genotypic coefficients of variation do not estimate the variations that are heritable (Falconer, 1960), and estimation of heritability becomes necessary. Genotypic coefficient of variation represents the total genetic variation whereas heritability measures the proportion to which the variability of a character is transmitted to offspring (Lush, 1949). Burton and De Vane (1953) suggested that genetic coefficients of variability, along with heritability estimates, would provide a reliable indication of expected degree of improvement through selection.

Heritability in broad sense is a parameter of tremendous significance to the breeders as its magnitude indicates the reliability with which a genotype can be recognized by its phenotypic

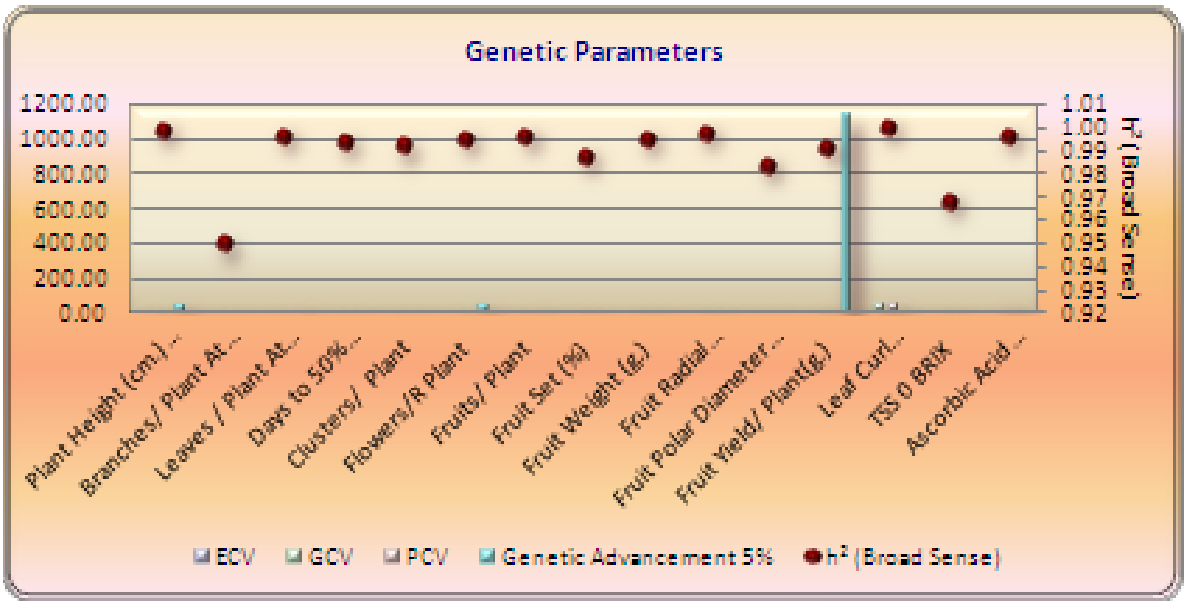

Figure 2. Range, mean, variance, coefficient of variations, heritability, genetic advance and genetic advance as percent of mean for fifteen characters of indeterminate tomato genotypes.

expression. The estimates of heritability were high for all the traits and ranged from 95 to 100 percent, suggested that selection based on phenotypic expression could be relied upon as there is major role of genetic constitution in the expression of these characters. The heritability estimates worked out in the present investigation are in consonance with earlier reports by Haydar et al. (2007) and Mohamed et al. (2012) for plant height, fruit weight, number of branches per plant and days to flowering in different genotypes of tomato; Kumar (2010) for days to flowering, polar diameter, TSS, plant height, fruits per plant, average fruit weight, yield per plant; Saleem et al. (2013) for plant height, fruit yield per plant, number of fruits per plant; Kumar et al. (2006) for fruit weight for all characters studied; Singh et al. (2006) for number of fruits per plant; Saeed et al. (2007) for number of fruits per plant and number of flowers per plant; Mehta and Asati (2008) also found high heritability in broad sense for plant height and TSS; Singh (2009), Kumar et al. (2013) for plant height, number of fruits per plant, fruit diameter, fruit weight, fruit yield per plant; Islam et al. (2012) for fruit weight, days to flowering and number of fruits per plant; Osekita and Ademiluyi (2014) also found high heritability in broad sense for days to flowering and plant height.

The estimate of genetic advance showed a wide range from 1.35 for TSS °B to 1129.78 for fruit yield per plant. Generally, genetic advance as percent of mean (GAM) at 5% selection intensity was high (>20%) for all characters studied except number of leaves per plant. The highest GAM was recorded for leaf curl incidence percentage (81.83), ascorbic acid (56.80), fruit yield per plant (37.65), average fruit weight (33.22), number of flowers per plant (32.20), number of fruits per plant (31.67), showed that these characters are governed by additive genes and selection will be rewarding improvement of such traits. This is in confirmation with the findings of Shashikanth et al. (2010) who reported high GAM for fruits per plant and fruit yield per plant; Islam et al. (2012) for fruit weight and number of fruits per plant; Kumar et al. (2013) for number of fruits per plant, fruit weight, yield per plant.

Heritability coupled with genetic advance is more effective and reliable in predicting the results and the effect of selection (Dudley and Moll, 1969). High heritability accompanied with high genetic advance were noted for fruit yield per plant (1129.78), plant height (43.37), number of flowers per plant (40.35), number of leaves per plant (25.48) and ascorbic acid (21.68) indicating that these characters are under additive gene effects and that these traits could be considered as reliable indices for selection and higher responses of this trait could be expected from selection. This result is in agreement with the findings of Patil et al. (2013) for fruit yield per plant. High heritability with low genetic advance was observed for TSS (1.35), number of branches per plant (3.92) and number of flower clusters per plant (5.80). Since, these characters are governed by non-additive gene action hybridization followed by selection may be used for improvement (Liang and Walter, 1968; Ara et al., 2009).

Similar results were also reported by Singh et al. (2006) for number of branches per plant and TSS. Johnson et al. (1955) has suggested that traits with high heritability coupled with high genetic advance would respond to selection better than those with high heritability and low genetic advance. High heritability and genetic advance as per cent for the trait suggested the possibility of selecting

high yielding cultivars from the present collection (Singh, 2009). The high heritability was associated with high genetic advance as per cent of mean for all the yield contributing characters except number of leaves per plant. The parallelism between the magnitude of heritability and degree of genetic gain has been due to the additive gene playing a predominant role and therefore, these were more reliable for effective selection. Similar finding were also reported by Singh (2009) for number of fruits per plant, fruit weight, plant height and fruit diameter.

Heritability, genetic advance as percent of mean and genotypic coefficient of variation together could provide the best image of the amount of advance to be expected from selection (Johnson et al., 1955). The characters *viz.*, leaf curl incidence percentage and ascorbic acid with high genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance as percent of mean. Similar results were noticed by Singh et al. (2006) for ascorbic acid. Therefore, this observation indicated that these characters are under additive gene effects and more reliable for effective selection.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Adebooye OC, Adeoye GO, Tijani-Eniola H (2006). Quality of fruits of three varieties of tomato (*Lycopersicon esculentum* (L.) Mill.) as affected by phosphorus rates. J. Agron. 5:396-400.

Agong SG, Schittenhelm S, Friedt W (2000). Genotypic variation of Kenyan tomato (*Lycopersicon esculentum* L.) germplasm. Plant Genet. Resour. Newsl. 123:61-67.

Ahmed N, Muhammad A, Khaliq I, Masahiko M (2007). The inheritance of yield and yield components of five wheat hybrid populations under drought conditions. Indones. J. Agric. Sci. 8:53-59.

Allard RW (1960). Principles of plant breeding. J. Wiley and Sons, New York.

AOAC (1975). In:Horowitz W (ed), Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.

Ara A, Narayan R, Ahmed N, Khan SH (2009). Genetic variability and selection parameters for yield and quality attributes in tomato. Indian J. Hortic. 66:73-78.

Bai Y, Lindhot P (2007). Domestication and breeding of tomatoes:what have we gained and what can we gain in the future. Ann. Bot. 100:1085-1094.

Burton GW, De Vane EH (1953). Estimating heritability in tall fescue (*Festuca arundiancea*) from replicated clonal material. Agron. J. 45:478-481.

Dar RA, Sharma JP, Nabi A, Chopra S (2012). Germplasm evaluation for yield and fruit quality traits in tomato (*Solanum lycopersicon* L.). Afr. J. Agric. Res. 7:6143-6149.

Dudley JW, Moll RH (1969). Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci. 9:257-262.

Falconer DS (1960). Introduction to quantitative genetics. Oliver & Boyd, Edinburgh/London.

Grandillo S, Zamir D, Tanksley SD (1999). Genetic improvement of processing tomatoes: A 20 years perspective. Euphytica 110:85-97.

Hamdi A, El-Chareib AA, Shafey SA, Ibrahim MAM (2003). Genetic variability, heritability and expected genetic advance for earliness and seed yield from selections in Lentil. Egypt J. Agric. Res. 81:125-137.

Haydar A, Mandal MA, Ahmed MB, Hannan MM, Karim R, Razvy MA, Roy UK, Salahi M (2007). Studies on genetic variability and interrelationship among the different traits in tomato (*Lycopersicon esculentum* Mill.). Middle-East J. Sci. Res. 2:139-142.

Islam MS, Mohanta HC, Ismail MR, Rafii MY, Malek MA (2012). Genetic variability and trait relationship in cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme* (Dunnal) A. Gray). Bangladesh J. Bot. 41:163-167.

Johnson HW, Robinson HF, Comstock RE (1955). Estimates of genetic and environmental variability in soybean. Agron. J. 47:314-318.

Joshi GC, Choudhury B (1981). Screening of *Lycopersicon* and *Solanum* species for resistance to leaf curl virus. Veg. Sci. 8:45-50.

Kaushik SK, Tomar DS, Dixit AK (2011). Genetics of fruit yield and it's contributing characters in tomato (*Solanum lycopersicum*). J. Agric. Biotech. Sustain. Dev. 3:209-213.

Krumbein A, Schwarz D, Klaering HP (2006). Effects of environmental factors on carotenoid content in tomato (*Lycopersicon esculentum* Mill.) grown in a greenhouse. J. Appl. Bot. Food Qual. 80:160-164.

Kumar D, Kumar R, Kumar S, Bhardwaj ML, Thakur MC, Kumar R, Thakur KD, Dogra BS, Vikram A, Thakur A, Kumar P (2013). Genetic variability, correlation and path coefficient analysis in tomato. Int. J. Veg. Sci. 19:313-323.

Kumar R, Mishra NK, Singh J, Rai GK, Verma A, Rai M (2006). Studies on yield and quality traits in tomato *(Solanum lycopersicon* (Mill.) Wettsd.). Veg. Sci. 33:126-132.

Kumar R, Ram CN, Yadav GC, Deo C, Vimal SC, Bhartiya HD (2014). Studies on correlation and path coefficient analysis in tomato (*Solanum lycopersicon* L.). Plant Archives 14:443-447.

Kumar S (2010). Genetic variability and interrelationship of traits in F_3 progenies of tomato (*Lycoperscion esculentum* Mill.) under cold desert of Leh-Ladakh. Crop Improvement 37:66-72.

Liang GHL, Walter TL (1968). Heritability estimates and gene effect for agronomic traits in grain sorghum. Crop Sci. 8:77-80.

Lush JL (1949). Heritability of quantitative traits in farm animals. Proc 8th Inst Cong Genetics, Herides: pp. 336-357.

Mangi S, Sial M, Ansari B, Arain M, Laghari K, Mirbahar A (2010). Heritability studies for grain yield and yield components in F_3 segregating generation of spring wheat. Pak. J. Bot. 42:1807-1813.

Mehta N, Asati BS (2008). Genetic relationship of growth and development traits with fruit yield in tomato (*Lycopersicon esculentum* Mill.). Karnataka J. Agric. Sci. 21:92-96.

Mohamed SM, Ali EE, Mohamed TY (2012). Study of heritability and genetic variability among different plant and fruit characters of tomato (*Solanum lycopersicon* L.). Int. J. Sci. Tech. Res. 1:55-58.

Narolia RK, Reddy RVSK, Sujatha M (2012). Genetic architecture of yield and quality in tomato (*Solanum lycopersicon*). Agric. Sci. Digest 32:281-285.

Nwosu DJ, Onakoya OA, Okere AU, Babatunde AO, Popoola AF (2014). Genetic variability and correlations in rainfed tomato (*Solanum* spps.) accessions in Ibadan, Nigeria. Greener J. Agric. Sci. 4:211-219.

Osekita OS, Ademiluyi AT (2014). Genetic advance, heritability and character association of component of yield in some genotypes of tomato *Lycopersicon esculentum* (Mill.) Wettsd. Acad. J. Biotechnol. 2:006-010.

Pandey P, Pandey VR, Tiwari DK, Yadav SK (2015). Studies on direct selection parameters for seed yield and its component traits in pigeonpea [*Cajanus cajan* (L.) Millsp.]. Afr. J. Agric. Res. 10:485-490.

Panse VG, Sukhatme PV (1985). Statistical methods for agricultural

workers (2nd Edn.), Indian Council of Agricultural Research, New Delhi. P. 381.
Patil S, Bhalekar MN, Kute NS, Shinde GC, Shinde S (2013). Genetic variability and interrelationship among different traits in F3 progenies of tomato (*Solanum lycopersicum* L.). Bioinfolet 10:728-732.
Rick CM (1976). Tomato. Evaluation of Crop Plant. London. pp. 268-273.
Sadiq SM, Saleem, Igbal J (1986). Genetic variability and selection in hexaploid triticale. Proceedings of the International symposium, Sydney, Austr. Inst. Agric. Sci. 182-185.
Saeed A, Hayat K, Khan AA, Iqbal S, Abbas G (2007). Assessment of genetic variability and heritability in *Lycopersicon esculentum* Mill. Int. J. Agri. Biol. 9:375-377.
Saleem MY, Iqbal Q, Asghar M (2013). Genetic variability, heritability, character association and path analysis in F_1 hybrids of tomato. Pak. J. Agri. Sci. 50:649-653.
Senapati BK, Kumar A (2015). Genetic assessment of some phenotypic variants of rice (*Oryza* spp.) for some quantitative characters under the Gangatic plains of West Bengal. Afr. J. Biotechnol. 14:187-201.
Shankarappa KS, Sriharsha, Rangaswamy KT, Aswathanarayana DS, Prameela HA, Kulkarni RS, Muniyappa V, Rao AM, Maruthi MN (2008). Development of tomato hybrids resistant to tomato leaf curl virus disease in South India. Euphytica 164:531-539.
Shashikanth, Basavaraj N, Hosamani RM, Patil BC (2010). Genetic variability in tomato (*Solanum lycopersicon* [Mill].Wettsd.). Karnataka J. Agric. Sci. 23:536-537.
Shi J, Le-Maguer M (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. Crit. Rev. Food Sci. Nutr. 40:1-42.
Singh A, Singh AK, Singh V, Singh N, Singh VN, Shamim M, Vikram P, Singh S (2014). Genetic variability among traits associated with grain yield of rice (*Oryza sativa* L.) exposed to drought at flowering stage. Afr. J. Agric. Res. 9:1252-1264.
Singh AK (2009). Genetic variability, heritability and genetic advance studies in tomato under cold arid region of Ladakh. Indian J. Hortic. 66:400-403.
Singh PK, Singh B, Pandey S (2006). Genetic variability and character association analysis in tomato. Indian J. Plant Genet. Resour. 19:196-199.
Songsri P, Joglloy S, Kesmala T, Vorasoot N, Akkasaeng CPA, Holbrook C (2008). Heritability of drought resistant traits and correlation of drought resistance and agronomic traits in peanut. Crop Sci. 48:2245-2253.
Tasisa J, Belew D, Bantte K, Gebreselassie W (2011). Variability, heritability and genetic advance in tomato (*Lycopersicon esculentum* Mill.) genotypes in West Shoa, Ethiopia. Am-Euras J. Agric. 11(1):87-94.

