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Table of Contents: Volume 13 Number 28, 12 July, 2018

ARTICLES

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African Journal of Agricultural Research

Full Length Research Paper

Technical efficiency and yield gap of smallholder wheat producers in Ethiopia: A Stochastic Frontier Analysis

Tadele Mamo[1*], Wudineh Getahun[1], Ali Chebil[2], Agajie Tesfaye[1], Tolessa Debele[1], Solomon Assefa[2] and Tesfaye Solomon[1]

[1]Ethiopian Institute of Agricultural Research (EIAR), Ethiopia.
[2]International Center for Agricultural Research in the Dry Areas (ICARDA), Ethiopia.

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Improving technical efficiency of smallholder farmers is one of the options to increase wheat yield in developing countries. This paper assesses technical efficiency, factors for inefficiency and the yield gap due to technical inefficiency in major wheat producing regions of Ethiopia, where the support to agricultural research for development of strategic crops (SARD-SC) wheat project has been implemented using primary data collected from 946 sample households operating 1616 wheat plots. One-step stochastic frontier approach with a Translog production form was used for econometric analysis. The results show that the mean technical efficiency of the overall sample is 0.769 meaning about 23% technical inefficiency in the system implying that the sample wheat producers are producing at a yield gap of 659 kg/ha. Different input variables contribute for wheat yield. It also reveals that education, oxen ownership, credit, soil fertility, using tractor, and using improved seed (in Tigray) were found to improve technical efficiency of wheat producers either for the overall or for some regions. On the contrary, family labor negatively affects efficiency in Oromia and in overall sample, while using improved seed (in Amhara and SNNP), plot distance and crop rotation (in Oromia) had a negative effect on technical efficiency.

Key words: Technical efficiency, wheat, Ethiopia.

INTRODUCTION

Increasing wheat production and productivity could usefully reduce the burden of wheat imports by Ethiopia. To this end, the country has been implementing several research and development strategies. The attention given to improved wheat variety generation and dissemination is one of the attempts made by the government.

According to Ministry of Agriculture (MoA, 2014), 67 and 33 bread and durum wheat varieties, respectively, have been released from different federal and regional agricultural research centers and disseminated for production. As a result, wheat revealed steady growth in production and productivity over recent years even though it did not grow on par with growth of demand. According to the Central Statistical Agency (CSA, 2005, 2015) reports, the domestic production of wheat increased from 2.2 million tons in 2004/2005 to 4.2 million tons ten years later (2014/2015), which is a 91% growth. Similarly, productivity has increased from 1.56 tons/ha in

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

2004/2005 to 2.54 tons/ha in 2014/2015, which is a growth of 63%. This swift growth of productivity could largely be attributed to the use of improved technologies of wheat. Within the same period of time the area coverage for wheat has also increased from 1.4 million hectares in 2004/2005 to 1.6 million hectares in 2014/2015, which is a growth of 14%.

Basically, increasing domestic wheat production can be achieved through two options other than increasing area cropped. One is through making more intensive use of inputs and/or technologies while the other is improving the level of efficiency of the wheat producers, given fixed level of inputs at the current available technology so that the producers produce at the frontier to obtain maximum output without using additional input. The former option needs high investment in expensive inputs which is difficult for smallholder farmers, especially if credit is not available on time. In developing countries, resource use is often scanty as farmers are highly constrained with cash to purchase input on one hand and the option of frequently generating new technologies can be rare on the other hand, (Yadav and Sharma, 2015). Another reason which makes this option less probable is that wheat technology adoption is already fairly high in Ethiopia (Chilot et al., 2013) which means the probability of increasing wheat production through wheat technology adoption could not work in areas where the adoption level is at its climax and there is only small room to increase wheat productivity through technology adoption. Different governmental and NGOs are intensively working on dissemination of improved wheat varieties to cover the farmers who have not yet adopted the technology. In order to make its own contribution in bridging-up of wheat supply and demand gaps, the SARD-SC wheat project was launched in Ethiopia in 2013 with four main components, namely, technology generation; technology dissemination and adoption; capacity building; and project management. The project follows the innovation platform (IP) approach in four major wheat producing regions, namely, Tigray, Amhara, Oromia and SNNP regions to bring all stakeholders together to achieve its broad objectives.

While increasing wheat yield through improved technologies is one option, increasing wheat yield by improving efficiency of producers seems the most appropriate strategy in Ethiopia since it does not need additional investment in input use but improving the producers' knowledge to wisely produce at the production frontier with a given technology and limited inputs. The focus of this paper is therefore, unpacking the second option through assessing the efficiency of wheat producers in the IP sites of the project.

Efficiency analysis is one of the important fields of production economics. Economic efficiency is composed of technical efficiency and allocative efficiency. Mathematically, economic efficiency is the product of the two. Technical efficiency is dealing with attaining the maximum attainable level of output for a given level of inputs, under a given technology or it can be seen as producing a given level of output from the minimum amount of inputs for a given technology, given the current range of alternative technologies available for the farmer (Battese and Coelli, 1992; Ellis, 1993; Farrell, 1957; Kalirajan and Shand, 1999). On the other hand, allocative efficiency is the ability of a producer to use the inputs in optimal proportions (Farrell, 1957; Kalirajan and Shand, 1999). Based on the hypothesis of Schultz's (1964) 'poor-but-allocatively efficient' stating that 'small farmers in traditional agricultural settings are reasonably efficient in allocating their resources by responding positively to price incentives', Ethiopian smallholder wheat producers are considered as more or less allocatively efficient. Regardless of the challenges exerted on it from opponents (Shapiro, 1983; Ball and Pounder, 1996; Duflo, 2006; Ray, 2006), the hypothesis has been well accepted by both economists and policy makers over the past half of a century. Therefore, this paper deals with assessing the technical efficiency of wheat producers focusing on four major wheat producing regions of Ethiopia where the SARD-SC wheat project has been implemented.

Objectives

(1) To assess the level of technical efficiency of wheat producers,
(2) To assess determinants of inefficiency of wheat producers,
(3) To estimate yield gap due to inefficiency.

METHODOLOGY

The study area

The study was conducted in six districts of support to agricultural research for development of strategic crops (SARD-SC) IP sites selected from four major wheat producing regions of Ethiopia. Two districts each from East Gojjam zone of Amhara region and Bale zone of Oromia region and one district each from South Tigray zone of Tigray region and Gurage zone of SNNP region were purposively selected for the following reasons. First, these districts were selected by each of the regions themselves for the SARD-SC wheat project Innovation Platform (IP) intervention. Second, the districts did not receive enough attention and supports from other development projects to enhance wheat production and productivity. Third, the districts have a high potential of wheat production even though Enemay and Shebel Berenta districts are not as high potential as others. The selected sites represent the African Highlands hub of the SARD-SC wheat project of Ethiopia (Figure 1).