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Full Length Research Paper

Modeling the effects of different irrigation schedules and drain depths for soil salinity management: A case study from Southern Iraq

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Soil-water-atmosphere-plant (SWAP) relationship model is used to evaluate the impact of current irrigation practices on groundwater table depth, soil salinity and crop yields and to determine optimal irrigation requirements and drain depth for the study area. The results indicate that current irrigation practices of applying 600 mm to wheat and 1000 mm to maize are wasting more than 30% of applied irrigation water as deep percolation, which causes rise in groundwater table, increase in profile salinity and reduction in crop yields. The simulation results reveal that in the absence of an effective drainage system in the study area, a groundwater table depth of approximately 200 cm together with an irrigation application of 5000 m^3 ha^{-1} for wheat and 6000 m^3 ha^{-1} for maize will be the most appropriate combination for obtaining optimum yields of wheat (3.0 t ha^{-1}) and maize (1.80 t ha^{-1}). However, to achieve potential yields, leaching of excessive salts from the root zone through freshwater application would be essential. Therefore a drainage system in these areas should be installed to maintain groundwater table depth around 200 cm. Installation of deeper drains would not be feasible as it will increase the costs and without much gains in crop yields.

Key words: Irrigation management, drain depth, soil salinity, crop yields, transient modeling.

INTRODUCTION

In arid and semi-arid regions, accumulation of salts in soil and groundwater are the greatest threats to the sustainability of irrigated agriculture (Qureshi et al., 2010). Ideally, salts added through irrigation water must be removed from the soil system at the same rate at which they are added. If leaching of salts does not occur, salts build up in the soil hampered plant growth as plants are restricted in their capacity to extract water under saline conditions. In shallow and saline groundwater areas, even if leaching occurs, salts enter the top soil layer through capillary rise as a result of high soil evaporation during the summer. This vertical recycling of

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salts ultimately increases the soil salinity to intolerable levels. The southern irrigated areas of Iraq are located between the Tigris and Euphrates Rivers, which produces more than 70% of the total cereal production in the country (Qureshi et al., 2013).

Excessive use of irrigation water and poor drainage conditions are the major factors contributing to rising groundwater tables in southern Iraq. To overcome the problems of waterlogging and soil salinity, a drainage network of open field drains consists of collector drains and branch and secondary drains was installed in the area.

However, due to poor maintenance, this drainage network has been partially destroyed or become non-functional. This has resulted in rising groundwater table in most areas with serious consequences of soil salinization and reduction in crop yields (FAO, 2011).

In the absence of an effective drainage system, precise irrigation applications could be a feasible solution to control groundwater table rise and soil salinity in southern Iraq. The drainage requirements of (semi-) arid areas are largely dependent on the irrigation component. Therefore the groundwater table should be maintained at a depth which can maximize groundwater contribution to the crops through capillary rise without permanently accumulating salts in the root zone (Hendrickx et al., 1990).

In the areas where groundwater quality is of concern, water table should be kept deep enough to minimize capillary rise to avoid secondary soil salinization (Prathapar and Qureshi, 1999). This makes irrigation and groundwater table management of (semi-) arid regions much more complex than in other irrigation conditions (Sarwar and Feddes. 2000). This necessitates the calculation of precise irrigation amounts and determination of suitable groundwater table depths to halt environmental degradation and foster crop production.

The complex interaction between irrigation, crop production, and soil salinity under variety of climatic and physical conditions can be better explained by transient simulation models. These models can be used to evaluate long-term effects of different irrigation regimes on groundwater table depth, soil salinity, and crop growth.

In this study, the Soil-Water-Atmosphere-Plant (SWAP) relationship model (Van Dam et al., 1997) was used to determine optimum irrigation requirements and groundwater table depth for maximizing wheat and maize crops in Al-Dujaila project area located in the southern Iraq. Before application, SWAP model was calibrated for the soil, crop and climatic conditions prevailing in the area.

MATERIAL AND METHODS

The SWAP model

SWAP is a field scale one-dimensional agro-hydrological model, which was developed by Feddes et al. (1978) and modified by Belmans et al. (1983) and Van dam et al. (1997). SWAP is designed to simulate unsaturated flow and solute transport and has successfully been applied in the field of agriculture and water management under variety of climatic and environmental conditions (Qureshi et al. 2010, 2013).

SWAP is designed to simulate unsaturated flow, solute transport, heat flow and crop growth in the soil–plant–atmosphere environment at the field scale. The model has been successfully applied to evaluate the effects of different irrigation and drainage conditions on crop production and soil salinity in the Bhakra irrigation system of India (Bastiaanssen et al., 1996).

Sarwar (2000) used the SWAP model to re-evaluate drainage design criteria for the Fourth Drainage Project of Pakistan and also to investigate the effects of conjunctive management of surface and groundwater on soil salinity and crop production. Qureshi et al. (2010) used the SWAP model to determine optimum groundwater table depth for maximizing cotton production in the Syrdarya Province of Uzbekistan.

The model applies Richard's equation for soil water flow in the soil matrix described as below:

$$C(h)\frac{\partial h}{\partial t} = \frac{\partial}{\partial z}\left[K(h)\left(\frac{\partial h}{\partial z} + 1\right)\right] - S(h)$$

Where h is the soil water pressure head (cm), K is the hydraulic conductivity (cm/day), C is the soil water capacity ($d\theta/dh$) (cm^{-1}), S is the soil water extraction rate by plant roots, z is the vertical coordinate positive in the upward direction and t is the time (d). SWAP solves the above partial differential equation using an implicit finite difference mechanism.

The upper boundary of the system is described by potential evapotranspiration rate (ET_{pot}), irrigation and precipitation. ET_{pot} is divided into potential transpiration rate (T_{pot}) and potential soil evaporation rate (E_{pot}) based either on the leaf area index (*LAI*) or the soil cover fraction (*SC*), both as a function of crop development. Reduction of the E_{pot} into actual soil evaporation (E_{act}) is calculated by an empirical function following Boesten and Stroosnijder (1986) model. Irrigations may be prescribed at fixed times or scheduled according to a number of criteria. The bottom boundary conditions of the model can be described with various options (Van Dam et al., 1997).

These include groundwater level as a function of time, flux to/from semi-confined aquifers, flux to/from open surface drains, an exponential relationship between bottom flux and groundwater table or zero flux, free drainage and free outflow (Van Dam et al., 1997). Irrigations in SWAP may be prescribed at fixed times or scheduled according to a number of criteria. The scheduling options allow the evaluation of alternative application strategies.

Under water limiting conditions, it is important to know the minimum amount of irrigation water needed to ensure the maximum production of a certain crop. For this study, a linear relationship between relative yield and relative transpiration was assumed. The validity of linear relationship in field experiments was confirmed by several researchers in different climates [(Hanks, 1974; Hanks 1983; Stewart et al., 1977; Feddes, 1985). Further details of SWAP are described by Van Dam et al. (1997) and the program use is documented by Kroes et al. (1999).

The potential root water extraction rate is equal to the potential transpiration rate, which is governed by atmospheric conditions. Stresses due to dry or wet conditions and/or high salinity concentrations may reduce water extraction. Water stress in SWAP is described by the function proposed by Feddes et al. (1978). For salinity stress the response function of Maas and Hoffman (1977) is used. They found that the reduction in crop yield due to salinity can be linearly related to the soil solution electrical conductivity. Crops

Figure 1. Geographic location of Al-Dujaila project area in Iraq.

can tolerate increases in soil salinity up to a threshold value, after which yield reduces linearly with increasing salt concentration.

$$\frac{Y_{act}}{Y_{pot}} = 1 \quad \text{for } 0 \le EC_e \ge EC_e^*$$

$$\frac{Y_{act}}{Y_{pot}} = 1 - a(EC_e - EC_e^*) \quad \text{for } EC_e > EC_e^*$$

Where EC_e is the electrical conductivity of the soil saturation extract (dS m^{-1}), EC_e^* is the electrical conductivity of the soil saturation extract at which yield begins to decrease (dS m^{-1}) and *a* is the slope which equals the fraction yield decrease per unit of electrical conductivity increase. Salt tolerance data have been listed for a number of crops by Maas (1990).

Water stress in SWAP is described by the function proposed by Feddes et al. (1978). For salinity stress the response function of Maas and Hoffman (1977) is used, which considers a linear relationship between reduction in crop yield due to salinity and EC_e. For this study, a linear relationship between relative yield and relative transpiration was assumed.

The validity of linear relationship in field experiments was confirmed by several researchers in different climates (Hanks 1974; Feddes, 1988). Further details of SWAP are described by (Van Dam et al., 1997).

Description of the study area

This study was conducted in the Al-Dujaila project area, which is one of the oldest irrigation projects in Iraq and is located on the right bank of the Tigris River. The study area falls under the Mesopotamian plain and represents the typical climate and environment of the southern Iraq. Average annual rainfall is 135 mm, which mainly occurs in winter from December to February. Summers are dry and hot to extremely hot and long season with day temperature of over 44°C and dropping at night to 26°C. Location of the study area is shown in Figure 1.

The total area of the Al-Dujaila project is 72,500 ha with net irrigated area of 22,418 ha. The project lands are irrigated from the right side of Tigris River. Irrigation water to the fields is supplied through a network of unlined canals. During the 1950s, the project area was equipped with a surface drainage network which consists of open field drains, collector drains and branch and secondary drains connected to the main outlet drains of the project. Due to years of neglect and poor maintenance and the on-going war in Iraq, the drainage network has been partially destroyed or become non-functional. This has led to rising groundwater tables with serious consequences of soil salinization and reduction in crop yields in most of the project area. Currently, groundwater table varies between 45 and 200 cm. Groundwater salinity is extremely high with seasonal variations of 4 to 43 dS m^{-1}. The major crops cultivated in the area are wheat, barley, corn, and winter/summer vegetables. The cropping intensity is 80 percent in winter and 20% in the summer.

The gravity run irrigation system is owned by the Government, where fix water duties are fixed at the beginning of a cropping season. The irrigation duty is 3 mm d^{-1} for gross cultivated area. Water distribution in the fields is entirely the responsibility of farmers. The soils of the Mesopotamian plain are rich in calcium carbonate, moderate in lime (25 to 30% lime is quite common and less than 20% is rare) and low in organic matter (Al-Jaboory, 1987; Buringh, 1960; Boumans et al., 1977). Large tracts of the irrigated lands of the project area are salinized.

The degree of salinization varies along the latitude, depending on various factors which include quality of irrigation water, irrigation practices, soil types, natural drainage and the status of groundwater table. Irrigation applications without proper drainage facility have added huge amounts of salts in the soil profile.

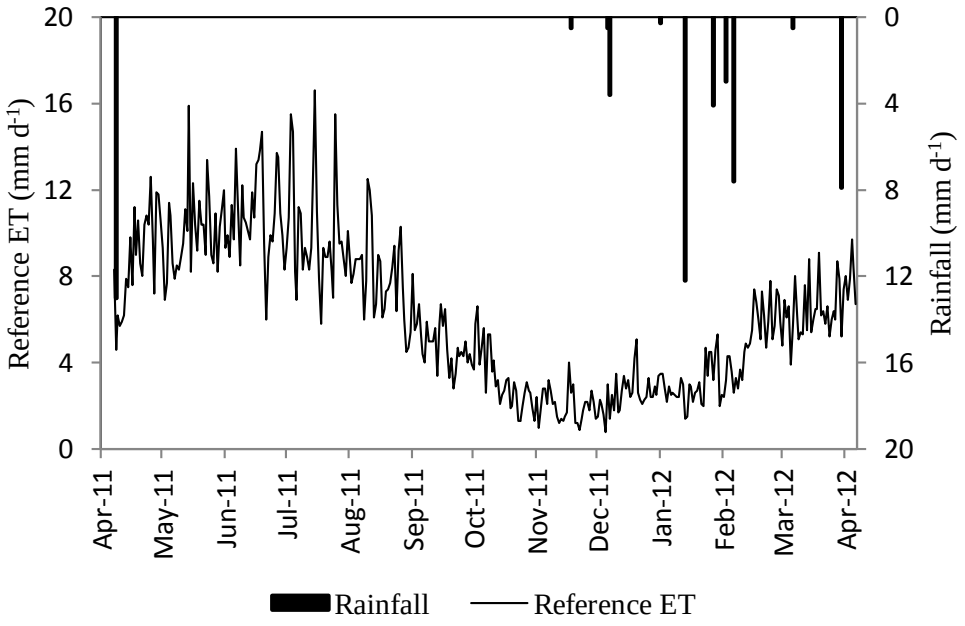

Figure 2. Rainfall and reference evapotranspiration during the calibration period.

Main crops cultivated in the project area are wheat, barley and maize with small proportions of clover, sunflower, and winter/summer vegetables.

The cropping intensity is 80 percent in winter and 20 percent in the summer (Al-Zubaidi, 1992). Irrigation water use efficiencies are low, which waste a considerable amount of water as deep percolation. This causes groundwater table to rise resulting in increased soil salinity and low crop yields. The average yields of wheat and maize are around 2.0 t ha^{-1} compared to the production potential of up to 4-5 t ha^{-1}.

Data collection and model inputs

To collect data for model calibration, a 0.5 ha farmer field was extensively monitored during April 2011 to May 2012. Wheat and maize crops were grown during the monitoring period. Reference evapotranspiration (ET_o) was calculated by the Penman-Monteith (PM) method (FAO, 1998) using daily climatic data obtained from the nearby meteorological station. ET_{pot} was calculated by multiplying ET_o by the crop coefficient (K_c). The K_c values were taken from (Al-Falahi and Qureshi, 2011).

Soil samples were collected at depths of 0-30, 30-60 and 60-90 cm and analyzed in the laboratory for the determination of electrical conductivity of the saturation extract (EC_e) values. These values were then used to compare model simulated EC_e values. Precipitation and ET_o values during the calibration period is shown in Figure 2.

Data on rooting depth, leaf area index (LAI) and soil cover values as a function of crop development stage were taken from Qureshi et al. (2013). The threshold values for salinity stress for wheat and maize were taken as 6.0 and 1.7 dS m^{-1}, respectively (Mass and Hoffman, 1977).

Crops react differently to soil water limitations and their sensitivity to matric potential needs to be specified in the model as input. The *h1* to *h4* values refer to the sink term theory of Feddes et al. (1978). The sink term values for this study were taken from Qureshi et al. (2013). The agronomic and crop parameters used in this study are summarized in Table 1.

Irrigations were applied to bring soil moisture up to 70% of the field capacity. In this study, good quality canal water (EC = 0.80 dS m^{-1}) was used for all irrigations. During the study period, farmers applied 7 irrigations (600 mm) to wheat and 9 irrigations (1000 mm) to maize crop. Amount and date of all irrigations to wheat and maize during the calibration period is given in Table 2.

Groundwater table depth was monitored on a bi-weekly basis with the help of three observation wells which were installed in the monitoring field. The average value of these three observation wells was used in the model as input. The analysis shows that there was very little variation in groundwater table values recorded by the three observation wells. The bottom boundary condition of the soil profile was described as "*free drainage*" and model was set to simulate daily groundwater table depths.

The simulated groundwater table depth was compared with the observed groundwater table depth data for model calibration. The salinity parameters in the classical convection–dispersion equation that describe salt transport are dispersivity, $D_{dis,}$ and diffusion, D_{dif}. The model is more sensitive to dispersion than to diffusion. The value of D_{dis} typically ranges from 0.5 cm, or less, for laboratory-scale experiments involving disturbed soils, to about 10 cm or more for field-scale experiments (Nielsen et al., 1986). The values for D_{dis} and D_{dif} that gave the best results during model calibration were 0.48 and 15 cm^2 day^{-1}, respectively. For salinity stress the response function of Maas and Hoffman (1977) was used. The threshold values for salinity stress for wheat and maize were taken as 6.0 and 1.7 dS m^{-1}, respectively.

The 300 cm soil profile was divided into three layers based on laboratory analysis of samples. For each soil layer, soil hydraulic properties were described by the Van Genuchten-Mualem (VGM) parameters (Mualem, 1976; Van Genuchten, 1987). These parameters are saturated soil moisture content (θ_{sat}), residual soil moisture content (θ_{res}), saturated hydraulic conductivity (K_{sat}), empirical shape parameters (λ, α, n). Soil hydraulic functions were taken from pedo-transfer functions (Wösten et al., 1998) and were slightly adjusted during the calibration process. Final calibrated VGM parameters are given in Table 3.