Data collection techniques and target groups

A structured questionnaire was used to collect input and output data from wheat producer households. The comprehensive data

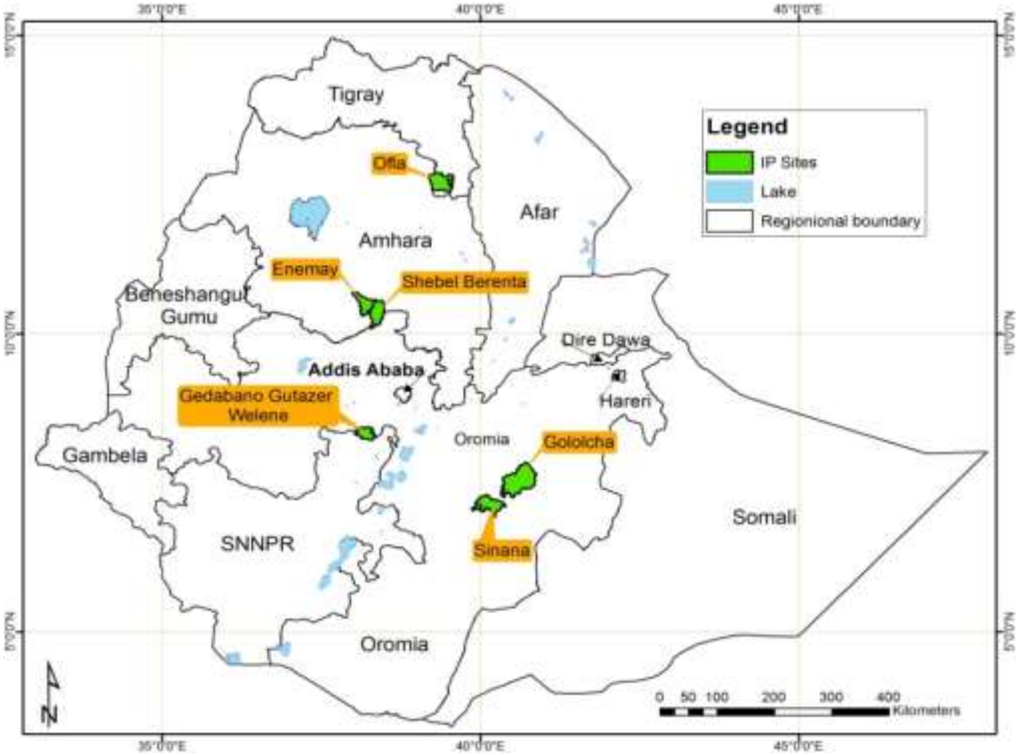

Figure 1. Map of the study area, 2014.

were collected from December 2013 to January 2014 through trained enumerators and supervisors using structured questionnaire. The data collection was implemented using Computer Aided Personal Interview (CAPI) to improve the quality of the data.

Sampling frame and sampling procedure

The sampling frame of the study is the list of wheat producer households in the study districts. Stratified multistage sampling technique was employed to select the required samples of households. First, four wheat growing regions were identified purposively to represent the diverse socio-economic and biophysical environment of wheat producers in Ethiopia. Second, in the stakeholder consultation workshop in each region, six districts which are considered representative of the respective regions were selected based on wheat growing potential, and status of previous wheat research and development interventions. Then, three kebeles from each of the districts were selected based on their level of participation in SARD-SC wheat project. Finally, from the household list available at each kebele, a total of 946 sample households were randomly drawn for interview (Table 1).

Methods of data analysis and synthesis

Information and dataset collected were analyzed and synthesized using different statistical and econometric tools. Descriptive statistics were utilized to analyze the data and summarize the information. An econometric model was also employed for the analysis of wheat producers' technical efficiency and determinants of inefficiency.

Specification of the Stochastic Frontier Production and Translog Model

Based on Aigner et al. (1977), Battese and Corra (1977), Battese and Coelli (1995), Bravo-Ureta and Evenson (1994) and Meeusen and Broeck (1977), the Translog stochastic production function of wheat producing sample households aimed to analyze both the production frontier and determinants of inefficiency simultaneously can be specified as:

$$\ln Y_i = \beta_0 + \sum_{j=1}^{6} \beta_{jk} \ln X_{ij} + \sum_{j<k=1}^{6} \beta_{jk} \ln X_{ij} \ln X_{ik} + (V_i - U_i) \quad (1)$$

where ln = stands for the natural logarithm; Y_i = the amount of wheat production in Kg of the i^{th} plot of the farmer; i= stands for the i^{th} wheat plot of the sample household; $\beta_0,..,\beta_{jk}$ = vectors of unknown parameters to be estimated in the SFPF; X_{ij} = represents the j^{th} farm input variables used for wheat produced on i^{th} plot in such a way that: X_1= is the size of plot of land allocated for wheat in hectares;

X_2 =is the amount of expense in agrochemicals (inorganic fertilizer, herbicides, pesticide and fungicides) in Ethiopian Birr; X_3 =is the amount of wheat seed used (in kg) on the plot of land; X_4 =is oxen labor in oxen days (one oxen day=eight hours of ploughing); X_5 =is human labor in man days (one man day=eight working hours); X_6 =is the amount of expense in tractor hiring in Ethiopian Birr;

$\ln X_{ij} \ln X_{ik}$ are squares (second order) and interaction terms of the input variables; V_i = a symmetrical two sided random-error assumed to be independently and identically distributed with zero

Table 1. Distribution of sample sizes by IP districts and region.

Region	Zone	District of IP sites	Sample size
Tigray	South Tigray	Ofla	128
SNNP	Gurage	Gedebano-Gutazer-Wolene	100
Amhara	East Gojjam	Enemay	218
		Shebel Berenta	189
Oromia	Bale	Sinana	166
		Gololcha	145
Total			946

means N $(0, \sigma_v^2)$ associated with random factors beyond the control of the farmer (like: measurement errors, weather, diseases); U_i=the one-sided ($U_i \geq 0$) inefficiency component assumed to be independently distributed such that U_i follows a truncated (at zero) normal distribution N$\left(\mu_i, \sigma_u^2\right)$.

It is assumed that the two error components (U_i and V_i) are independent of each other.

The technical inefficiency effect, U_i is defined as:

$$U_i = \delta_0 + \sum_{j=1}^{15} \delta_j Z_{ij} + W_i \tag{2}$$

where $\delta_0, .. \delta_j$ are parameters to be estimated that assumed to affect inefficiency; Z_{ij} are factors determining the inefficiency of wheat producers and specified as: Z_1= is a dummy variable for sex of a household head (1=male and 0=female); Z_2 =is a continuous variable to represent the age of household head in completed years; Z_3 =is a continuous variable of education level of household head in completed school years; Z_4 =is a continuous variable stands for a labour force in man equivalent; Z_5 =is a dummy variable for ownership of at least a pair of oxen (1=Yes, 0=No); Z_6 =is a dummy variable for access to extension service (1=Yes, 0=No); Z_7 =is a dummy variable for access to credit (1=Yes, 0=No); Z_8 =is a continuous variable stands for the number of wheat plots operated by a household; Z_9 =is a dummy variable for the use of improved seed (1=Yes, 0=No); Z_{10} =is a continuous variable for an area allocated to wheat (in ha); Z_{11} =is plot distance from homestead of farmers (in minutes of walk); Z_{12} =is a dummy variable for fertility level of plot (1=fertile and 0=otherwise); Z_{13} =is a dummy variable for crop rotation practice (1=Yes 0=No); Z_{14} =is a dummy variable for soil and water conservation practice (1=Yes 0=No); Z_{15} =is a dummy variable for tractor use to plough wheat plot (1=Yes, 0=No); The frontier production function is estimated from a sample of observed yield of each farm that is operating at the best practice farm to indicate the maximum potential output for a given set of inputs, X_i which is expressed as:

$$Y^* = f(X_i; \beta).\exp(V_i) \tag{3}$$

Equation 3 is an output produced with full efficiency and each farm's performance is then compared with the estimated frontier. Estimating this frontier is served to estimate the level of technical efficiency (TE) of each observation that is given as:

$$TE = \frac{Y_i}{Y_i^*} = \frac{f(X_i; \beta).\exp(V_i - U_i)}{f(X_i; \beta).\exp(V_i)} = \exp(-U_i) \tag{4}$$

where exp ($-U_i$) takes values between zero and one and is inversely related to the level of the technical inefficiency effect. The parameters of the stochastic frontier production function (SFPF) model and determinants of inefficiency were estimated by the method of maximum likelihood.

For the model proposed in Equation 1, Battese and Corra (1977) proposed the log Likelihood (LL) function assuming that the distribution of technical inefficiency effect has a half normal. According to these authors, the LL function model can be specified as:

$$\ln \ell = -\frac{N}{2}\left(\ln\left(\frac{\pi}{2}\right) + \ln \sigma_s^2\right) + \sum_{i=1}^{N} \ln\left[1 - \Phi\left(\frac{\varepsilon_i \sqrt{\gamma}}{\sigma_s^2} \sqrt{\frac{\gamma}{1-\gamma}}\right)\right] - \frac{1}{2\sigma_s^2} \sum_{i=1}^{N} \varepsilon_i^2 \tag{5}$$

where $\varepsilon_i = \ln X_i \beta - \delta_k \ln Z_{ik}$ is the residual of Equation 1; N is the number of observations (number of wheat plots in our case); Φ(.) is the standard normal distribution; $\sigma_s^2 = \sigma_v^2 + \sigma_u^2$ are variance parameters; $\gamma = \sigma_u^2 / \sigma_s^2$ is a variance parameter having a value of zero to one and it measures the technical inefficiency.

Minimizing Equation 5 with respect to $\beta, \sigma_s^2, \delta$ and solving their partial derivation simultaneously results in the maximum likelihood estimates of $\beta, \sigma_s^2, \delta$. Moreover, γ parameter is used to test the existence of inefficiency which is expressed as the percentage of the variation in output due to technical inefficiency. Similarly, the generalized likelihood ratio test which is given as:

$$LR = -2[\ln(H_0)/L(H_1)] \sim \chi(n)^2 \tag{6}$$

is used to test whether the conventional average production function adequately represent the data or not, where: $L(H_0)$ is the likelihood value for the restricted estimate, $L(H_1)$ is the likelihood value for the unrestricted estimate, and n is the number of restrictions imposed by the null hypothesis.

RESULTS AND DISCUSSION

Descriptive analysis of wheat producers technical efficiency

Descriptive results of technical efficiency are shown in Table 2. The result shows that there is a variation in technical efficiency of wheat producers among the IP sites with a smallest and largest mean technical efficiency of 0.682 (68.2%) and 0.837 (83.7%) in Gurage zone of the SNNP IP site and Bale zone of Oromia IP sites, respectively. The overall technical efficiency of the whole sample is 76.9% implying that about 23% of the potential wheat yield is lost due to technical inefficiency in the study area. Within an IP sites, the minimum technical efficiency is as low as 9.7% (in Amhara IP site), while the maximum technical efficiency of the same is as high as 91.9%. Therefore, besides adopting improved wheat varieties, raising the technical efficiency of wheat producers can play a great role in increasing wheat yield. Improving technical efficiency of wheat producers is important to make them produce an optimum yield that can be produced with the current level of production technology and without additional input. This may be possible by arranging experience sharing and formal and informal education and improving access to better agricultural services on time.

Ranges of technical efficiency

Table 3 shows ranges of technical efficiency of wheat producing farmers in percent. The result shows that 25% of the overall sample falls in a technical efficiency range of more than or equal to 85% efficient, while 13% of the wheat producers fall in 50% or less technically efficient group. However, there is variation among the IP sites in terms of ranges of technical efficiency proportion. In SNNP IP site where the mean technical efficiency is the lowest (0.682), wheat producers whose technical efficiency is less than or equal to 50% is the highest (25%) as compared to other IP sites while the opposite is true in Oromia IP (4%) site where the mean technical efficiency is the highest (0.837). The level of technical efficiency is directly related to the average wheat productivity in the respective IP sites. Therefore, raising the proportion of farmers with high technical efficiency would have a great impact on increasing wheat production without incurring additional cost of production but adopting the practice of best performing farmers in the study area. This could be in effect through arranging frequent training and experience sharing mechanisms.

Yield gap due to technical inefficiency

The average yield gap between the potential and the actual yield is shown in Table 4. The result indicated that there is a maximum yield gap of 845 kg/ha in Tigray and a minimum yield gap of 527 kg/ha in Amhara with an average yield gap of 659 kg/ha for the overall sample households. The result implies that there is a large yield gap that could be captured by raising the level of technical efficiency of wheat producers in the study area. This in turn helps the country to be more self-sufficient in wheat from domestic production.

Econometric analysis of wheat producers' technical efficiency

The maximum likelihood estimates of the parameters of the stochastic frontier production and inefficiency model are estimated using Stata software computer program version 13. Before analyzing the parameter estimates and factors that determine the inefficiency of the wheat producers, the validity of the model used for the analysis was investigated using appropriate tests of hypotheses. The results of the tests of hypotheses are shown in Table 5. Testing of the hypotheses that the Translog SFPF can be reduced to Cobb-Douglas and the null hypothesis that technical inefficiency is not influenced by any variable included in the model were conducted using the log likelihood-ratio (LR) statistics using a formula: $LR = -2 \ln [L(H_0) / L(H_1)]$, where $L(H_0)$ and $L(H_1)$ represent the values of the log likelihood function under the specifications of the null and alternative hypotheses, respectively. The LR test statistic is given by an asymptotic chi-square distribution with degrees of freedom equal to the difference between the number of parameters in the unrestricted and restricted models. Another hypothesis that technical inefficiency effects are not in the model was tested using the t-statistic.

The LR test results indicated that the null hypothesis that states the Translog production function can be reduced to Cobb-Douglas (the null hypothesis that second order and the interaction terms in the Translog functions are not different from zero) was rejected at 5% level of significance. Hence, the Translog SFPF is more suitable to the wheat producers' survey data in the study areas (Table 5). Similarly, the null hypothesis states that there is no inefficiency (technical inefficiency effects are not in the model) among wheat producers ($\gamma=0$) was also rejected as ($\gamma=0.59$) was statistically significant at 5% level of significance implying that the existence of inefficiency in the model and hence the traditional average response function was not an adequate representation of the data. Another hypothesis that states there were no factors that contribute for technical inefficiency ($\delta_1= \delta_2= \delta_3=,\ldots= \delta_{15}= 0$) was also rejected at 5% level of significance implying that one or more variables jointly affect the technical inefficiency of wheat producers and hence important to include them in the inefficiency model.

Table 2. Descriptive result of technical efficiency of wheat producers in SARD-SC wheat project IP site in 2012/2013.

IP site	Observation	Mean	Std. Dev	Minimum	Maximum
Tigray	128	0.73	0.13	0.28	0.92
SNNP	100	0.68	0.22	0.12	0.97
Amhara	407	0.75	0.12	0.10	0.92
Oromia	311	0.84	0.12	0.14	0.95
Average	946	0.77	0.13	0.14	0.94

Table 3. Ranges of frequency of technical efficiency of wheat producers in SARD-SC project IP sites in 2012/2013 (% of farmers fall in different categories of technical efficiency).

IP site	<50% efficient	50%-74.9% efficient	75%-84.9% efficient	>=85% efficient
Tigray	6	41	39	13
SNNP	25	21	21	33
Amhara	5	31	47	18
Oromia	4	9	24	63
Overall	13	31	31	25

Table 4. Average yield gap due to technical inefficiency of wheat producers in SARD-SC IP sites in 2012/2013.