Table 1. Agronomic and crop parameters used for simulations with the SWAP model.

Parameter	Wheat	Maize
Sowing date	05-11-2011	10-05-2011
Harvesting date	17-04-2012	28-09-2011
Number of irrigations	6	9
Total irrigation depth (mm)	600	1000
Maximum rooting depth (cm)	100	120
Maximum crop factor	1.15	1.2
Limiting pressure heads (cm)	h_1 = -0.1; h_2 = -20.0; h_3^h = -500; h_3^l = -900; h_4 = -16000	h_1 = -10; h_2 = -20.0; h_3^h = -325; h_3^l = -600; h_4 = -8000

Table 2. Irrigation schedule followed for wheat and maize crops during the calibration period.

Wheat		Maize	
Date	**Irrigation depth (mm)**	**Irrigation date**	**Irrigation depth (mm)**
01-01-2012	80	23-07-2012	100
17-01-2012	85	01-08-2012	100
02-02-2012	85	12-08-2012	120
18-02-2012	95	23-08-2012	120
06-03-2012	90	03-09-2012	120
22-03-2012	85	14-09-2012	120
07-04-2012	80	25-09-2012	120
		06-10-2012	100
		17-10-2012	100

Table 3. Calibrated Van Genuchten-Mualem (VGM) parameters.

Parameter	Al-Dujaila		
	Layer 1	**Layer 2**	**Layer 3**
Depth of layer (cm)	0 - 60	60 - 150	150 - 300
Soil texture	Loam	Silt Loam	Silt Loam
Residual moisture content θrs (cm^3/cm^3)	0.01	0.01	0.01
Saturated water content θsat (cm^3/cm^3)	0.4500	0.4692	0.4698
Saturated hydraulic conductivity K_{sat} (cm day^{-1})	21.25	21.12	24.38
Shape parameter α (cm^{-1})	0.099	0.075	0.068
Shape parameter λ (-)	1.98	1.60	1.74
Shape parameter n (-)	1.0426	1.0394	1.0342

RESULTS AND DISCUSSION

Model calibration

Comparison of measured and simulated groundwater table depths

Figure 3 shows a comparison of observed and simulated groundwater table (GWT) depth for the study area during the calibration period. The simulated values are on daily basis whereas observed values are on bi-weekly basis. It is pertinent to note that irrigation has a significant effect on the groundwater table depth as the amount of precipitation during the calibration period was only 35 mm. It is evident that current irrigation practices led the groundwater rise to 70 cm below soil surface at the end of wheat season, which made the root zone saturated causing increase in salinity and reduction in crop yields. This implies that in the absence of drainage system, reduction in irrigation amounts may help in keeping

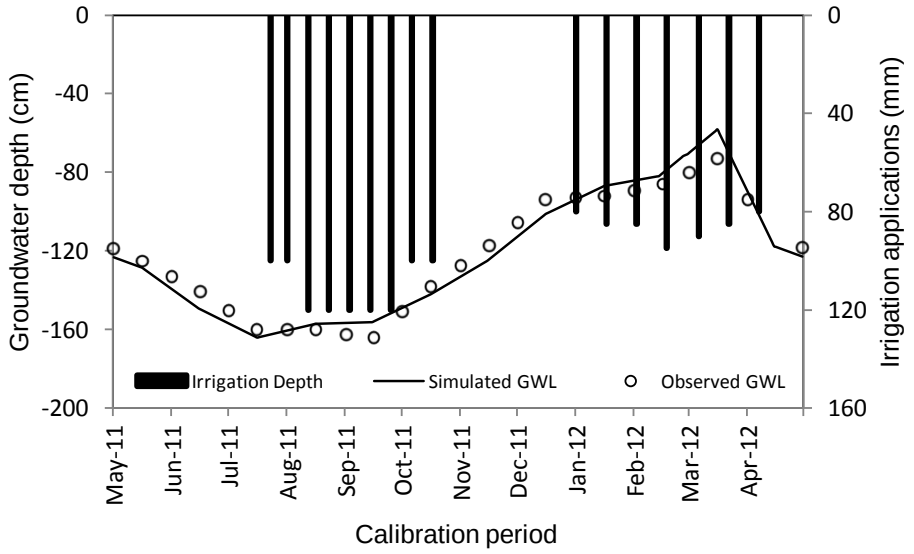

Figure 3. Observed and simulated groundwater depths in the study area.

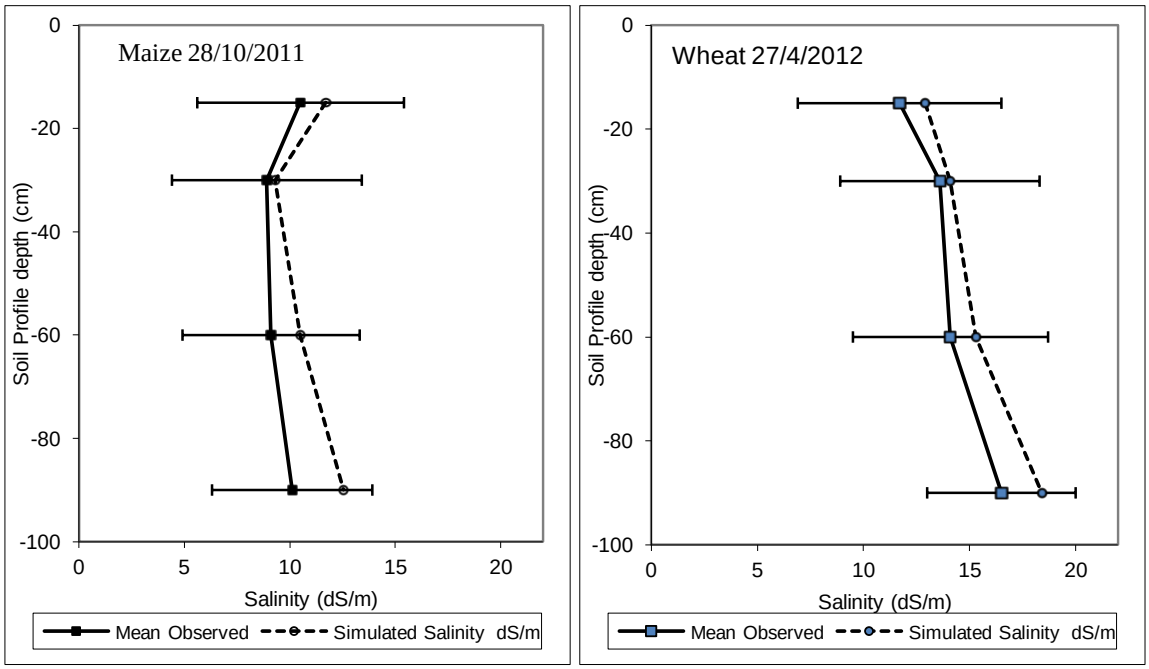

Figure 4. Observed and simulated *ECe* at different depths in the study area.

groundwater table below root zone. A good agreement between observed and simulated groundwater depths gives confidence on calibrated parameters to represent processes in the unsaturated zone.

Comparison of measured and simulated soil salinity profiles

The measured EC_e values were available only for 2 days during the study period therefore a comparison could only be accomplished for those days (Figure 4). The simulated values are within the standard deviations of the observed salinity values. The close proximity between measured and simulated values reveals that the calibrated model is good enough to represent salinity at the field scale. The high standard deviation values show that there are large variations in salinity within same field. These differences are attributed to non-uniform application of irrigation water in the field due to poor land leveling.

Table 4. Simulated water balance components for wheat and maize crops.

Water balance component	Al-Dujaila	
	Wheat	Maize
Irrigation (mm)	600	1000
Rainfall (mm)	35	0
Actual Evapotranspiration, ET_{act} (mm)	175	307
Potential Evapotranspiration, ET_{pot} (mm)	609	1080
Actual Transpiration, T_{act} (mm)	135	235
Potential Transpiration, T_{pot} (mm)	495	830
Relative Transpiration, T_{act} / T_{pot} (-)	0.27	0.28
Measured Yield, $Y_{measured}$, (t ha^{-1})	1.20	0.90
Simulated Yield $Y_{simulated}$, (t ha^{-1})	1.08	0.85
Bottom flux, q_{bot} (mm)	188	255
Salt Storage Change, *SSC*	0.910	0.775

SSC ($\Delta C/C_{initial}$) is the salt storage change in top one meter of the soil profile. ΔC is the salt concentration change over the crop growing period and $C_{initial}$ is the initial salt concentration.

This uneven distribution of water produces patches of low and high water infiltration, which in turn produces patches of low and high salinity within the same field. Relatively high groundwater table conditions during the wheat season are the probable reason for higher root zone salinity and reduction in wheat yield as compared to maize.

Simulated soil water balance components

Table 4 summarizes simulated water and salt balance components for the wheat and maize crops. The calibrated soil hydraulic parameters, measured irrigation depths and other input data were used in the SWAP model to simulate the salt and water balance components. The simulated water balance components include ET_{act}, E_{act}, T_{act}, salt storage change (*SSC*) and bottom flux (q_{bot}). The positive value of qbot represents addition of water to the soil profile from the groundwater. Table 3 reveals that 25 to 30% of the applied irrigation water was wasted as deep percolation which causes groundwater table to rise and affect T_{act} and reduced relative transpiration (T_{act}/T_{pot}). In the study area, T_{act}/T_{pot} ratio was significantly low (that is, 0.30) mainly due to high soil and groundwater salinity. T_{act}/T_{pot} ratio is considered equivalent to relative crop yields because it takes into account the effect of both soil water and salinity and reflects overall conditions in the unsaturated zone and their effect on crop yields. The maximum attainable yields of wheat and maize for Al-Dujaila area are taken as 4.0 and 3.0 t ha^{-1}, respectively (Qureshi and Al-Falahi, 2011). Using this criterion, simulated yields for wheat and maize were 1.08 and 0.85 t ha^{-1}, respectively. These simulated yields were within 5% of the measured yields, which confirms the validity of agronomic and crop parameters used for model calibration. The significant addition of salts over the calibration period reflects that salt stress was also the major factor in reducing crop yields.

Table 3 shows that during the calibration period, addition of salts in the root zone is considerably small, which does not affect the crop yield. This suggests that saturated root zone conditions caused by an intensive rise in the groundwater table were the major factors in reducing crop yields. In addition to water and salt stress under field conditions, other factors such as nutrition deficiency, pests and diseases may affect crop yields. However, SWAP does not consider these factors and assumes optimum nutrition conditions without any pest or disease stress.

Determination of optimum irrigation requirements

The calibrated SWAP model was used to perform simulations for the determination of optimal irrigation amounts and optimal groundwater table depth under the current situation to maximize crop yields and control soil salinization. The model simulations were performed to evaluate the effect of four different groundwater table depths (that is, 150, 175, 200 and 250 cm) and four irrigation regimes for wheat (600, 550, 500 and 450 mm) and six irrigation regimes for maize (that is, 1000, 700, 600, 500, 400 and 300 mm) on root zone salinity and crop yields. The groundwater table depth was maintained at different depths by setting the bottom boundary condition. However, the groundwater table was allowed to fluctuate during the growing season based on irrigation and evapotranspiration activity. The results of these simulations are presented in Figure 5.

Figure 5 shows the relationship between groundwater table depth, irrigation application and crop yields in the Al-Dujaila area. It seems that wheat is very sensitive to groundwater table depth condition. Under existing

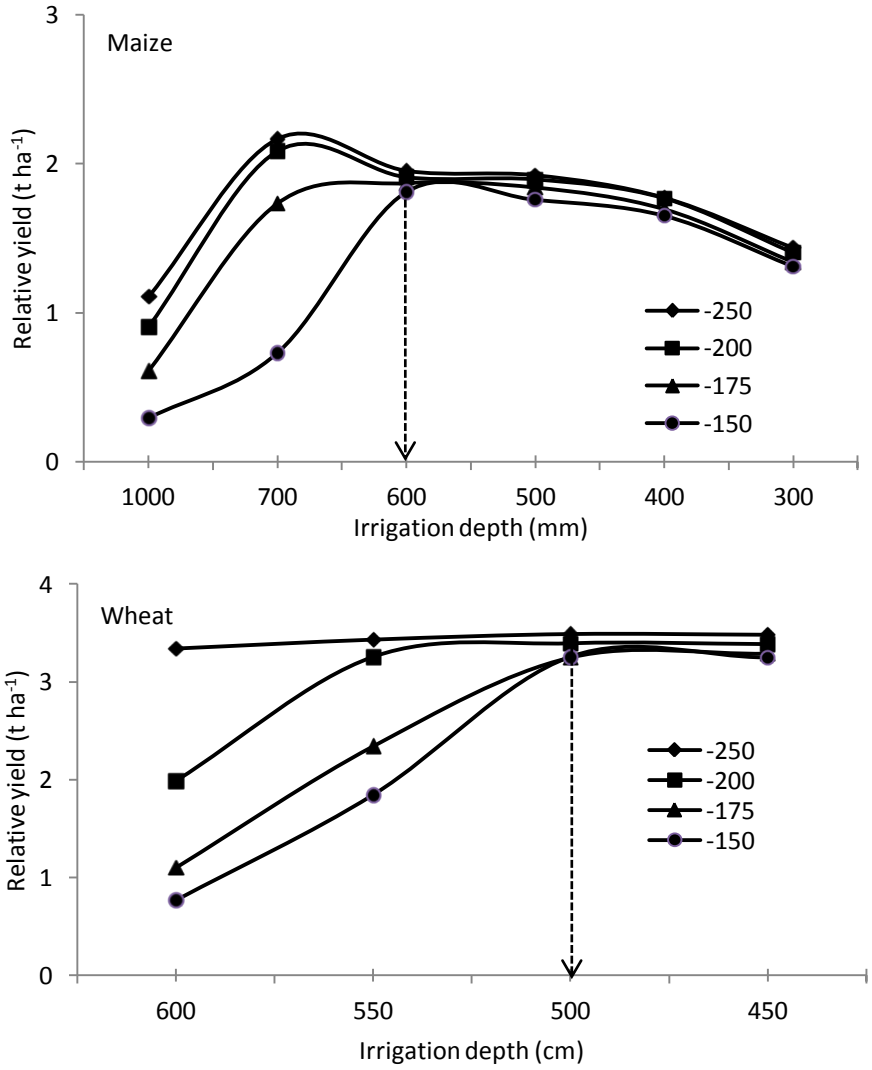

Figure 5. Wheat and maize yields as affected by different groundwater table depths and irrigation regimes.

conditions, reduction in wheat yield is almost inevitable with the irrigation application of 600 mm. It is evident that increasing groundwater table depth will have positive impact on wheat yields. Wheat yields will be increased to 1.10 t ha^{-1} at groundwater depth of 175 cm and 2.0 t ha^{-1} at or below 200 cm. Reducing irrigation applications to 500 mm to wheat will lower the groundwater table depth, which will increase the wheat yield to 3.39 t ha^{-1}.

However, further reduction in irrigation amounts could result in yield reductions.

The maize yields obtained under existing irrigation and drainage conditions are far below than the potential of 3 t ha^{-1} (FAO, 2011), Figure 5 illustrates that reduction in irrigation amounts to 600 mm would almost double the maize yield (regardless of groundwater table depth). Irrigation amounts lower than 600 mm seems insufficient to meet crop water requirements and maintain favorable salt balance in the root zone resulting in drastic reductions in maize yield. This suggests that in Al-Dujaila area, the situation is much more fragile therefore keeping groundwater out of root zone is of extreme importance to control soil salinization especially because drainage systems in the area are non-functional.

However, this must be realized that these management measures are for short-term benefits and does not guarantee long-term improvements in the soil health. To ensure long-term sustainability of irrigated agriculture in these areas, rehabilitation of existing drainage systems should be done on priority basis. These results are consistent with the findings of Qureshi et al. (2013).