S/N	Variable	Tigray	SNNP	Amhara	Oromia	Overall
1	Actual yield (kg/ha)	2239	1560	1589	3175	2195
2	Mean technical efficiency	0.726	0.682	0.751	0.837	0.769
3	Potential yield (kg/ha) (1/2)	3084	2287	2116	3793	2854
4	Yield gap (kg/ha) (3-1)	845	727	527	618	659

Table 5. Result of hypotheses tests for some important assumptions.

Hypothesis	H_0	LR test or t-calculated	Critical value: χ^2 or t	Df	Decision
Testing the null hypothesis that the translog Stochastic Frontier Production Function (SFPF) can be reduced to a Cobb-Douglas SFPF	H0; $\beta ij = 0$	386.88	32.67	21	Reject H_0
The null hypothesis that technical inefficiency is not influenced by any variable included in the model	H0: $\delta 0=\delta 1=\ldots=\delta 15=0$	127.41	25	15	Reject H_0
The null hypothesis that technical inefficiency effects are not in the model (all farmers are efficient)	H0: $\gamma = 0$	19	6.31	1	Reject H_0

Parameter estimates

Table 6 shows the coefficients of the maximum likelihood estimates of the parameters in the Translog stochastic frontier for each IP sites of the wheat producers of the SARD-SC project. The result shows that different production inputs contribute to wheat yield in different IP sites. The coefficient of land (in hectares) was positive and significant at 5% in Oromia and not significant in all other IP sites. The result implies that increasing land allocated for wheat production would increase wheat output in Oromia. This result has been supported by the

Table 6. Coefficients of the maximum likelihood estimate of the Translog stochastic frontier production function by IP sites.

Variable	Tigray	Amhara	Oromia	SNNP	Overall
Constant	-3.430	6.706	3.214	19.087	3.849**
lnland	-6.434	2.412	0.210**	9.697	0.067
lnagrochemicals	-0.047	-0.029	0.428	2.585	-0.075
lnseed	0.314	-0.070	0.609	-1.245	0.368
lnoxen	7.399**	-1.095	0.337	-1.300	0.110
lnlabor	-0.531	0.414	-0.324	-9.519***	0.229
lntractor	-	-	-0.076	-	0.251***
lnlandsquare	-1.325*	0.469	0.058	1.614	0.001
lnagrochemsquare	0.014**	0.013***	0.006**	-0.091	0.004**
lnseedsquare	0.241	-0.044	0.007	0.043	-0.020
lnoxensquare	-1.027*	0.291	-0.013	0.517	0.022*
lnlaborsquare	0.200**	-0.070	0.010	0.087	0.062***
lntractorsquare	-	-	0.045***	-	0.042***
Lnland*lnAgchemical	0.005	-0.013	0.087**	0.521	-0.018
Lnland*lnseed	0.305	-0.077	-0.064	-0.151	0.032
Lnland*lnoxen	2.574**	-0.723	-0.012	-1.035	0.014
Lnland*lnlabor	-0.405	0.051	0.026	-2.417***	0.003
Lnland*lntractor	-	-	-0.019	-	0.026**
lnAgrochemical*lnseed	0.075**	0.012	-0.097**	-0.169	0.024*
lnAgrochemical*lnoxen	-0.014	0.007	0.011	-0.172	0.008
lnAgrochemical*lnlabor	-0.065***	-0.019	0.008	0.134	-0.014
lnAgrochemical*lntractor	-	-	0.005	-	-0.002
Lnseed*lnoxen	-0.461	-0.060	-0.054	-0.816	0.058
Lnseed*lnlabor	-0.341**	0.174	0.048	1.130***	-0.060
Lnseed*lntractor	-	-	-0.009	-	-0.050***
Lnoxen*lnlabor	0.258	-0.100	0.008	0.930	-0.067***
Lnoxen*lntractor	-	-	-0.022	-	-0.044***
Lnlabor*lntractor	-	-	0.006	-	0.011**
σ^2_u =	0.127571	0.099745	0.039335	0.196338	0.133405
$\sigma^2 = \sigma^2_v + \sigma^2_u$	0.219634	0.232344	0.104349	0.233956	0.224986
$\lambda = \sigma_u / \sigma^v$ =	1.177153	0.867314	0.777838	2.284577	1.206936
$\gamma = [\lambda^2 / (\lambda^2 + 1)]$	0.580834	0.4293	0.37696	0.83921	0.592949

*, ** and *** means significant at 10, 5 and 1% level of significance, respectively.

findings of Yami et al. (2013). This might be due to the fact that tractors are widely employed in Bale zone of the Oromia IP site and using tractors is efficient in larger plot size than smaller ones leading to amalgamating plots of land would have a positive effect on wheat output.

In Ofla district of the Tigray IP site, the coefficient of oxen (in oxen days) was found to increase wheat yield positively and significantly at 5%. However, the coefficient of the second order of oxen is negative and the level of significance falls from 5 to 10% although the coefficient of the second order of the overall sample is positive and significant. The result implies that increasing the oxen power would increase wheat yield up to some level and increasing oxen power beyond that level will decrease wheat productivity. The coefficient of the interaction between oxen and wheat land was also positive and significant showing that expanding wheat land as far as oxen are available to cultivate land would have a positive contribution to wheat yield in Tigray IP site. Therefore, ensuring the ownership of a pair of oxen in areas where cultivation is mainly operated by oxen would have a positive effect on wheat yield.

The coefficient of labor (in man days) was found to have an inverse relationship to wheat yield in SNNP IP site and not significant at other IP sites. Similarly, the interaction effect of labor with wheat land was also negative and significant at 1% in the same IP site. On the contrary, the interaction effect of labor with wheat seed was found to have a positive effect on wheat yield in SNNP IP site. In this IP site therefore, increasing labor force for wheat production and expanding wheat area without using appropriate wheat seed penalizes the farmers in wheat production.

The result of the estimate shows that the coefficient of the second order of labor in Tigray IP site as well as the overall sample (probably due to Tigray IP site) was positive and significant at 5% showing that increasing labor force in wheat production activities such as plowing, weeding, harvesting and threshing would increase wheat yield in the Tigray IP site. This is because almost all activities including weeding are conducted using human labor in Ofla district of Tigray IP site.

The overall sample result shows that the coefficient of expense on tractor/combine harvester and the coefficient of the second order of the overall sample as well as Oromia IP site were positive and significant at 1% each suggesting that an increase in investment in hiring more tractor and combine harvester for wheat production would increase the wheat output. This is mostly related to timely conducting land preparation and sowing at appropriate sowing date and harvesting at minimum post-harvest loss that otherwise reduce wheat output. Therefore, in areas where topography is suitable for tractor/combine harvester, increasing the access to such machines at appropriate time has to be ensured. One of the options to ensure this is by pursuing farmers' cooperatives to own and rent tractors, as it is difficult and costly to own tractors at individual level. Currently, the use of tractor and combine harvester is practiced only in Oromia IP site.

The coefficient of the second order of expense on agrochemical inputs (inorganic fertilizer, herbicides, pesticides and fungicides) was found to contribute positively and significantly to wheat yield in all IP sites except SNNP IP site as well as the overall sample. Therefore, higher investment in agrochemical inputs would contribute to wheat yield in Tigray, Amhara and Oromia IP sites of the SARD-SC wheat project.

The estimation result shows that the coefficient of the second order of expense on hiring tractor and combine harvester was found to contribute positively and significantly to wheat yield in Oromia IP site. Therefore, there is a need of improving access to machinery to increase wheat yield in Bale zone. Similarly, the coefficient of interaction between wheat area and expenses on tractor/combine harvester was positive and significant at 5% level of significance indicating that expansion of wheat land as far as tractor is available would increase wheat production. This is an expected result as these two inputs have a complementary relationship. The result therefore implies that, by keeping other factors constant, increasing both land allocated for wheat and expenses to hire tractor increases the wheat yield. Hence, ensuring the sustainable access to tractors is again important for increased wheat production, especially in areas where suitable for cultivation by tractor.

The coefficient of the interaction between wheat area and expense on agrochemical costs on wheat plots was significant and positive in Bale zone of the Oromia IP site implying that expanding wheat area needs to simultaneously invest in agrochemicals (fertilizer and crop protection chemicals) to increase wheat yield in wheat belt areas such as in Bale zone.

Another important result of the estimate is that the coefficient of interaction between agro-chemical costs (cost of inorganic fertilizer and crop protection chemicals like herbicide, pesticide and fungicide) and wheat seed was positive in both Tigray IP site and the overall sample and statistically significant at 5 and 10%, respectively while it was negative and significant at 5% in the Oromia IP site. The positive result is expected as these two inputs are usually recommended as a package during variety release and hence have a complementary nature. That is, inorganic fertilizer is a key input in wheat production by providing important nutrients required for crop production. Similarly, crop protection agrochemicals such as herbicides are very important to control weeds on time while pesticides and fungicides are important to control diseases. The result further implies that, other factors held constant, an increase in both wheat seed and investment in agro-chemical expenses, increases the wheat productivity in the study area. However, it should be noted that application of these agrochemicals may not increase wheat productivity beyond some limit. Nevertheless, the contrary result in Oromia IP site may be due to the fact that farmers of Oromia IP site are using high seed rates and using additional seed rate would likely penalize farmers in reducing wheat yield.

The coefficients of the interaction between expenses on agrochemical inputs (fertilizer in the case of Ofla district) and labor and interaction between quantity of wheat seed and labor were both negative and significant at 1 and 5%, respectively in Ofla district of the Tigray IP site showing that there is no apparent substitution nature between these inputs.

The result of the maximum likelihood estimates demonstrated that the coefficient of the interaction between wheat seed and expenses on tractor/combine harvester was significant at 1% level of significance but negative in sign in the overall sample. This might be due to the fact that tractors are used only in Bale zones where average seed rate is about 185 kg/ha and this is higher than the recommended rate for most of the wheat varieties released so far, and using seed beyond this maximum level would penalize the yield even if tractors are employed for wheat production. The result hence implies that increasing seed rate of wheat plots cultivated by tractor would result in decrease in wheat yield in these areas, which means double penalty by incurring additional unnecessary cost of wheat seed and loss of wheat yield due to low performance in plant stand caused by inappropriate seed rate. Therefore, there should be some sort of awareness creation on appropriate seed rates.

Another interesting result of the estimate is that the coefficient of interaction between oxen labor (in oxen days) and human labor (in man days) was significant at 1% level of significance carrying negative sign in the overall sample. This is due to the fact that there is a fixed proportion relationship (one person to a pair of oxen) for land cultivation and some additional labor for planting and fertilizer application only during the planting time. A combination of these two inputs below or above the optimum would result in lower labor productivity and hence lower yield per hectare.

The coefficient of interaction between oxen labor (in oxen days) and expenses on tractor/combine harvester was significant at 1% level of significance but with negative sign in the overall sample. The result implies that using oxen labor in combination with tractor is not economical in wheat production in places where there is access for tractors for wheat production. That is, using tractor increases wheat productivity as stated earlier when used solely but the yield decreases if tractor is used in combination with oxen. It is a usual practice of Bale zone wheat producers that using tractor for the first time and using oxen for the second and third time to cultivate (level) their lands. However, this result suggests that using tractor is more advisable than oxen in these areas.

Finally, the results of the estimation shows that the coefficient of interaction between human labor (in man days) and tractor was positive and significant at 5% level of significance for the overall sample data implying the supplementary relationship between these two inputs. This is true especially as human labor is used where tractors cannot be used for some activities such as weeding that are usually implemented by human labor.

The value of gamma (γ) for each region of the SARD-SC wheat IP site was statistically significant at 1 to 5% level of significance and varies from region to region with a lowest and highest values at Oromia (0.38) and SNNP(0.84), while the value of the overall sample is 0.59. The value of gamma of each region imply that about 58, 43, 38, 84 and 59% of the yield variation from optimum production frontier in wheat production was due to farm specific technical inefficiency in Tigray, Amhara, Oromia, SNNP IP sites and for the overall sample, respectively while the rest 42, 57, 62, 16 and 41% of the respective IP sites and the overall sample result was due to external factors that are out of farmers' control that include diseases and other environmental factors (such as erratic rainfall) or due to measurement errors. The low value of the gamma in Oromia IP site implies that more of the yield variation was due to external factor (diseases outbreak observed in the area during the study period). The result therefore, suggests that there is a room to increase wheat productivity by improving the technical efficiency of wheat producers without adding any additional input in the study area by expanding the best practices of well-performing farmers using experience sharing and training mechanisms.

Determinants of technical inefficiency of wheat producers

Table 7 shows the coefficients of estimates of factors determine technical inefficiency of wheat producers in the study area. As expected, the overall sample result shows that education level of the household head (in number of grade completed) significantly influenced wheat producers technical inefficiency negatively at 1% of significance level implying that education reduces inefficiency or improves efficiency of wheat producers. Therefore, arranging education opportunities like adult education would improve technical efficiency of farmers. Previous findings (Ahmad et al., 2002; Hassan and Ahmad, 2005; Kaur et al., 2010; Sekhon et al., 2010; Wassie, 2014; Yami et al., 2013, Wudineh and Endrias, 2016) are in line with this finding.

The result of the overall sample and the Oromia IP site indicates that labor (in man days) found to affect wheat producers technical inefficiency positively and significantly at 10% level of significance each indicating that the amount of labor force beyond the required level decreases labor productivity and hence wheat output per labor force. This result has been supported by the findings of Kaur et al. (2010).

Another important result is that ownership of at least a pair of oxen significantly and negatively influenced technical inefficiency of wheat producers at 1% level of significance for the overall sample. This is an expected result especially in areas where most of farmers depend on oxen and the ownership of a pair of oxen enables farmers to plough their plot timely and at the recommended sowing date. In most areas, oxen are used for wheat production not only for ploughing but also for threshing. Therefore, policies that enable farmers to own such kind of productive assets using different methods such as arranging oxen purchase credit would have a positive effect in reducing farmers' inefficiency or enhancing their efficiency so that they can produce wheat at a maximum possible frontier using the same level of resource that they are currently using.

Access to credit in Tigray IP site was found to have a significant and negative effect on technical inefficiency or positively contributes to farmers' efficiency of wheat production as expected. The explanation for this result is that availability of credit enables farmers to purchase inputs and plant wheat within appropriate sowing dates that can positively contributes to wheat yield. This result is consistent with the finding of Ahmad et al. (2002).

As expected adoption of improved wheat variety was found to improve the efficiency of wheat producers in Ofla district of the Tigray IP site. This result has been supported by the finding of Wassie (2014). However, contrary to our expectation, adoption of improved seed negatively contributes to farmers efficiency in Amhara and SNNP region IP sites. One possible reason for the contradiction in Amhara and SNNP IP sites is that

Table 7. Coefficients for factors affecting technical inefficiency of wheat producers in SARD-SC study area disaggregated by IP sites.