They also found that reduction in irrigation application amounts can help keeping groundwater table depth below root zone which have positive impact on the yields of maize and wheat in the Al-Mussayab area in Central

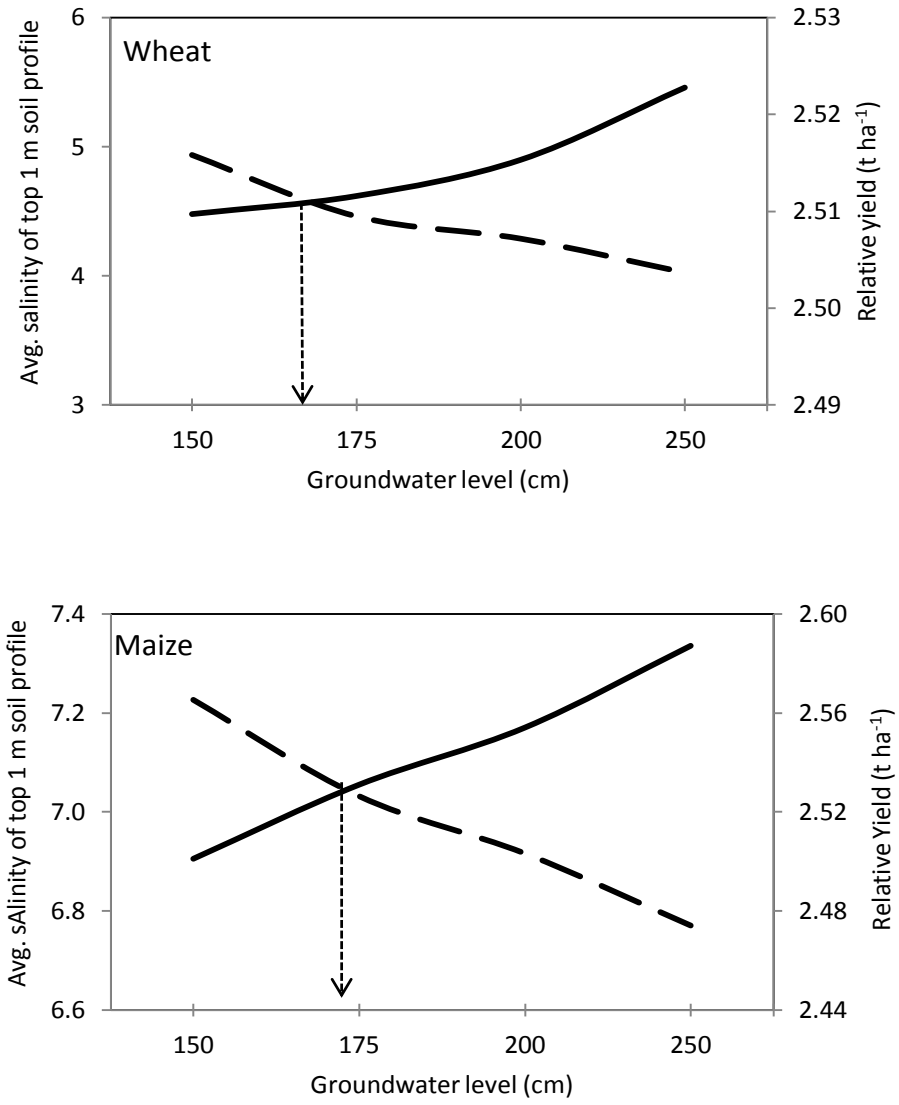

Figure 6. Relationship between root zone salinity and crop yields as affected by different groundwater table depths.

Iraq. This clearly indicates that in the Al-Dujaila area, reducing irrigation applications would be a useful strategy to control rising groundwater tables and incipient soil salinization which ultimately affect crop yields. However, for long-term sustainability of irrigated agriculture, rehabilitation of existing drainage systems would be needed.

Determining optimal groundwater table depth

Before finalizing groundwater table depth for optimal crop production and soil salinization, the effect of optimal irrigation requirements on profile salinity under different groundwater table conditions was evaluated. For this purpose, additional simulations were performed using optimal irrigation amounts (that is, 500 mm for wheat and 600 mm for maize) and their effect on salinity development in the top 1m of the soil profile was evaluated.

Figure 6 shows that application of 500 mm of irrigation water to wheat will maintain groundwater table at 200 cm, salinity around 4.3 dS m^{-1} and will produce 2.52 t ha^{-1} of wheat yield. As maize is more sensitive to root zone salinity, salinity levels above 5.0 dS m^{-1} will cause significant reduction in yields. Therefore maximum achievable yield under optimal irrigation schedule will be restricted to 1.80 t ha^{-1}. The simulated maize yield is almost double the yield obtained under current irrigation practices although they still remain well below the potential yields of the area. Qureshi et al. (2010) have also used the SWAP model to determine optimal drain depths for maximizing cotton production in the Syrdarya Province of Uzbekistan. Using a similar approach, they

also found that maintaining drain depths at 200 cm would be the most viable option to maximize crop production and control soil salinity. Using SWAP model, Sarwar and Feddes (2000) also found an optimal drain depth of 220 cm for the semiarid conditions of the Fourth Drainage Project of Pakistan. This shows that for semi-arid areas of Al-Dujaila area, a drain depth of 200 cm is suitable for maximizing crop production and controlling soil salinization.

The modeling results reveal that under the shallow and saline groundwater conditions of the study area, a groundwater table depth of approximately 200 cm and irrigation amounts of 5000 m^3 ha^{-1} to wheat and 6000 m^3 ha^{-1} to maize will be adequate to get optimum yields of wheat (2.52 t ha^{-1}) and maize (1.80 t ha^{-1}).

However, to achieve potential yields, leaching of excessive salts from the soil profile through freshwater application will be unavoidable. This will require rehabilitation of existing drainage system on priority basis and installation of new drainage systems wherever necessary. The network of surface drains also need to be cleaned to improve their efficiency in transporting saline drainage effluent away from irrigated areas. This requires substantial financial resources and time. Under the existing geo-political situation of the country, this seems difficult in the immediate future. Till then, managing irrigation to optimize crop production and control rising groundwater table and soil salinity could be a useful strategy to sustain irrigated agriculture.

Conclusions

The modeling results reveal that under the shallow GWT conditions prevailing in the southern Iraq, current irrigation practices are detrimental to crop growth because they lead to extensive groundwater table rise.

Therefore precise calculations of irrigation amounts could be beneficial in stabilizing groundwater table, conserving irrigation water and reducing drainage needs. The modeling results suggest that optimum yields of wheat (2.52 t ha^{-1}) and maize (1.80 t ha^{-1}) can be obtained by applying an irrigation amount of 5000 m^3 ha^{-1} to wheat and 6000 m^3 ha^{-1} to maize and maintaining groundwater depth at 200 cm. For potential yields, leaching of excessive salts from the soil profile will be inevitable. This will require rehabilitation of existing surface and subsurface drainage network. Under the existing geo-political and economic situation of the country, this seems difficult in near future. Till then, managing irrigation to optimize crop production by controlling rising groundwater table and soil salinity could be a useful strategy.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Al-Falahi A, Qureshi AS (2011). Understanding linkages between groundwater table depth, soil salinity and crop production in Al-Gujaila and Al-Mussaib areas of Iraq. Internal Report, Salinity Management in Iraq Project. Baghdad Iraq P. 15.

Al Zubaidi A (1992). Internal report on land reclamation. Ministry of Higher Education and Scientific Research: Baghdad, Iraq P. 200.

Bastiaanssen WGM, El-Deen MSK, Mirabile C, Korani M, Abdel G (1996). Data management related to the application of two crop–water–environment models in Argentina and Egypt. In Proceedings of the 16th ICID Congress, Cairo, Egypt pp. 54–68.

Belmans C, Wesseling JG, Feddes RA (1983). Simulation of the water balance of a cropped soil: SWATRE. J. Hydrol. 63:217–286.

Black TA, Gardner WR, Thertell GW (1969). The prediction of evaporation drainage and soil water storage from a bare soil. Soil Sci. Soc. Am. J. 33:655–660.

Boesten JJTI, Stroonsnijder L (1986). Simple model for daily evaporation from fallow tilled soil under spring conditions in a temperate climate. Nether. J. Agric. Sci. 4:75–90.

Boumans JH, Hulsbos WC, Lindenbergh HL, Van der Sluis PM (1977). Reclamation of Salt Affected Soils in Iraq. Soil Hydrological and Agricultural Studies. International Institute for Land Reclamation and Improvement ILRI: Wageningen.

Buringh P (1960). Soils and soil conditions in Iraq. Ministry of Agriculture, Baghdad, Iraq. P. 43.

Belmans C, Wesseling JG, Feddes RA (1983). Simulation of the water balance of a cropped soil: SWATRE. J. Hydrol. 63:217-286.

Boesten JJTI, Stroonsnijder L (1986). Simple model for daily evaporation from fallow tilled soil under spring conditions in a temperate climate. Nether. J. Agric. Sci. 4:75-90.

FAO (1998). Crop evapotranspiration-guidelines for computing crop water requirements. FAO Irrigation and Drainage paper 56. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO (2011). Crops. FAOSTAT: http://faostat.fao.org/site/567/default.aspx#ancor. 12.02.2012.

Feddes RA, Kavalik PJ, Zaradny H (1978). Simulation of water use and crop yield. Simulation Monogr, PUDOC, Wageningen, P. 188.

Feddes RA (1985). Crop water use and dry matter production: state of the art. Technical Bulletin № 63. Institute for Land and Water Management Research (ICW), Wageningen, The Netherlands pp. 221-234.

Feddes RA (1988). Effects of drainage on crops and farm management. Agric. Water Manage. 14:3-18.

Hanks RJ (1974). Model for predicting plant yield as influenced by water use. Agron. J. 66:660-665.

Hanks RJ (1983). Yield and water use relationships: an overview. In: Limitations to efficient water use in crop production, Taylor HM, Jordan WR, T.R. Sinclair (Eds.) Am. Soc. Agron. pp. 393–411.

Hendrickx JMH, Choudhry MA, Kijne JW, Sadiq M, Raza ZI (1990). Soil physical measurements for drainage design in arid regions. Symposium on Land Drainage for Salinity Control in Arid and Semi-Arid Lands. Cairo, Egypt 2:121-134.

Kroes JG, van Dam JC, Huygen J, Vervoort RW (1999). Uses guide of SWAP version 2.0. simulation of water flow, solute transport and plant growth in the Soil-Water-Atmosphere-Plant environment. Technical document 53, DLO-Winand Staring Center, Wageningen, Report 81, Deportment of Water Resources, Wageningen Agricultural University, the Netherlands.

Maas EV, Hoffman GJ (1977). Crop salt tolerance-current assessment J. Irrig. Drain. Div. ASCE 103:115-134.

Maas EV (1990). Crop salt tolerance. In: K.K. Tanji (ed.) Agricultural salinity assessment and management. ASAE manuals and reports on engineering practice No. 71. ASCE, New York, USA. pp. 262-304.

Mualem Y (1976). A new model for predicting the hydraulic conductivity of unsaturated porous media. Water Resour. Res. 12(3):513-521.

Nielsen DR, van Genuchten MTH, Bigger JW (1986). Water flow and solute transport in the unsaturated zone. Water Resour. Res. 22:89S–108S.

Prathapar SA, Qureshi AS (1999). Modeling the effects of deficit irrigation on soil salinity, depth to water table and transpiration in Semi-arid Zones with Monsoonal Rains. Wat. Resour. Dev. 15(½):141-159.

Qureshi AS, Eshmoratuv D, Bezbrodove G (2010). Optimal groundwater table depth for maximizing cotton production in Syrdarya province of Uzbekistan. Irrig. Drain. 59:1-12.
Qureshi AS, Waqas A, Al-Falahi A (2013). Optimal groundwater table depth and irrigation schedules for controlling soil salinity in central Iraq. Irrig. Drain. 62:414-424.
Qureshi AS, Al-Falahi A (2015). Extent, characterization and causes of soil salinity in Central and Southern Iraq and Possible Reclamation Strategies. Int. J. Engin. Res. Appl. 5(1):84-94.
Sarwar A (2000). A transient model approach to improve on-farm irrigation and drainage in semi-arid zones. PhD Dissertation, Wageningen Agricultural University, the Netherlands P. 147.
Sarwar A, Van Dam JC, Boers THM (2000). Evaluating Drainage Design Parameters for the Fourth Drainage Project, Pakistan by Using SWAP Model: Part I-Calibration. Irrig. Drain. Syst. 14:257-280.
Sarwar A, Feddes RA (2000). Evaluating drainage design parameters for the fourth drainage project, Pakistan by Using SWAP Model: Part II-Modeling Results. Irrig. Drain. Syst.14:281-299.
Stewert JI, Cuenca RH, Pruitt WO, Hagan R, Tosso J (1977). Determination and utilization of water production function for principal California crops, W-67 Calf.contrib. Proj. Rep. University of California. Davis USA.
Van Dam JC, Huygen J, Wesseling JG, Feddes RA, Kabat P, Van Walsum PEV (1997). Simulation of transport processes in the Soil-Water-Air-Plant environment. SWAP users manual. DLO-Winand String Center, Wagenngen, the Netherlands.
Van Genuchten MTH (1980). A closed from equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci. Soc. Am. J. 44:892-898.
Wösten JHM, Lilly A, Le Bas C (1998). Using existing soil data to derive hydraulic parameters for simulation models in environmental studies and land use planning. Report 156, Alterra Green Worlds Research, Wageningen, the Netherlands P. 106.

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Bermudagrass fertilization with human urine as a tool to close nutrient cycles: The use of micronutrients

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Segregating human urine from wastewater may significantly contribute to diminish the nitrogen and phosphorous problem, considered to be one of the major planetary limits already exceeded. Application of urine diluted in water in agriculture contributes to both sides of the problem. On one hand,it allows reduction of nutrient discharges into receiving bodies,on the other, it reduces the need for reactive nitrogen and its energy demand. Advantages of application of urine inbermudagrassand macronutrients accumulation are shown in the previous paper. This paper presents the results of the evaluation of accumulation of micronutrients (B, Fe, Mn, Cu and Zn) in soil and in leaves of bermudagrass irrigated with different dilutions of human urine in water. The experiment was conducted in a greenhouse in a completely randomized design with six treatments consistingof six urine doses (0 - control, 5, 10, 15, 20 and 25 ml of urine per liter of water), with four replicates. As for concentrations in the leaves, there was a significant difference between treatments for all nutrients, with the largest accumulations, of B, Fe and Mn, 120 days after planting, observed in the 10 mL L^{-1} human urine dilution in water. There were significant differences among treatmentsin the soil layer 0-20 cm, for Fe, Mn and pH, concentrations as well as the levels of B and Mn in the soil layer 20-40 cm, and for B, Mn and pH between the layers analysed, in treatments 15, 20 and 25 ml L^{-1}. Dilutions between 10 and 20 ml L^{-1} induced a greater accumulation of micronutrients in the plant tissue. The use of urine diluted in water provided adequate levels of micronutrients in the leaves in most of treatments, and it did not cause metal accumulation in the soil above the recommended levels for the bermudagrass cultivation.

Key words: *Cynodondactylon,* nutrient recycling, water reuse.

INTRODUCTION

The impoverishment of the soil, which increases the need fortheapplication of fertilizer, is accentuated by

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conventional sanitation systems because the nutrients excreted primarily in urine and the feces are mixed with water and other waste streams and discharged into water bodies. This final discharge causes not only a deficit of nutrients in the soil, but it also presents environmental and sanitary risks.

Human excreta are potential sources of nutrients for plants, but are usually considered as waste and disposed of, causing an increase in demand for energy and chemicals to remove nitrogen and phosphorus in sewage treatment plants (Spångberg et al., 2014). The main source of these nutrients in the domestic sewage is human urine (Tidåker et al., 2007), which could be collected at source and exploited in agriculture. By this mean, other significant energy savings may also be obtained as the the process of transforming inert nitrogen (N_2) from the atmosphere, to reactive nitrogen (Nr) demand about 13 kWh kg^{-1} of Nitrogen.

The use of human excreta in agriculture is a potential alternative in the pursuit of ensuring the sustainability of agricultural and conservation of natural resources as it enables recycling of nutrients. The high concentration of these elements in human excreta and the means to separate them at the source, has increased researchers interest in recent years (Jonsson et al., 2004, Richert et al., 2010).

Studies show the benefits of application of diluted human urine, segregated from wastewater streams, in crop production (Ganrot, 2005; Lienert and Larsen, 2010; Karak and Bhattacharyya, 2011). Besides the large presence of nitrogen, other important nutrients, such as phosphorus, potassium and micronutrients (Jonsson et al., 2004), are also present in urine.

In earlier publication, accumulation of macronutrients from urine used to irrigate bermuda grass (*Cynodondactylon*) was presented (accepted for publication in 2015).Here, accumulation of micronutrients in plant and soil isinvestigated.

Research has been conducted in various parts of the world, to assess the fate of nutrients in the soil-plant system, however, there is little data available regarding the effect of the use of segregated excreta on grass and the use of micronutrients (Provin et al., 2008). Many factors influence the dynamics of these elements in the soil, such as pH, organic matter, vegetation and management factors, such as the addition of organic waste (McDowell, 2003).

Grassland species have different nutrient requirements as well as different levels of maintenance requiring different nutritional managements (Wiecko, 2008). Evaluation of the fate of nutrients in the soil, while receiving different compounds, is important because of the potential for leaching and runoff. Application of an alternative source can minimize the requirements for conventional lawn fertilization (Provin et al., 2008).

It would be more beneficial to plants if a small amount of fertilizer was applied everyday. However,this procedure is rarely used because it is impractical and costly. The use of fertilizers with the irrigation water becomes more viable and can be applied often enough to maintain adequate and balanced levels of nutrients in the soil (Wiecko, 2008).

Nutrients in urine can be applied with irrigation water. Grass cultivation is an attractive option for diluted urine fertilization becauseits high nutrient demand and its cultivation in urban centers. Furthermore, possible improvements in plant development can be obtained by fractionating nutrient supply, by applying urine in the irrigation water.

The presence of micronutrients in urine demands investigation about its accumulation in soil and utilization by plants. Micronutrients present in high concentrations in the soil solution can reach levels which may betoxic to plants and microorganisms and may affect functionality, biodiversity and the sustainability of ecosystems.

According to Levy et al. (2011), wastewater use in arid and semiarid soils also involves risk from toxic levels of boron accumulation in soil. On the other hand, plants that can often accumulate nutrients in their tissues, without harming or causing visible phytotoxic effects, could be an alternative for decontamination of soils (Santos, 2005).

The objective of this study was to evaluate the accumulation of micronutrients in the soil and in the leaves of bermudagrass irrigated with different dilutionsof human urine.

MATERIALS AND METHODS

Research site

The assay was carried out in a greenhouse in the experimental area of the Soil and Water Engineering Group at the Federal University of Recôncavo of Bahia (NEAS/UFRB), located in Cruz das Almas, Bahia, at latitude 22° 42' S, longitude 47° 38' W and altitude of 220 m. Climate is classified as humid to sub-humid, with a mean annual temperature and relative humidity of 80% and 24ºC, respectively, and average annual rainfall of 1,143 mm (D' Angiolella et al., 1998).

The soil, Oxisolof low fertility, was collected in0-20 cm depth from the UFRB campus. According to the results of the analysis, the chemical compositionof the soil is shown in Table 1. The granulometric composition of the soil was 800, 13 and 187 gkg^{-1} of sand, silt and clay respectively, of sandy loam texture.

Experimental design

A completely randomized design (CRD) was used with six treatments which consisted of five different dilutions of human urine in water(T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), and a control (T0 - irrigation without urine and soil without any fertilization). Each plot consisted of a polyethylene container with a capacity of 100L and 0.41 m^2, with four replications, totaling 24 experimental plots.

Seeds of bermudagrass (*Cynodondactylon*) were used following the manufacturer's recommendations for an equivalent dose of 25 $kgha^{-1}$. The planting was carried out in plastic containers made of polyethylene with a capacity of 100 L and 0.41 $m^2$with drains and

Table 1. Chemical characteristics of the soil used in the experiment.

pH ($CaCl_2$)	M.O.	P (resin)	S-SO_4^{2-}	Na	K	Ca	Mg	Al	H+Al	Base saturation (BS) - %	B	Cu	Fe	Mn	Zn
	g dm^{-3}	mg dm^{-3}		$mmol_c dm^{-3}$							mg dm^{-3}				
6.2	13	<2	<3	1.9	18.4	10	<1	28	30	58.0	0.22	<0.3	15	11.1	4.7

Table 2. Characteristics of human urine and water used in irrigation.

Property	Unit	Water	Urine
pH		8.40	8.70
Electrical conductivity	dS m^{-1}	0.78	24.35
N-total	mg L^{-1}	nd	6,937.50
P-total	mg L^{-1}	nd	923.33
K_2O	mg L^{-1}	0.18	1,483.75
Ca^{2+}	mg L^{-1}	0.26	65.00
Mg^{2+}	mg L^{-1}	0.70	10.00
S	mg L^{-1}	nd	1,655.00
Fe - total	mg L^{-1}	nd	1.63
Mn - total	mg L^{-1}	nd	1.63
Cu	mg L^{-1}	nd	0.88
Zn	mg L^{-1}	nd	1.13
Na	mg L^{-1}	4.65	2,937.50
B	mg L^{-1}	nd	0.50
Cl^-	mg L^{-1}	0	4,093.75

nd, not determined.

filled with aforementioned soil over a 3 cm thick layer of gravel and a geotextile blanket.

Fertilization, collection and application of human urine

Human urine used in the experiment was collected in the males' toilets of a student residence, and stored in a 20Lblack reservoir. The students voluntarily contributed by urinating directly into the reservoir. The urine was collected for up to 3 days and was used within 4 days after collection. Single samples of urine used in each treatment were stored under refrigeration. A composite sample was produced each month using the weighted average of the volumes of water applied in each treatment. Irrigation management was performed using the values of class A evaporation pan, installed inside the greenhouse, applying 100% of the evaporated depth.

Treatments with urine received additional doses of phosphorous and potassium so that all treatments were fertilized with the same dose of these nutrients. In order to gaina better understanding of the effect of nitrogen from the urine, all the nitrogen provided to the plants came from this source. In order to ensurethat all treatments contained the same concentration of phosphorus and potassium, five samples of human urine where analysedbeforeassembly of the experiment to determine their mean chemical composition (Table 2), which was taken into account for the calculation of fertilization.

The supply of nutrients throughout the stage of establishment of grass was estimated, based on predicted values of evaporation using historical data, and this was used to manage the amount of irrigation water. Chemical fertilization was performed based on the value obtained by the difference between the amount of nutrients supplied by urine and the recommendation for the crop.

Commercial fertilizers,composed of simple superphosphate and potassium chloride, were applied based on the recommendations of Godoy and Boas (2003). Each treatment was fixed to supply an equivalent of 150 $kgha^{-1}$ of phosphorus and 150 $kgha^{-1}$ of potassium. The potassium fertilizer was applied at planting and 60 days after planting (DAP).

Soil was raised to field capacity before planting, establishing an irrigation interval of two days. Irrigation was performed manually, using a watering can and the distance between the plots was 50 cm.

Grass management and evaluation

Grass was maintained at a height of approximately 2.0 cm. Pruning/cuttingwas performed when at least one of the treatments achieved a height of 10 cm with a frequency of 7 to 14 days. After trimming, the material was placed in paper bags and taken to an oven with forced air circulation at 65°C for 72 h to reach a constant weight.

This same material was used to determine the concentration of boron, iron, manganese, copper and zinc in leaves, respecting the interval equal to 30 days. Analysis to determine the levels of micronutrientes were performed in the Laboratory of Mineral Nutrition of Plants at the College of Agriculture Luis de Queiroz

Table 3. Summary of analysis of variance, coefficient of variation (CV%) and estimation of parameters B, Fe, Cu, Mn and Zn, 120 days after planting (DAP) in leaves of bermudagrass.

Source of variation	Mean square					
	DF	B	Fe	Cu	Mn	Zn
Treatment	5	26.55**	2,134.34**	22.08**	3,112.21**	354.41**
Residue	18	3.04	168.51	1.43	618.04	24.62
Mean (mg kg^{-1})		7.76	118.31	11.14	187.83	61.68
CV (%)		22.47	10.97	10.74	13.24	8.04

*Significant at p <0.05 by F test; **Significant at p <0.01 by F test; DF, degree of freedom.

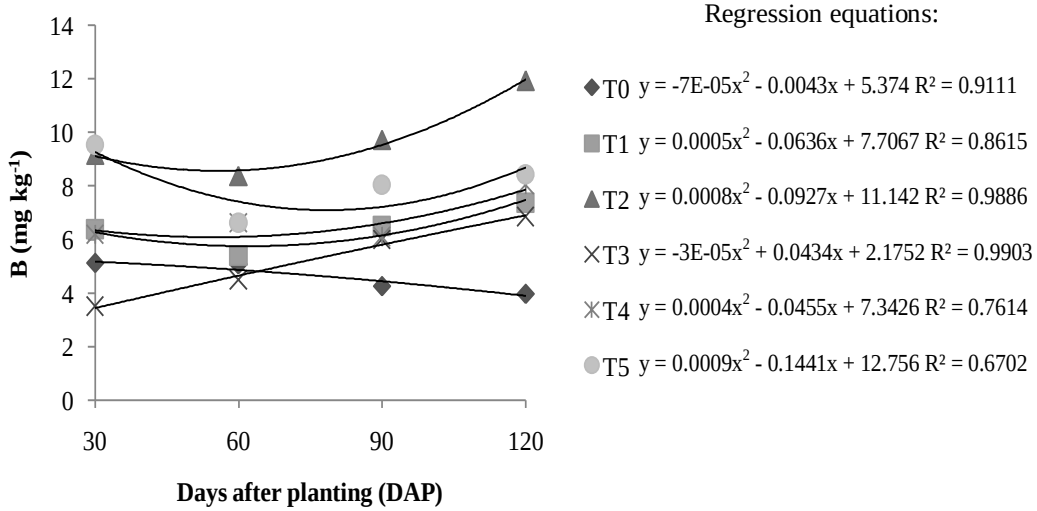

Figure 1. Concentration of B in leaves of bermudagrass (*Cynodondactylon*) as a function of dose in urine (T0 - 0 ml L^{-1}, T1 - 5 mL L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), evaluated to four periods.

(ESALQ/USP), by the method described by Malavolta et al. (1997). At the end of the experiment, soil samples were collected at 0-20 and 20-40 cm to determine the levels of B, Fe, Mn, Cu, Zn and pH. Analysis were performed at the ESALQ/USP laboratory, according to the methods described by Raij (2001).

Statistical analysis

Data collected were subjected to analysis of variance followed by regression analysis. For both analysis, SISVAR System software - Analysis of Variance, version 5.3 (Ferreira, 2010) was used.

RESULTS AND DISCUSSION

Concentration of micronutrients in the leaves

Boron

Micronutrients concentrations in plants' tissue of bermudagrass are presented in Table 3. Results show that nutrient concentrations 120 days after planting, were positively influenced by the urine application.

For concentrations of B in plant tissue at the end of the experiment (Figure 1), the highest values (11.9 $mgkg^{-1}$) were observed in samples that received dose of 10 ml L^{-1} of urine, greater than the 200% observed in the control treatment - T0 - (<4.0 $mgkg^{-1}$). In addition, urine use caused the plants to show adequate levels of B in theirtissues, which according to Wiecko (2006) is in the range of 6 to 30 $mgkg^{-1}$, a value which was observed in all treatments except the control.

Assessment of each treatment over time (Table 4) shows that, for boron, a significant effect was observed onlyin the treatment of 10 ml L^{-1}(T2 treatment).For this concentration of urine in water, the B concentration in the leaves rose from 9.2 to 11.3 $mgkg^{-1}$ in the interval of 30 to 120 days after planting (DAP), a 30% increase.

Boron translocation from roots to shoots may be influenced by the level of supply in the soil. Plants with adequate supply retain higher proportions in roots than plants subject to disabilities and short-term deprivation which can cause a sharp fall in levels in shoots (Dannel

Table 4. Summary of analysis of variancefor the effect of time on the concentration of B, Fe, Cu, Mn and Zn in the leaves, for each treatment.

Source of variation	DF	Treatment	Mean square				
			B	Fe	Cu	Mn	Zn
Period	3	T0	1.34^{NS}	448.34**	3.80^{NS}	12,237.18**	29.43 NS
Residue	12		2.48	24.07	1.57	332.23	20.17
Mean (mg kg^{-1})			4.60	115.71	12.65	162.65	63.56
CV (%)			34.19	4.24	9.93	11.21	7.07
Period	3	T1	2.59^{NS}	186.93^{NS}	13.72**	3,114.64*	150.76*
Residue	12		4.12	184.97	1.77	731.50	35.78
Mean(mg kg^{-1})			6.40	122.78	12.81	204.21	63.53
CV (%)			31.70	11.08	10.39	13.24	9.42
Period	3	T2	9.25*	497.18^{NS}	4.01**	5,566.64**	95.27NS
Residue	12		1.76	476.57	0.59	599.57	47.17
Mean(mg kg^{-1})			9.80	146.68	11.46	189.65	67.68
CV (%)			13.53	14.88	6.75	12.91	10.15
Period	3	T3	8.95^{NS}	186.30^{NS}	2.20^{NS}	7,814.47**	80.41*
Residue	12		3.10	85.21	0.76	962.06	15.64
Mean(mg kg^{-1})			5.20	98.21	10.37	181.46	44.75
CV (%)			33.87	9.40	8.40	17.09	8.84
Period	3	T4	3.20^{NS}	452.55^{NS}	13.80**	3,781.16NS	394.12**
Residue	12		1.14	255.10	0.42	1,551.72	29.53
Mean(mg kg^{-1})			6.73	111.21	10.78	171.37	49.37
CV (%)			15.92	14.36	6.02	22.99	11.01
Period	3	T5	5.79^{NS}	50.56^{NS}	10.47**	2,411.43*	155.79*
Residue	12		2.75	70.18	0.72	591.17	27.17
Mean(mg kg^{-1})			8.14	114.56	8.09	161.34	61.75
CV (%)			20.36	7.31	10.51	15.07	8.44

*Significance ($p < 0.05$) by F test; **Significance ($p < 0.01$) by F test; NS, not significant; DF, degree of freedom.

et al., 1998; Li et al., 2001; Noguchi et al., 2000). Although it is an essential element for plants, boron is also toxic when present in excess (Takano et al., 2008).

Grasses grown in soils with low concentrations of B may undergo slow growth and may not even complete their life cycle and may also present characteristic deficiency symptoms such as stunted leaves (Samples and Savoy, 2008). Results show that urine used as a source of nutrients led to an increase in boron concentrations in the leaves without causing excesses that could affect plant development.

Iron

Similar to boron, the highest concentrations of Fe in plants tissue, at 120 DAP,were also observed under the dose of 10 ml L^{-1}ofurine in water (T2) with gains of up to 35% compared to the control treatment (T0) (Figure 2).

In the case of this micronutrient, all treatments were within the recommended range which,according to Wiecko (2006), is 50 to 350 mgkg^{-1}. It is worthwhile to mention that highest dose of urine presented contenntsof Fe even lower than control.

Concentrations of Fe varied significantly over time (Table 4) just for the control treatment T0, with values of 100 and 120 mgkg^{-1}, at 30 and 120 DAP, respectively (Figure 2).

According to Foth (1990), acidic soils have sufficient Fe concentrations to meet the needs of plants. Analysing the soil's pH, it appears that this justifies the results obtained for the concentrations of Fe in the leaves.

The application of urine did not influence negatively the Fe concentration in the plant tissue. Application of urine, therefore, may represent a good alternative for grasses in the tropics which often suffer from iron deficiencies, especially in alkaline and sodic soils in arid and semiarid regions demanding foliar application of the element. Munshaw et al. (2006) claimed that the application of Fe to bermudagrass provides intense green color in spring and promotes better recovery of the lawn.