Variable	Tigray	Amhara	Oromia	SNNP	Overall
Constant	-0.165	-3.309***	-2.931**	-16.485	-2.545***
Sex of head (1=Male; 0=Female)	-0.602	0.497	-0.984	6.683	-0.197
Age of HH head in years	-0.017	0.007	0.011	0.033	0.005
Education of HH head	-0.087	-0.008	-0.045	0.010	-0.072***
Labor in man equivalent	0.213	-0.051	0.175*	-0.034	0.095*
pair of or more oxen (1=Yes, 0=No)	0.089	-0.130	-0.051	0.186	-0.441***
Access to extension (1=Yes, 0=No)	-0.860	0.405	0.382	4.848	0.273
Access to credit (1=Yes, 0=No)	-0.778*	-0.040	0.182	-0.242	-0.106
Number of wheat plots	0.048	-0.069	0.018	0.078	0.047
improved seed (1=Yes, 0=No)	-0.824**	0.449*	0.383	1.971***	0.052
Area allocated to wheat (ha)	3.576	0.093	-0.322	1.433	0.312
Plot distance (minutes of walk)	0.003	0.011**	0.014***	-0.017	0.007***
Soil fertility level (1=fertile; 0=not)	-0.479	0.061	-0.885***	0.005	-0.188
Crop rotation (1=Yes and 0=No)	0.291	0.159	0.476*	-0.623	0.403***
SWC (1=Yes and 0=No)	-0.044	0.062	-	0.428	0.086
Tractor used (1=Yes , 0=No)	-	-	-1.70***	-	-1.761***
Number of observations	331	559	617	109	1616
Wald chi2	543.89	420.99	1665.04	294.30	6460.49
Prob > chi2	0.0000	0.0000	0.0000	0.0000	0.0000
Log likelihood	-197.96	-372.855	-170.845	-54.7644	-981.541

*, ** and *** means significant at 10, 5 and 1% level of significance, respectively. SWC: Soil and water conservation practice.

adoption of improved wheat varieties is less in both IP sites as compared to other IP sites and farmers may not be aware of some important agronomic practices such as sowing dates of newly introduced wheat varieties.

The result also demonstrates that plot distance (in walking minutes from homestead of the wheat producers) significantly and positively influenced the technical inefficiency of wheat producers of the overall sample, Amhara and Oromia IP sites at 1, 5 and 1%, respectively, indicating that nearer plots can be efficiently operated as compared to farther plots. This is an expected result as far plots require additional time to travel before actually starting the operation. The finding of Ahmad et al. (2002) is also consistent with this result.

The estimation result indicates that planting wheat on fertile soils was found to significantly improve the technical efficiency (reduces technical inefficiency) of wheat producers in Bale zone of Oromia IP site. This is an expected result as fertile soil rewards more to farmers and initiated to perform farm activities with a great motive.

Another variable that significantly influenced the technical inefficiency of wheat producers is the crop rotation practice both for the overall sample and for the Oromia IP site. The result shows that a plot on which crop rotation was practiced was positively related to technical inefficiency of wheat producers in the study area. This might be because not only crop rotation but also appropriate (usually pulses) crop rotation is more important for farmers to be technically efficient in wheat production. Therefore, besides availing improved wheat variety choice, providing appropriate alternative crop used for crop rotation would contribute to wheat production efficiency in the study area.

As expected, using tractors for cultivating wheat plots and combine harvester for harvesting and threshing influenced the technical inefficiency of wheat producers negatively and significantly at 1% level of significance in Oromia IP site (also for the overall sample due to Oromia IP site). Using tractors for land preparation and combine harvesters for harvesting and threshing enables to perform agricultural activities in a timely manner and hence increase technical efficiency of wheat producers. Therefore, arranging for easy access to tractors where there is no problem of land topography for tractors would have a positive effect on improving wheat farmers' technical efficiency and hence increases wheat yield in the study area.

Conclusion

The results indicated that the technical efficiency of wheat producers is about 77% for the overall sample but varies from IP site to IP site. The result shows that the average yield gap due to technical inefficiency of farmers ranges from 527 kg/ha (in Amhara IP site) to 845 kg/ha (in Tigray IP site) with a yield gap of 659 kg/ha for the overall sample indicating domestic wheat production could be significantly increased only by improving farmers practices with the current amount of resource they are using. The Gamma (γ) value of the overall sample is 0.59 implying 59% of the variation in wheat yield is due to the existence of technical inefficiency in the system while the rest 41% is due to the external factors out of control of the farmers.

The results indicate that expanding land would result in increasing wheat yield in Oromia but decreasing yield in Tigray in its second order. Similarly, using more oxen labor would increase wheat yield to some limit in Tigray and then decreases. However, in its second order, the yield increment continues for the overall sample. Using more labor alone as well as with an interaction with land would lead to decrease in wheat yield in SNNP while it resulted in continuously increasing wheat yield in Tigray and in the overall sample. Additional expenses on tractor use in the overall sample and its second order increases wheat yield for the overall sample and both for Oromia and overall sample, respectively while its interaction effect with land also increases wheat yield in the overall sample. The result also indicates that the coefficient of the second order of agrochemical cost increases yield in all IP sites as well as in the overall sample except SNNP IP site where it is not significant. The result shows that the interaction effect of land and agrochemicals (fertilizer, crop protection chemicals); and oxen would result in increasing wheat yield in Oromia and Tigray IP sites, respectively while the interaction effect between agrochemicals and seed increases yield in Tigray as well as in the overall sample but decreases wheat yield in Oromia. The interaction effect between agrochemicals and labor; and seed and labor decrease wheat yield in Tigray while the interaction effect between seed and labor decreases yield in SNNP. The interaction effects between seed and tractor, oxen and human labor, oxen and tractor all found to decrease wheat yield while the interaction effect between labor and tractor increases yield of the overall sample.

As a factor of inefficiency, result indicates, education, oxen ownership, credit, soil fertility, using tractor, and using improved seed (in Tigray) were found to improve technical efficiency of wheat producers either for the overall or for some regions. On the other hand, family labor (in man equivalent) negatively affect efficiency in Oromia and overall sample using improved seed (in Amhara and SNNP), plot distance and crop rotation (Oromia) were found to negatively affect technical efficiency.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Direct shoot regeneration from hypocotyl explants of *Heracleum candicans* Wall: A vulnerable high value medicinal herb of Kashmir Himalaya

Mahroofa Jan[1*], Seema Singh[1], Farhana Maqbool[1] and Irshad Ahmad Nawchoo[2]

[1]Plant Tissue Culture Research Laboratory Department of Botany, University of Kashmir Hazratbal, Srinagar, 190006 J&K India.
[2]Plant Reproductive Biology, Genetic Diversity and Phytochemistry Research Laboratory Department of Botany, University of Kashmir, Hazratbal, Srinagar, 190006, India.

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Heracleum candicans **belongs to the family Apiaceae, and is categorized as a vulnerable Himalayan medicinal herb. Due to its diverse chemical constituents it is having an increased demand in pharmaceutical industries, especially in international market. This herb is commercially useful as a major source of Xanthotoxin which is widely used to treat leucoderma and to prepare suntan lotions. During the present study direct shoot regeneration has been achieved from hypocotyl explants on MS (Murashige and Skoog, 1962) medium fortified with different plant growth hormones. Shoot bud regeneration was achieved on media augmented with auxins like Indoleacetic acid (IAA), 2,4 -dichlorophenoxyacetic acid (2,4-D) and cytokinins like Kinetin (KN) and Benzyladenine (BA). Due to the presence of 2,4-D in the medium friable callus development has been reported in some cultures. Regenerated shoots were transferred to MS basal medium for root induction and later on successfully acclimatized in vermiculite under controlled conditions. This is the first report on plant regeneration from hypocotyl explants of** ***H. candicans*** **and could be used as an alternative for large scale propagation and conservation of this vulnerable plant species.**

Key words: Apiaceae, explants, callus, auxins, cytokinins, MS medium.