Copper

The highest concentrations of copper, at 120 DAP, were

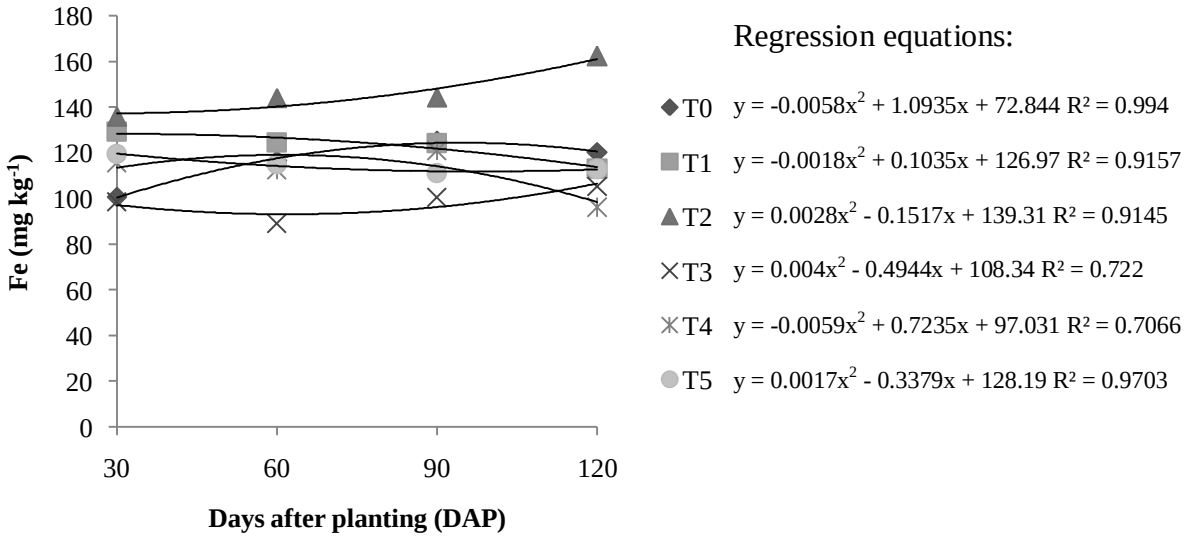

Figure 2. Concentration of Fe in leaves of bermudagrass (*Cynodondactylon*) as a function of dose in urine (T0 – 0 ml L^{-1}, T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), evaluated four periods.

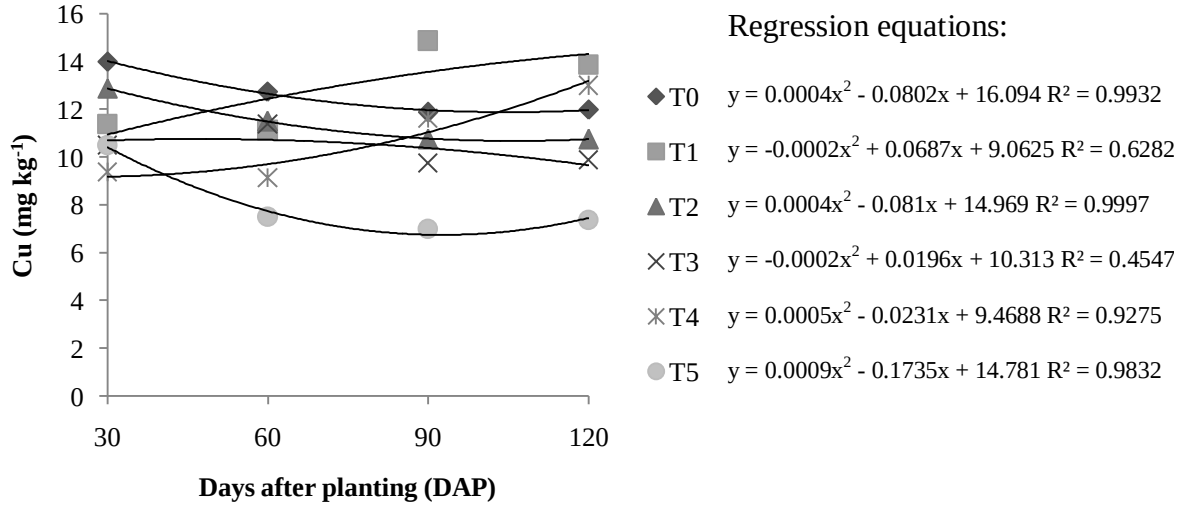

Figure 3. Concentration of Cu in leaves of bermudagrass (*Cynodondactylon*) as a function of dose in urine (T0 – 0 ml L^{-1}, T1 – 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 – 20 ml L^{-1}, T5 – 25 ml L^{-1}), valued over four periods.

observed in treatments with a dose of 5 ml L^{-1} of urine (Figure 3), while the lowest concentrations were observed in the T5 treatment (20 mL L^{-1}) with 7.4 $mgkg^{-1}$, a value 47% lower than that observed in the treatment T1. However, all the observed values are within the optimal range 5 to 50 $mgkg^{-1}$ (Wiecko, 2006). Accumulation over timeshows that copper had a significant effect in all treatments except contol (Table 4). Like many other micronutrients, symptoms of copper deficiency also appear in younger leaves of grass (Broyer, 1954). Although little research has been carried out on the impacts of copper fertilizers on lawns (Heydari and Balestra, 2008), studies have shown that copper affects the growth of some species of grassland and excessive application may cause root lesion in some grass species (Broyer, 1954). These findings reinforce the potential use of human urine, because of the adequate levels of micronutrients in leaf tissues obtained by its use.

Manganese

The highest concentrations of Mn in plant tissue were observed in T2, at 120 DAP, with 12.4% increases compared to the controltreatment (Figure4). All

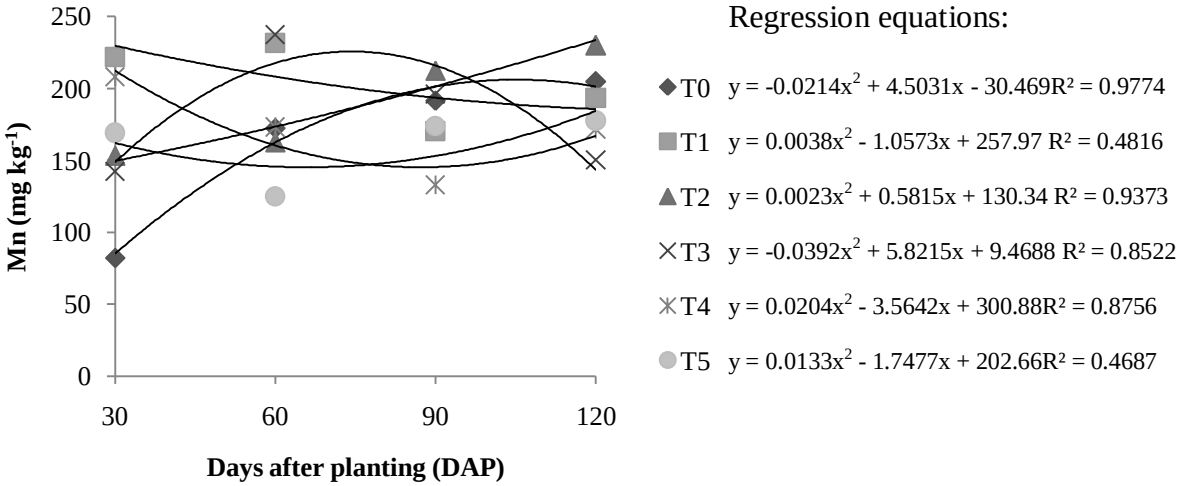

Figure 4. Concentration of Mn in leaves of bermudagrass (*Cynodondactylon*) as a function of dose of urine (T0 - 0 ml L^{-1}, T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 1 5 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), valued into four periods.

treatments also maintained adequate levels of this concentration in leaves - which, according to Wiecko (2006) is between 25 and 300 $mgkg^{-1}$ for grass.For Mn, there was a significant effect over time for all treatments except T4 (Table 4). T2 showed the highest concentration,at 120 DAP, which was 53.2% higher than in T3.

Manganese deficiency is not common in bermudagrass, however, if it occurs, it usually results in chlorosis of young leaves. Low absorption of Mn sometimes occurs with excessive application of nitrogen and potassium. Manganeseconcentrations can also be affected by pH values (Broyer, 1954; Snyder et al., 2008). In the experiment, high N concentration did not affect the absorption of Mn.

Evaluating the use of human urine in the cultivation of cabbage (*Brassica oleracea*), Pradhan et al. (2007) found that Mnbehaveddifferently with respect to other micronutrients undersimilar concentrations between plants fertilized with urine and commercial fertilizer.

Zinc

The highest concentrations of Zn were observed in T1 (72 $mgkg^{-1}$). There was a significant variation in Zn concentration overtime (Table 4) in relation to T3 , T4 and T5 doses with values 60.5, 12.5 and 23.4% lower than in T1(Figure 5). Zn deficiency is uncommon in grasses (Snyder et al., 2008) however, a deficiency can impair plant growth, generating small and stunted leaves (Wiedenhoeft, 2006).

These symptoms were not observed during the course of experiment. Contrary to that observed with the use of urine, in a study with bermudagrass and use of sewage sludge by Lane (1988), increasing Zn doses led to increasing concentrations of Zn in the leaves. Theauthor attributedthis fact to a greater growth of plants that received higher doses of nutrients.

Provin (2008) evaluated the influence of the use of sewage sludge on soil nutrient dynamics in growing bermudagrass observing that concentrations of Zn and Fe in leaves increased with increasing doses of these substances.

Concentration of micronutrients in the soil and pH

Boron

Concentrations of boron in the soil did not vary significantly with increasing concentrations of urine in the irrigation water, in layer 1 (0 to 20 cm), but there was significant difference between treatments in the layer 2-20 to 40 cm (Figure 6a and Table 5).

Differences in concentrations were observed between layers in all treatments with urine (Table 6). Apparently, boron did not leach from the first to the second layer of the soil. An appropriate concentration of B in the soil was maintained. For growing lawns, according to Wiecko (2006), B concentration in soil should be between 0.25 and 0.75 $mgdm^{-3}$.

Although this range does not represent toxicity for many plants in heavy soils, higher levels can cause problems, especially for trees and shrubs. Grasses are generally much more tolerant toboron than other plants if they are pruned and the clippings are removed regularly (Harivandi, 1983).

Iron and manganese

No significant effects were observed between treatments for the concentration of Fe in the soillayer 2 (Table 5).

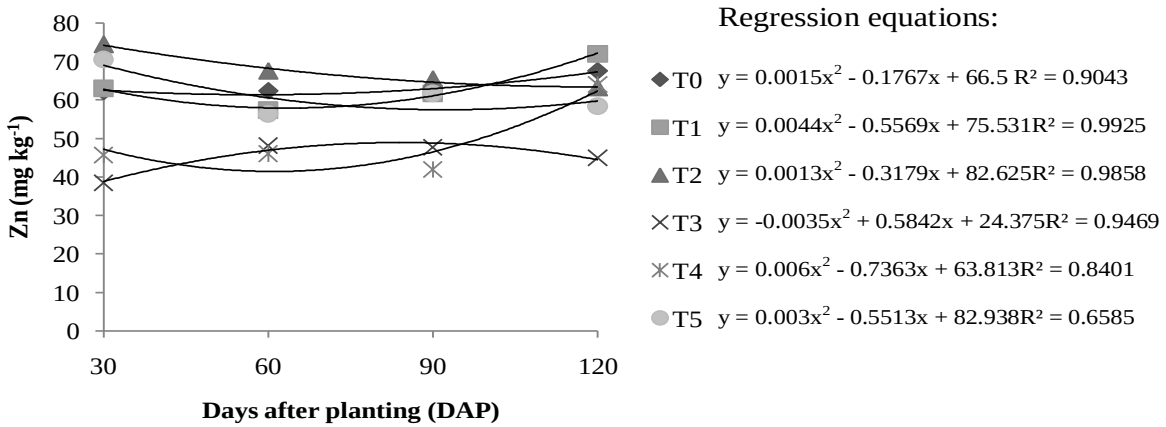

Figure 5. Concentration of Zn in leaves of bermudagrass (*Cynodondactylon*) as a function of dose of urine (T0 - 0 ml L^{-1}, T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), evalated in four periods.

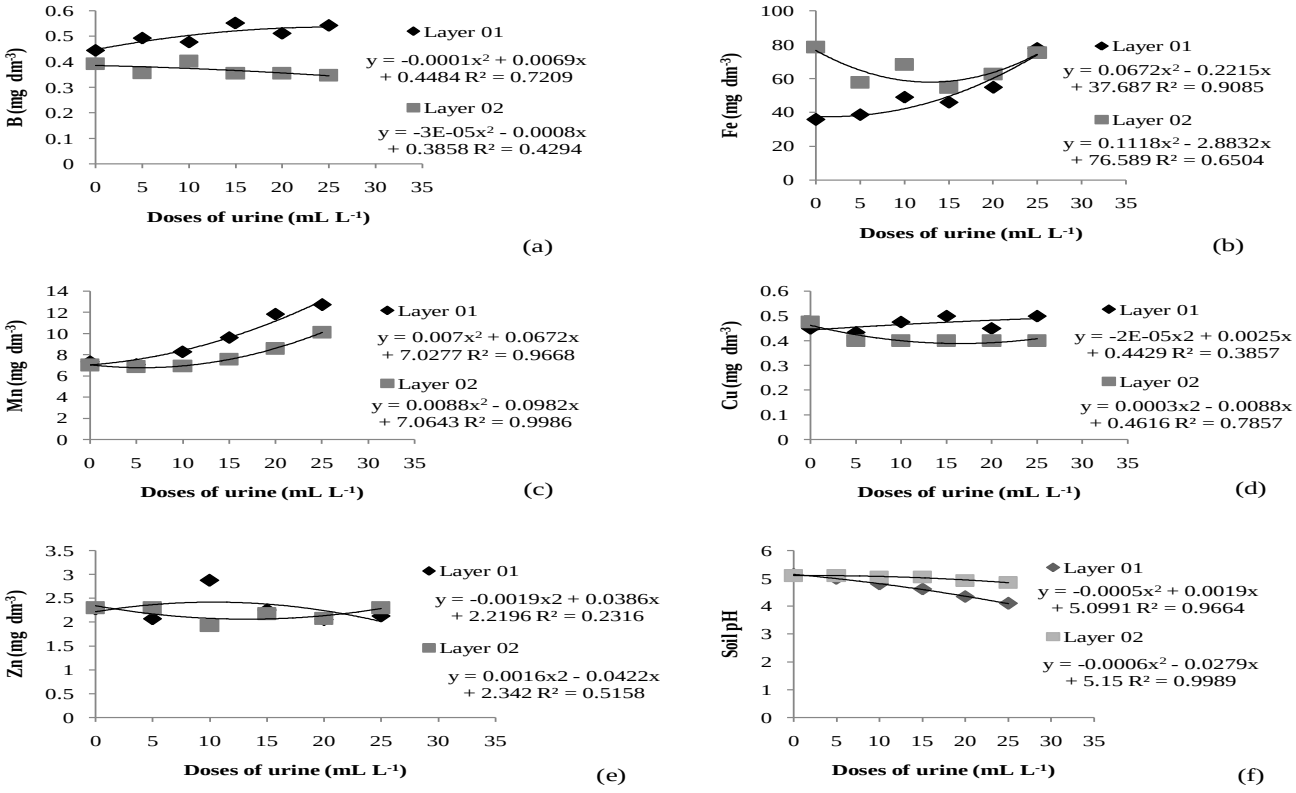

Figure 6. Content of B, Fe, Mn, Cu and Zn and pH in the soil depending on the doses of urine in water, in the different soil layers, 120 DAP.

However, a significant difference was observed between the two layers analyzed, for T0 and T1 treatments, as seen in Figure 6b and Table 6. Fe concentration in the deeper layer was more than twice than in the upper level before urine application (T0). As higher concentrations of urine in the irrigation water were applied, concentrations of this element in layer 1 increased and the differences between layers were reduced. For 25 mg L^{-1} (T5) values were almost the same.

Only T1 and T3 concentrations remained within the range recommended for growing lawns, which is 7.5 to 60 mg dm^{-3} (Wiecko, 2006).For all treatments there was a lower concentration of Fein the upperlayer of the soil,

Table 5. Summary of analysis of variancefor the concentrations of B, Fe, Cu, Mn, Zn and pH in the soil layers 0-20 and 20-40 cm.

Source of variation	DF	Mean square					
		B	Fe	Cu	Mn	Zn	pH
		(Layer 1- 0-20 cm)					
Treat	5	0.00657^{NS}	912.40*	0.00320^{NS}	22.381**	0.38144^{NS}	0.62841**
Residue	18	0.00251	225.82	0.00523	0.96606	0.49717	0.00819
Mean (mg dm^{-3})		0.50375	50.31	0.46791	9.48	2.27	4.67
CV (%)		9.96	29.86	15.46	10.37	31.01	1.94
		(Layer 2 – 20-40 cm)					
Treat	5	0.00212*	362.86^{NS}	0.00375^{NS}	6.61**	0.09541^{NS}	0.04175^{NS}
Residue	18	0.00071	477.83	0.004861	1.49	0.082361	0.04430
Mean (mg dm^{-3})		0.36830	66.16	0.41250	7.95	2.17	5.01
CV (%)		7.27	33.04	16.90	15.55	13.17	4.20

*Significance ($p < 0.05$) by F test; **Significance ($p < 0.01$) by F test; NS, not significant; DF, degree of freedom.

where most of the plant roots are found, causing greater absorption, as observed in the nutrient levels in the leaves.

For Mn, a significant effect of treatments on the two layers was observed for treatments T2, T3, T4 and T5 (Figure 6c and Table 6). No difference was observed between layers for the T1 treatment and control which showed 7 mg dm^{-3} in both layers. For all treatments with urine, there was a greater accumulation of Mn in the upper layers of the soil.

Copper and Zinc

No significant difference between treatments was observed for concentrations of Cu and Zn in soil or between the two layers (Figure 6d and e), except for the concentration of Cu in the treatment T5 (Table 6). Values observed were inside Wiecko's (2006) recommended range for growing lawns, 0.5 - 5 mg dm^{-3} for Cu and 2-5 mg dm^{-3} for Zn. In the case of T2 the identified value of 1.9 mg dm^{-3} at Layer 2, is below the range. However, in Layer 1 where most of the roots develop, all treatments showed adequate levels.

According to Foth (1990), copper tends to be found adsorbed in a fraction strongly complexed with inorganic or organic matter. As a result, copper is rather immobile in soil and concentrations in the solution tend to be very low. As for availability to plants, Zinc and copper exhibit similar behaviors.

Piedade et al. (2009) evaluated the effects of irrigation with wastewater in four species of grass. The values for most micronutrients were found to be superior to those observed at the start of the experimental phase because the application ofwaste water to the ground causes increases in the levels of micronutrients. As expected, similar results were observed when using human urine diluted in water, since the nutrients present in wastewater are mainly derived from human urine (Tidåker et al., 2007). The use of urine's micronutrients in the soil, 120 DAP, did not lead to levels above those recommended for growing grass for lawns.

pH

The values of soil's pH showeda significant difference between the layers for T3, T4 and T5 (Table 6), with the lowest values (4.1) observed inthe 0-20 cm layer in treatment T5. There were significant differences between treatments in layer 2, as can be seen in Figure 6f and Table 5. The use of urine reduced the soil's pH. OnlyT1 presented the same valueas the control case (5.1).

Pradhan et al. (2010) compared theeffect of human urine with that of commercial fertilizers in the cultivation of sugar beet and found no significant differences. For urine treatmentsthese authors observed values of 7.31 and 7.13 at the beginning and end of the experiment.For commercial fertilizers they found a pH 7.17.

The main influence of pH on plant growth is on the availability of micronutrientsas boron, copper,and zinc are leachable and can be deficient in leached, acid soils. On the other hand, they can become insoluble (fixed) and therefore

Table 6. Summary of the analysis of variance to the concentrations of B, Fe, Cu,Mn, Zn and pH for the 0-20 and 20-40 cm of soil layers, for each treatment.

Source of variation	DF	Treatment	Mean square					
			B	Fe	Cu	Mn	Zn	pH
Layer	1	T0	0.00551^{NS}	3,655.12*	0.00125^{NS}	0.21125^{NS}	0.00125^{NS}	0.00500^{NS}
Residue	6		0.00229	367.29	0.00291	1.37	0.24458	0.00833
Mean (mg dm^{-3})			0.41875	57.12	0.46250	7.18	2.28	5.12
CV (%)			11.44	33.55	11.68	16.30	21.62	1.78
Layer	1	T1	0.03645**	728.28**	0.00211^{NS}	0.07411^{NS}	0.10811^{NS}	0.02^{NS}
Residue	6		0.00109	41.56	0.00111	1.90	0.16444	0.03666
Mean (mg dm^{-3})			0.42500	48.20	0.41625	6.97	2.18	5.05
CV (%)			7.77	13.37	8.01	19.78	18.57	3.79
Layer	1	T2	0.01125**	741.12^{NS}	0.01125^{NS}	3.64**	1.80^{NS}	0.125^{NS}
Residue	6		0.03666	267.79	0.00458	0.84250	1.21	0.02500
Mean (mg dm^{-3})			0.44	58.62	0.43750	7.60	2.4	4.92
CV (%)			6.39	27.91	15.47	12.08	46.01	3.21
Layer	1	T3	0.07801**	153.12^{NS}	2.02^{NS}	8.40**	0.01125^{NS}	0.36120**
Residue	6		0.00106	238.45	0.01	0.92250	0.04291	0.00958
Mean (mg dm^{-3})			0.45375	50.37	0.45000	8.60	2.21	4.83
CV (%)			7.18	30.65	22.22	11.17	9.36	2.02
Layer	1	T4	0.04961**	120.12^{NS}	0.00500^{NS}	21.45**	0.00125^{NS}	0.66120*
Residue	6		0.00266	99.62	0.01166	1.26	0.02625	0.06291
Mean (mg dm^{-3})			0.43375	58.62	0.42500	10.21	2.06	4.63
CV (%)			11.90	17.03	25.41	11.03	7.86	5.41
Layer	1	T5	0.07605**	12.50^{NS}	0.02000**	13.52000**	0.06125^{NS}	1.12500**
Residue	6		0.00179	1,096.25	0.00	1.05	0.04	0.01
Mean (mg dm^{-3})			0.44500	76.50	0.45000	11.42	2.21	4.47
CV (%)			9.51	43.28	0.0	9.01	9.18	2.74

*Significance ($p < 0.05$) by F test; **Significance ($p < 0.01$) by F test; NS - not significant; DF, degree of freedom

unavailable for plants in alkaline soils (Foth, 1990). In acid soils, the presence of iron may provoke deficiencies of other elements, such as molybdenum, due to its reaction to form insoluble compounds. However, even at lower pH values, no deficiencies were observed for the micronutrients assessed, with appropriate levels found in leaves.

Conclusions

The results presented in this study allow us to conclude that:

1. Irrigation with diluted human urine positively influences the levels of micronutrients present in plant tissue and soil. Adequate levels of micronutrients were verified in bermudagrass when irrigated with human urine. This represents an additional advantage for nitrogen and phosphorous cycling through human urine use in agriculture;
2. The highest accumulation of B, Fe and Mn in leaves, 120 days after planting, were observed under 10 ml urine per liter of water;

3. Concentrations between 5 and 15 mL L^{-1} showed better results as they promoted good nutrient accumulation in the plant tissue;
4. As the use of urine promotes the recycling of nutrients which reduces the amount of industrial fertilizers needed, it can be a viable alternative for the discharge of excreta in rural and urban areas. It allows improvements in plant development, permitting a better fractionation of nutrient supply. Furthermore, the use of urine does not cause accumulation of micronutrients considered toxic for growing bermudagrass, both in the leaves and in the soil.

Conflict of Interest

The authors verify that there are no competing interests.

REFERENCES

Broyer TC, Carlton AB, Johnson CM, Stout PR(1954). Chlorine - A micronutrient element for higher plants. Plant Physiol 29: 526-532.

D'Angiolella GLB,Castro Neto MT,Coelho EF (1998). Climate trends for the Coastal Tablelands of Cruz das Almas region, BA [In Portuguese]. In: Congresso Brasileiro de Engenharia Agrícola, 27, Poços de Caldas. Annals... Lavras: SBEA1 pp. 43-45.

Dannel F, Pfeffer H, Romheld V(1998). Compartmentation of boron in roots and leaves of sunflower as affected by boron supply. J. Plant Physiol. 153:615-622.

Ferreira DF(2010).Computer programSisvar - UFLA, version5.3.

Foth HD(1990). Fundamentals of soil science. 8 ed. Toronto: John Wiley and Sons, P. 382.

Ganrot Z(2005). Urine processing for efficient nutrient recovery and reuse in agriculture. PhD Thesis.Göteborg:Department of Environmental Science and Conservation Faculty of Science, Göteborg University. Sweden: P. 170.

Godoy LJG, Villas Bôas RL(2003). Nutrition and fertilizer for lawns [In Portuguese]. In: Simpósio sobre gramados, 1, 2003, Botucatu. Annals... Botucatu: Departamento de Recursos Naturais, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista.

HarivandiMA(1983). The use of effluent water for turfgrassirrigation.California Turfgrass Culture32:1–4.

Heydari A, Balestra GM(2008). Nutritional disorders of turfgrasses. In: Pessarakli M. (ed) Handbook of turfgrass management and physiology. Boca Raton: CRC Press, pp. 211-235.

Jonsson H, Stinzing AR, Vinneras B, Salomon E(2004). Guidelines on the use of urine and faeces in crop production. EcoSanRes Publication Series Report 2004-2. Stockholm: Stockholm Environment Institute P. 43.

Karak T, Bhattacharyya P(2011). Human urine as a source of alternative natural fertilizer in agriculture: A flight of fancy or an achievable reality. Resour. Conserv. Recycle 55:400-408.

Levy GJ, Fine P, Bar-Tal A (2011). Treated wastewater in agriculture: use and impacts on the soil environment and crops.Oxford: Blackwell Publishing, P. 188.

Li C, Dannel F, Pfeffer H, Rimheld V, Bangerth F(2001). Effect of boron starvation on boron compartmentation, and possibly hormone-mediated elongation growth and apical dominance in pea (*Pisum sativum*) plants. Physiol. Plant 111:212-219.

Lane R(1988). The effect of sewage sludge application to bermudagrass on forage quality production, and metal mccumulation. Agric. Ecosyst. Environ. 20:209-219.

Lienert J, Larsen TA (2010). High acceptance of urine source separation in seven european countries: A review. Environ. Sci. Technol. 44:556-566.

Malavolta E, Vitti GC, Oliveira SA (1997). Avaliação do estado nutricional das plantas:princípios eaplicações.Piracicaba: Associação Brasileira para Pesquisa da Potassa e do Fosfato, P. 319.

McDowell WH (2003). Dissolved organic matter in soils–future directions and unanswered questions. Geoderma 113: 179-186.

Munshaw CG, Ervin EH, Shang C, Askew SD, Zhang X,LemusRW(2006). Influence of lateseason iron, nitrogen, seaweed extract on fall color retention and cold tolerance of four bermudagrass cultivars. Crop Sci. 46:273–283.

Noguchi K,Dannel E,Pfeffer H,Romheld V, Hayashi H, Fujiwara T(2000). Defect in root-shoot translocation of boron in *Arabidopsisthaliana* mutant bor 1-1. Plant Physiol. 156:751-755.

Piedade AR, Cruz RL, Campos S, Boas RLV(2009). Vegetative growth of four species of grass irrigated with wastewater [In Portuguese]. Irrig. 14:268-275.

Pradhan SK, Nerg AM, Sjöblom A, Holopainen JK, Heinonen-Tanski H (2007). Use of human urine fertilizer in cultivation of cabbage (*Brassicaoleracea*)-Impacts on chemical, microbial, and flavor quality. J. Agric. Food Chem. 55:8657-8663.

Pradhan SK, Holopainen JK, Weisell J, Heinonen-Tanski H (2010). Human urine and wood ash as plant nutrients for red beet (*Beta vulgaris*) cultivation: Impacts on yield quality. J. Agric. Food Chem. 58:2034–2039.

Provin TL, Wright AL, Hons FM, Zuberer DA, White RH(2008). Seasonal dynamics of soil micronutrients in compost-amended bermudagrass turf. Bioresour. Technol. 99:2672-2679.

Raij B (2001). Análise química para avaliação da fertilidade de solos tropicais. Campinas: IAC P. 285.

Richert A, Gensch R, Jönsson H, Stenström TA, Dagerskog L(2010). Practical guidance on the use of urine in crop production. Stockholm: Stockholm Environment Institute.(SEI), Sweden. EcoSanRes Series, 2009-1.

Samples T, Savoy H (2008). Turfgrass maintenance - Essential elements. Knoxville: The University of Tennessee. Available in: <https://utextension.tennessee.edu/publications/Documents/W161-D.pdf>. Accessed 13 dez. 2014.

Santos GCG (2005). Comportamento de B, Zn, Cu, Mn e Pb em solo contaminado sob cultivo de plantas e adicção de fontes de matéria orgânica como amenizantes do efeito tóxico. Thesis (doctorate in agronomy). Piracicaba: Escola superior de Agricultura Luiz de Queiroz. P. 150.

Snyder GH, Cisar JL, Park DM(2008). Warm-season turfgrass fertilization. In: Pessarakli M. (ed) Handbook of turfgrass management and physiology. Boca Raton: CRC Press, pp. 47-55.

Spångberg J, Tidåker P, Jönsson H(2014). Environmental impact of recycling nutrients in human excreta to agriculture compared with enhanced wastewater treatment. Sci. Total Environ. 493:209–219.

Sposito G(2008). The chemistry of soils. Oxford: University Press. P. 342.

Takano J, Miwa K, Fujiwara T(2008). Boron transport mechanisms: collaboration of channels and transporters. Trends Plant Sci. 13:451-457.

Tidåker P, Mattsson B, Jönsson H (2007). Environmental impact of wheat production using human urine and mineral fertilizers - a scenario study. J. Clean Prod. 15:52-62.

Wiecko G(2006). Fundamentals of tropical turf management . Cambridge: CABI Publishing P. 208.

Wiecko G(2008). Management of tropical turfgrasses. In: Pessarakli M. (ed)Handbook of turfgrass management and physiology. Boca Raton: CRC Press pp. 115-137.

Wiedenhoeft AC(2006). Plant nutrition. New York: Chelsea House Publishers P. 153.

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Full Length Research Paper

Milk production function and resource use efficiency in Jaipur District of Rajasthan

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The study was undertaken during 2013 and 2014 in Jaipur District of Rajasthan with the objectives to examine the input-output relationships and assess the resource use efficiency in milk production. The study covered 100 commercial dairy farms. The results of Cobb-Douglas production function revealed that expenditure on dry fodder and green fodder for small category of herd size were found to be positive and significant. The results for medium category of herd size revealed that partial regression coefficients for expenditure on green fodder and miscellaneous expenditure for were found positive and significant. The results for large category of herd size revealed that the partial regression coefficient for expenditure on concentrate and miscellaneous expenditure were found positive and significant. In case of small category of herd size, it was observed that dry fodder, green fodder, labour and miscellaneous expenses were optimally utilized. In case of medium category it was observed that dry fodder, green fodder and miscellaneous expenses were optimally utilized. In case of large category of herd size, dry fodder, concentrate and miscellaneous expenditure were optimally utilized.

Key words: Concentrate, dry fodder, green fodder, labour, milk, mvp and resource use efficiency.

INTRODUCTION

One of the most significant changes in India's agricultural economy over the past three and a half decades has been the rising contribution of livestock sector in the agricultural gross domestic product. Between 1970 and 2012, the share of livestock in agricultural gross domestic product has risen from 17 to 26% (Govt. of Rajasthan, 2014). The milk production is influenced by various genetic and non-genetic factors. The non-genetic factors influencing the milk production are quantity and quality of feeds and fodders fed, order of lactation, stage of lactation, herd size, labour use etc. Hence the selection of suitable variables to study the milk production is very essential. To ensure the optimal use of various inputs used by the milk producers is matter of primary concern. It is important to know whether the inputs owned by commercial dairy farmers are used efficiently or not. An empirical assessment of determinants of milk production and resource use efficiency are important for planning, projecting and formulating dairy development policies in a particular region of the country. The input-output relationship in milk production and resource use efficiency have been studied by several researchers in

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the various parts of the country and found different for different areas depending upon the type of milch animals and the milk production technology. No study has been carried out to investigate the milk production function and resource use efficiency in respect of commercial dairy farms in Jaipur district of Rajasthan.