INTRODUCTION

Heracleum candicans Wall. Commonly known as Patrala, is a perennial herb endemic to the northwest Himalayas. It is found distributed in mountains and alpine zones of Bhutan, Afghanistan, south-west China, West Pakistan, Nepal and India at an altitude of 2000 to 4300 m asl. It is one amongst the rare Himalayan resources and the most valued species of genus *Heracleum* and produces optimum quantity of Xanthotoxin (Kaul, 1989). Almost each and every part of this plant has the ability to cure various diseases and its medicinal efficacy is well recognized by indigenous communities as well as modern systems of medicine (Rawat et al., 2013). In Himalayan region, its juvenile shoots and leaves are eaten mostly by shepherds, and also used as fodder for

*Corresponding author. E-mail: mahroofa1989@gmail.com.

increasing the milk production of cows (Badola and Butola, 2005). The native people use its roots for treating various skin diseases like eczema and itches (Gaur, 1999). The roots contain 0.1% essential oil and bergapten. Its roots also yield xanthotoxin which is used to treat leucoderma and in preparation of suntan lotions (Kaul, 1989). Its fruits are used as an aphrodisiac and nerve tonic (Satyavati et al., 1987). Activity-guided isolation has also shown heraclenin to be the anti-inflammatory principle present in *H. candicans.* The plant possesses potent stimulatory effect on melanogenesis with significant enhancement of cell proliferation (Matsuda et al., 2005). Extracts of root and shoot showed antibacterial activity (Kaur et al., 2005). Twenty-eight compounds were isolated and identified from essential oil using gas chromatography–flame ionization detection and GC–mass spectrometry (GC–MS) analysis (Chauhan et al., 2014). These compounds are very useful for the pharmaceutical, flavor and fragrance industries. This plant species propagates by seed, but seed viability is very low (Butola and Badola, 2004). This poor seed germination together with overharvesting of plants from natural habitats for commercial utilization puts this plant species under severe threat. Owing to its unsustainable harvesting in nature, this plant species is categorized as endangered for northwestern Himalayas (Anonymous, 1998; CAMP, 2003), and vulnerable for the state of Himachal Pradesh and Jammu and Kashmir (Ved et al., 2003). Only a few reports are available on *in vitro* plant regeneration from leaf, petiole and shoot tip explants of *H. candicans* (Xing, 2006; Sharma and Wakhlu, 2001; Sharma and Wakhlu, 2003) and to date, there are no reports on plant regeneration from hypocotyl explants of *H. candicans.* This study describes a rapid protocol for direct shoot regeneration from hypocotyl explants of *H. candicans.*

MATERIALS AND METHODS

Raw material

Seeds were obtained from the plants growing in Kashmir university botanical garden and washed under running tap water for about 30 min. Detergent Labolene 1% v/v (LOBA Chemie- Laboratory reagents and fine chemicals) containing few drops of surfactant, Tween 20 (Hi Media) were then added to the seeds for washing. This was followed by washing with tap water to remove the detergent and finally washed with double distilled water under laminar air flow cabinet. Finally the seeds were sterilised with 2% sodium hypochlorite solution (Hi Media) for 30 min. After 30 min disinfectant solution was decanted and the seeds were washed 5-6 times with double distilled water so as to remove any traces of the sterilant. The sterilized seeds were then transferred on to petriplates filled with sterilized cotton.

Implementation of plant propagation protocol

Hypocotyl explants were taken from seedlings and cultured on MS medium (Hi Media) containing auxins, cytokinins and auxin-cytokinin combinations. The cultures were maintained at a temperature of 25±2°C, light intensity of 3000 lux and a regular photoperiod of 16 h. Each experiment was repeated at least twice and data was analysed by calculating Standard Error (SE) of various treatments and means were analyzed by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The hypocotyl explants inoculated on MS medium without added growth hormones showed no response for shoot bud formation. However an interplay of auxin and cytokinin individually and in combinations showed enhanced shoot bud proliferation. Adventitious shoot buds formed from hypocotyl explants directly within 15-30 days without an intervening callus phase. Among auxins 2,4-D showed shoot bud proliferation with best response obtained on 2,4-D 3 mg/l (Figure 1). After 2,4-D 3 mg/l the number of shoots per explant declined (Table 1). Among cytokinins, BAP was more effective as compared to Kn in shoot bud induction (Table 2). The best response, however, was obtained at BAP 3 mg/l (Figure 2a) where an average of 4.5 shoots formed per explant within 20 days of inoculation onto the medium. Number of shoots formed per explant declined after BAP 3 mg/l. When explants were inoculated on MS medium augmented with Kn, there occurs a gradual increase in the number of shoots upto 4 mg/l (Figure 2b) which is directly proportional to increasing Kn concentration. After 4 mg/l there occurs a decline in the number of shoots per explant. Among the auxin and cytokinin combinations used, BAP 3 mg/l and IAA 2 mg/l (Figure 3) gave best results with an average of 9.4 shoots per explant, being much higher than that obtained when BAP, Kn and 2,4-D were used individually (Table 3). Roots were obtained when regenerated shoots were transferred to MS basal medium within a period of 10 days (Figure 4a). Rooted plantlets were transferred to pots containing vermiculite and were successfully acclimatized under green house (Figure 4b).

In vitro regeneration of plants is influenced by many factors such as environment around cultures, media composition, source of explant, plant growth hormones and genotype (Zhang et al., 1998; Bano et al., 2010; Jana and Shekhawat, 2011; Dhir and Shekhawat, 2014). The results obtained in the present study indicate clearly that high frequency direct shoot regeneration is achieved from hypocotyl explants of *H. candicans* on MS medium supplemented with BAP 3 mg/l + IAA 2 mg/l. Similar results were obtained in *Cuminum cyminum* L. were hypocotyl was also reported as the most responsive explant in terms of shoot organogenesis (Tawfik and Noga, 2002). Hypocotyl explant was also used for shoot proliferation in *Ferula assa-foetida* L. on MS medium supplemented with auxin cytokinin combinations (Zare et al., 2010). It has also been reported that in *Dorema ammoniacum* D. Don. BAP alone or a combination of

Figure 1. Shoot regeneration: MS+2,4-D 3 mg/l.

Table 1. Effect of Auxins on multiple shoot formation from hypocotyl explant.

Treatments	Mean number of shoots±SE	Mean height of shoots(cm)±SE	Mean Number of Days	% Culture response
MS basal	-	-	-	-
2,4-D 1 mg/l	1.0±0.2^{a}	1.9±0.1^{a}	32	30
2,4-D 2 mg/l	1.9±0.3^{a}	1.8±0.2^{a}	29	50
2,4-D 3 mg/l	3.2±0.2^{b}	2.9±0.2^{b}	23	60
2,4-D 4 mg/l	1.7±0.2^{a}	1.8±0.1^{a}	27	40
2,4-D 5 mg/l	1.6±0.2^{a}	1.4±0.1^{a}	30	30

(10 replicates per treatment).*Means followed by different superscripts are significantly different from each other at 5% level.

Table 2. Effect of Cytokinins on multiple shoot formation from hypocotyl explant.