The present study was undertaken to fill this vital gap with the specific objectives to (i) examine the input-output relationships in milk production across different categories of herd sizes of commercial dairy farms, and (ii) Study the resource use efficiency in milk production across different herd size categories of commercial dairy farms.

REVIEW OF LITERATURE

Mahajan (2010) in his study economic analysis of rural and pere urban farm in Ludhiana district of Punjab indicated that Cobb Douglass function was best fit. In production function of peri urban dairy farm, the partial regression coefficient of expenditure on concentrate for crossbred cattle were found positive and significant with total explained variation, that is, R^2 as 84.3%.

Singh (2008) in his study on economic analysis of milk production of milk production in Varanasi District of Utter Pradesh concluded that green fodder; dry fodder and concentrate were underutilized indicating that feeding of more quantity of green fodder and concentrate will further increase the productivity of milch buffaloes in the study area.

Wani et al. (1992) studied input-output relationship in milk production and estimated the marginal value product of relevant input variables separately for non descript and crossbred cows in the Kashmir valley.

METHODOLOGY

The study was conducted during 2013 to 2014 in Jaipur district of Rajasthan. The sampling design consisted of selecting the ultimate sampling unit, that is, dairy commercial farms using multistage random sampling method. The tehsils, villages and sample commercial dairy farms consisted the first, second and third stage of sampling. Jaipur district has 13 tehsils namely Amer, Chomu, Jamwaramgarh, Phagi, Phulera, Bassi, Sanganer, Muzamabad, Viratnagar, Kotputli, Chaksu, which were classified on the basis of water and type of animal into different zones. Out of thirteen tehsils, six tehsils namely Amer, Bassi, Shahpura, Sanganer, Phuleraand Jaipur thesil were selected consisting of 590 commericial dairy farms. Among 590 commercial dairy farms 100 commercial dairies were selected on the basis of probability proportional to size.

The selected 100 commercial dairies were post stratified into three categories using Cumulative Square Root Frequency Method on the basis of number of milch animals. The commercial dairy farms were thus categorized into three herd size categories namely small (up to 31 milch animals), Medium (32-45 milch animals) and large (above 45 milch animals). The distribution of sampled commercial dairy farms in the small, medium and large herd size categories were found to be 43, 41 and 16 respectively. The primary data of commercial dairy farms were collected with help of well structured pre-tested schedule by personal interview/enquiry method. The data were collected on milk production, quantity of green fodder, dry fodder, concentrate and miscellaneous expenditure along with their monetary values.

Analytical framework

Specification of milk production function

The specification of milk production function used in the present study for functional analysis is as follows:

$Y=f(X_1, X_2, X_3, X_4, X_5)$

Where, Y = Income from milk per farm per day (Rs.); X_1 = Expenditure on green fodder per farm per day (Rs.); X_2 = Expenditure on dry fodder per farm per day (Rs.); X_3 = Expenditure on concentrate per farm per day (Rs.); X_4 = Value of labour used per farm per day (Rs.); X_5 = Miscellaneous expenses per farm per day (Rs.).

Four types of functional forms, viz., Cobb-Douglas, Linear and Semi log (both linear-log and log-linear models) were tried which are as follows:

Linear:

$$Y = a + \sum_{i=1}^{n} bi\, Xi + \mu$$

Cobb Douglas:

$$Y = a \prod_{i=1}^{n} X_i^{bi}\, e^{\mu}$$

Semi Log(Log-Lin):

$$lnY = a + \sum_{i=1}^{n} b_i X_i + \mu$$

Semi log(Lin-Log):

$$Y = \ln a + \sum_{i=1}^{n} b_i \ln X_i + \mu$$

Where, Y = value of output; X_i = value of i^{th} input used; a = constant term; b_i = partial regression coefficient of the i^{th} input to be estimated; μ = random error distributed normally with zero mean and constant variance, and e = base of natural log.

The best function will be selected on the following economic and statistical criteria:

1. The higher value of coefficient of multiple determination (R^2)
2. Significant level of individual regression coefficients, and
3. The ability of the function to provide economically meaningful results.
4. The minimum value of Root Mean Square Error (RMSE).

Cobb-Douglas production function was found best fit for all categories of commercial dairy farms because it has high value of R^2 and low value of Root Mean Square Error (RMSE) among all other fitted production functions (Table 1).

Ideally, the output (Y) and inputs (X_i) in the above production functions were measured in monetary values rather than their

Table 1. Root mean square error (RMSE) and R^2 (%) values of different fitted production functions.

Type of function	Category of commercial dairy farm					
	Small		Medium		Large	
	RMSE	R^2 (%)	RMSE	R^2 (%)	RMSE	R^2 (%)
linear	0.629	23.520	18.180	84.360	0.123	87.780
log lin	0.253	21.910	0.062	84.760	0.047	87.790
Cobb-Douglas	0.235	32.420	0.066	82.740	0.043	89.630
lin log	0.586	33.260	0.197	81.600	0.114	89.600

Table 2. Various fitted production function and their marginal value product

Type of function	Marginal value product
Linear	b_i
Cobb Douglas	$b_i \times \frac{\bar{Y}}{\bar{X}}$
Semi log (lin log)	$\frac{b_i}{\bar{X}}$
Semi log (log lin)	$b_i \times \bar{Y}$

b_i = partial regression coefficient of the i^{th} input to be estimated; $\bar{Y}$ = geometric mean of output Y, and $\bar{X}$ = geometric mean of input X

physical quantities. This was done because the quality of different feeds and fodders differ from one respondent to the other and can be more appreciably reflected in value terms. The monetary values of inputs in production functions have been preferred over physical quantities by many of earlier researchers e.g. Sharma and Singh (1993), Shiyani and Singh (1996) etc.

Marginal value product

Marginal value productivity of inputs was estimated from the fitted production function (Table 2).

Recourse use efficiency

Recourse use efficiency of an input measures whether or not the input is used optimally. The inputs are used optimally if the MVP of the input is equal to its price, that is,

$MVP_i = P_i$

Where P_i is the unit price of input

In order to examine the resource use efficiency, the marginal value productivity of various inputs was worked out for significant regression coefficients in the estimated milk production function. Any deviation of MVP of input from its unit price may be termed as resource use efficiency. The higher the difference between MVP of an input and its unit price, the higher is the resource use inefficiency and vice versa.

Further, t-statistics given below was used to test the statistical significance of the difference between MVP and its unit price. If the difference between MVP of an input and its unit price is statistically non significant, it indicates that the inputs is being utilized efficiently. A significant higher MVP of an input than its unit price shows that the input can be used further to increase productivity, while a significantly lower MVP of an input is being used in excess and hence needs reduction.

t statistic:

$$t = \frac{MVPx_i - Px_i}{S.E.(MVPx_i)}$$

S.E (MVP_i) = Standard error of MVP, and P_i is the unit price of input.

RESULTS AND DISCUSSION

The Cobb-Douglas production function for all categories of commercial dairy farms has been presented in Table 3. A close perusal of the table revealed that the coefficient of multiple determination (R^2) for the small, medium, large and overall category was 32.42, 82.74, 89.63 and 17.73% of total variation in income from milk per farm per day, respectively, were explained by the variables included in the selected regression model.

It was revealed from Table 3 that partial regression coefficients of expenditure on dry fodder and green

Table 3. Root mean square error (RMSE) and R^2 (%) values of different fitted production functions.

Type of function	Category of commercial dairy farm					
	Small		Medium		Large	
	RMSE	R^2 (%)	RMSE	R^2 (%)	RMSE	R^2 (%)
linear	0.629	23.520	18.180	84.360	0.123	87.780
log lin	0.253	21.910	0.062	84.760	0.047	87.790
Cobb-Douglas	0.235	32.420	0.066	82.740	0.043	89.630
lin log	0.586	33.260	0.197	81.600	0.114	89.600

Table 4. Estimated coefficients of milk production function for different categories of commercial dairy farms.

Category	No. of dairy farms	Constant	Regression Coefficients					R^2 (%)
			G.F (X_1)	D.F (X_2)	Conc. (X_3)	Labor (X_4)	Misc. (X_5)	
Small	43	2.571 (1.977)	0.155* (0.062)	0.487** (0.151)	0.736 (0.410)	-0.499* (0.229)	-0.293* (0.121)	32.42
Medium	41	3.100 (1.074)	0.631** (0.152)	-0.086* (0.136)	0.0966 (0.303)	0.191 (0.097)	0.297* (0.124)	82.74
Large	16	-8.967 (3.629)	-0.200** (0.022)	0.185 (0.281)	3.986*** (0.765)	-1.655** (0.417)	1.188** (0.240)	89.63
Overall	100	3.423 (1.193)	0.043 (0.083)	0.112 (0.099)	0.605* (0.242)	-0.344** (0.113)	-0.009 (0.083)	17.73

Figures in parenthesis indicate the standard error of regression coefficient. *Significant at p<0.05, * significant at p<0.01.

fodder for small category of herd size were found to be positive and significant. The production function analysis indicated that milk production could be increased through effective feeding of concentrates. The table further revealed that partial regression coefficients for labour and miscellaneous expenditure were negative and significant. The concentrate, was, thus, found to have no impact on income from milk.

The partial regression coefficients for expenditure on green fodder and miscellaneous expenditure for medium category of herd size were found to be positive and significant with total explained variation, that is, R^2 as 82.74%. The production function analysis indicated that milk production could be increased through effective feeding of green fodder and miscellaneous expenditure.

The partial regression coefficient for expenditure on concentrate and miscellaneous expenditure for large category of herd size were found to be positive and significant. The production function analysis indicated that milk production could be increased through effective feeding of concentrate and by increasing miscellaneous expenditure. The table further revealed that partial regression coefficient for labour and green fodder, it was negative and significant which implied that by increasing expenditure on green fodder and labour income from milk production will decrease. The partial regression coefficient for dry fodder, was, thus, found to have no impact on income from milk. The study is similar to Mangesh (2003).

The partial regression coefficients for expenditure on concentrate and labour for overall were found to be positive and significant which implies that income from milk production could be increased through effective feeding of concentrate and by decreasing use of labour. The table further revealed that partial regression coefficients for rest of variables were found statistically non-significant which implied that these variables not have impact on income from milk. The study is similar to Das (2004)

In order to find out whether or not the significant inputs viz. green fodder, dry fodder, labor and miscellaneous in case of small category of herd size and green fodder, dry fodder and miscellaneous expenditure in case of medium herd size and green fodder, concentrate, labour and miscellaneous in case of large category, concentrate and labour in case of overall farm were used efficiently, Marginal value of productivities (MVP) of these inputs has been worked out (Table 4).

Resource use efficiency in milk production

In order to examine the resource use efficiency, the marginal value productivities (MVP) of inputs whose

Table 5. Resource use efficiency in milk production.

Small category	MVP	Input Price	Difference	S.E	t-value
Dry fodder	1.51	1.00	0.51	0.06	0.34
Green fodder	7.29	1.00	6.29	0.15	0.86
Labour	2.69	1.00	1.69	0.23	0.63
Miscellaneous	17.66	1.00	16.66	0.12	0.94
Medium category					
Dry fodder	3.57	1.00	2.57	0.15	0.72
Green fodder	7.65	1.00	6.65	0.14	0.87
Miscellaneous	23.25	1.00	22.25	0.12	0.96
Large category					
Dry fodder	0.57	1.00	-0.43	0.15	-0.11
Concentrate	1.95	1.00	0.95	0.30	1.23
Labour	8.14	1.00	7.14**	0.10	3.78
Miscellaneous	49.22	1.00	48.22	0.12	1.89
Overall					
Dry fodder	1.03	1.00	0.03	0.80	0.03
Green fodder	5.66	1.00	4.66	4.87	0.96
Concentrate	1.44	1.00	0.44	0.58	0.76
Labour	-5.03	1.00	-6.03**	1.66	-3.62
Miscellaneous	-1.52	1.00	-2.52	14.22	-0.18

**Significant at p< 0.01.

regression coefficients were found statistically significant in estimated milk production function were compared with their acquisition cost, that is, marginal factor cost (MFC).

The inputs viz., green fodder, dry fodder, labour and miscellaneous expenditure in case of small category, green fodder, dry fodder and miscellaneous expenditure in case of medium, green fodder, concentrate, labour and miscellaneous in case of large and concentrate and labour in case of overall herd size category of commercial dairy farm were found to be statistically significant. The marginal value productivity of all the significant inputs was computed at their geometric mean level. The results of the different herd size categories are presented in Table 5 along with their prices. Since all the inputs were expressed in monetary terms in the production function, the acquisition cost of the inputs was taken as Re.1. The estimated marginal value productivity was, therefore, compared with unity to examine the resource use efficiency. t-statistic was used to test the significance of deviation of MVP of an input from its unit price. A significant higher difference of MVP of an input from its unit price shows that more of that input can be used to increase productivity, while a significant lower difference MVP of an input from its unit price indicates that the input is used in excess and needs curtailment. The marginal value productivity (MVP) of significant inputs for all categories of commercial dairy farms, their difference with unit price of respective inputs (MFC) and t-statistic are given in the Table 5.

In case of small category of herd size, it can be observed that marginal value productivity of dry fodder, green fodder, labour and miscellaneous expenses were found to be positive but statistically non-significant. Similar findings were also observed in case of medium category of herd size, it can be revealed that marginal value productivity of dry fodder, green fodder and miscellaneous expenses were found to be positive but statistically non-significant.

The marginal value productivity of dry fodder, concentrate and miscellaneous expenses were found to be statistically non-significant in case of large category of herd size while it was positive and statistically significant for labour. The marginal value productivity of labour was under utilized as the difference between its MVP and unit price was positive and significant in the study area.

The marginal value productivity of concentrate was found to be statistically non-significant in case of overall category of herd size. The marginal value productivity of labour was negative and significantly lower than their acquisition cost which indicated that labour was over-utilized in the study area.

Conclusions

The green fodder, dry fodder, labour and miscellaneous

expenditure were found to be statistically significant in case of small category of commercial dairy farm, green fodder, dry fodder and miscellaneous expenditure in case of medium, green fodder, concentrate, labour and miscellaneous in case of large and concentrate and labour in case of overall herd size category. The results of resource use efficiency indicated that none of the marginal value productivity of all inputs was statistically significant across and overall herd size category except labour in large category and overall herd size category in the study area.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Das S (2004). Economic efficiency of milk production and marketed surplus in rural area of Burdwan District (W. Bengal). M.Sc. Thesis, NDRI (Deemed University), Karnal, Haryana, India.

Government of Rajasthan (2014). Economic Survey of Rajasthan 2013-14, Directorate of Economics and Statistics, Planning Department, Jaipur (Rajasthan).

Mahajan S (2010). Economic analysis of rural and pere urban farm in Ludhiana district of Punjab. M.Sc. Thesis, NDRI (Deemed University), Karnal, Haryana, India.

Mangesh GM (2003). Economics of milk production and disposal pattern in Wardha District of Maharastra. M.Sc. Thesis, NDRI (Deemed University), Karnal, Haryana, India.

Sharma VP, Singh RV (1993). "Resource productivity and allocation efficiency in milk production in Himachal Pradesh". Indian J. Agric. Econ. 48 (2):201-215.

Shiyani RL, Singh RV (1996). An economic analysis of technical efficiency in milk production. Indian J. Dairy Sci. 49(9):572-578.

Singh S (2008). Economic analysis of milk production in Varanasi District of Uttar Pradesh. M.Sc. Thesis, NDRI (Deemed University), Karnal, Haryana, India.

Wani SA, Mathur SC, Singh CB (1992). Resource productivity and allocation in dairy farming with different breeds of cows in temperate regions of Kashmir. J. Dairying Foods Home Sci. 11(1):25-30.

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