Treatments	Mean number of shoots±SE	Mean height of shoots(cm)±SE	Mean Number of Days	% Culture response
MS basal	-	-	-	-
BAP 1 mg/l	-	-	-	-
BAP 2 mg/l	2.6±0.3^{ab}	2.1±0.1^{a}	28	70
BAP 3 mg/l	4.5±0.3^{c}	4.1±0.1^{b}	20	100
BAP 4 mg/l	2.9±0.2^{b}	2.5±0.3^{a}	29	80
BAP 5 mg/l	1.7±0.2^{a}	2.3±0.1^{a}	30	40
Kn 1 mg/l	1.7 ±0.2^{a}	2.5±0.1^{a}	32	60
Kn 2 mg/l	1.9±0.2^{a}	2.8±0.2^{a}	28	70
Kn 3 mg/l	2.8±0.4^{a}	2.9±0.2^{a}	26	80
Kn 4 mg/l	4.2±0.4^{b}	4.1±0.3^{b}	23	90
Kn 5 mg/l	2.5±0.4^{a}	2.8±0.3^{a}	29	40

(10 replicates per treatment), *Means followed by different superscripts are significantly different from each other at 5% level.

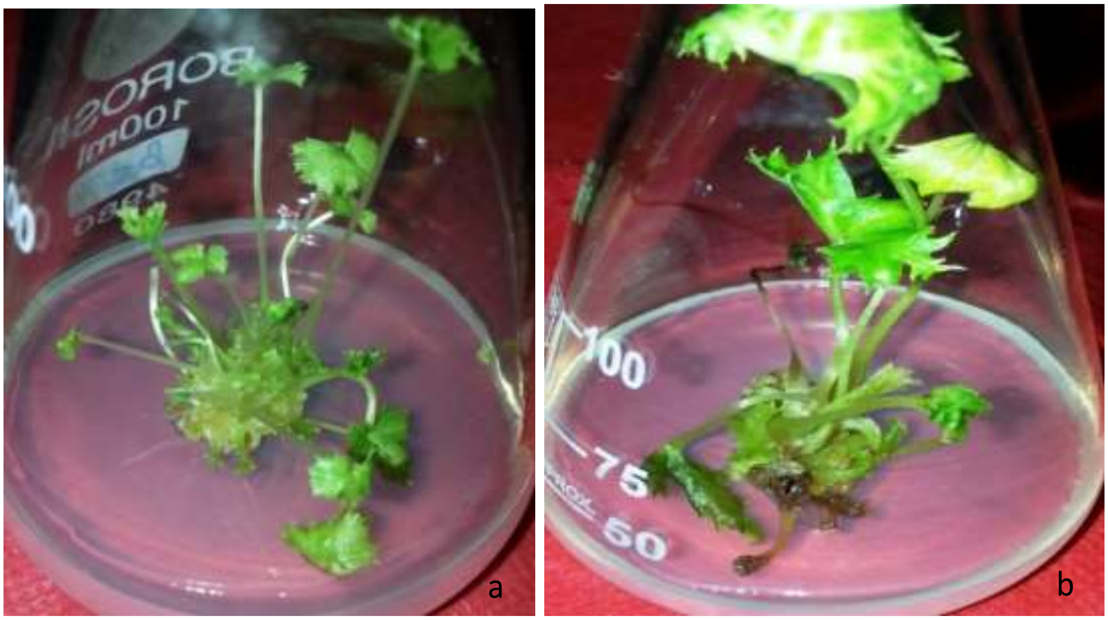

Figure 2. Shoot regeneration: a) MS+ BAP 3 mg/l, b) MS + Kinetin 4 mg/l.

Figure 3. Shoot regeneration: MS + BAP 3 mg/l + IAA 1 mg/l.

auxins proved effective for shoot regeneration (Irvani et al., 2010). Direct adventitious shoot bud formation from hypocotyl explant was also obtained in *Millettia pinnata* (L.) on MS medium supplemented with 8.88 µM BAP (Nagar et al., 2017). Results obtained for rooting were in accordance with the results obtained in *Daucus carota* where in the regenerated shoots showed best root regeneration on MS medium without any added growth

Table 3. Effect of Auxin cytokinin combinations on multiple shoot formation from hypocotyl explant.

Treatments	Mean number of shoots±SE	Mean height of shoots(cm)±SE	Mean Number of Days	% Culture response
MS basal	-	-	-	-
BAP 3 mg/l + IAA 1 mg/l	6.9 ± 0.5^{a}	2.9 ± 0.3^{a}	22	70
BAP 3 mg/l + IAA 2 mg/l	9.4 ± 0.8^{b}	5.0 ± 0.4^{b}	15	100
BAP 3 mg/l + IAA 3 mg/l	6.7 ± 0.4^{a}	2.5 ± 0.2^{a}	25	40

(10 replicates per treatment), *Means followed by different superscripts are significantly different from each other at 5% level.

(a) (b)

Figure 4. (a) Root regeneration, (b) Acclimatization of regenerated shoots.

regulators.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Use of millimeter ruler as an alternative tool in the phenotyping of potential descriptors of soybean

Ronaldo Machado Junior[1*], Guilherme Ferreira Alves[2], Victor Afonso Reis Gonçalves[1], Sylas Clemente Oliveira[2], Ronaldo Silva Gomes[3], Paulo Roberto Cecon[4], Silvana da Costa Ferreira[5] and Eder Matsuo[6]

[1]Departament of Genetic and Breeding, Federal University of Viçosa, Viçosa-MG, Brazil.
[2]Institute of Plant Science, Federal University of Viçosa -Rio Paranaíba, Rio Paranaíba-MG, Brazil.
[3]Departament of Plant Science, Federal University of Viçosa, Viçosa-MG, Brazil.
[4]Departament of Estatistics, Federal University of Viçosa, Viçosa-MG, Brazil.
[5]Institute of Biological Sciences and Health, Federal University of Viçosa - Rio Paranaíba, Rio Paranaíba-MG, Brazil.
[6]Institute of Exact Sciences and Technological, Federal University of Viçosa - Rio Paranaíba, Rio Paranaíba-MG, Brazil.

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The soybean stands out in the Brazilian agribusiness and part of this success stems from the development of improved cultivars. One of the requirements for the concession of protection to a given cultivar is that it should be distinct from others cultivars. Potential additional descriptors of soybean have been studied. Thus, the aim of this study was to evaluate the performance of the millimeter ruler in the measurement of the lengths of hypocotyl and epicotyl in seedlings of soybean and to compare the methods based on the use of millimeter ruler and the method based on the use of caliper. The hypocotyl and epicotyl of 1.084,0 plants in the stage V2 and 1.252,0 plants in the stage V3 were measured by digital caliper and millimeter ruler in 2014 and 2015. The plants were kept in a greenhouse. The linear regression ($Y = \beta X$), bias, concordance index, performance index, sample standard deviation and confidence interval were used to evaluate the performance. The millimeter ruler overestimated the length of epicotyl and hypocotyl by 0.0508 and 0.0147 mm, respectively. The millimeter ruler had an optimum performance in the measurement of the lengths of hypocotyl and epicotyl in seedlings of soybean in V2 and V3. A millimeter ruler is an alternative tool in the measurement of seedlings of soybean in most of the developmental stages.

Key words: Epicotyl, *Glycine max,* hypocotyl, additional descriptors.

INTRODUCTION

Soybean (Glycine max (L.) Merr.) stands out as one of the most important crops in the Brazilian agribusiness. The production of this crop in the 2015/2016 harvest was estimated at 101.18 million tons, which corresponded to an increase of 5.1% as compared to the previous harvest (CONAB, 2016). Part of this success is due to the

*Corresponding author. E-mail: ronaldo.juniior@ufv.br

programs of genetic breeding kept by different institutions of research and universities in Brazil (Oda et al., 2015).

Three basic requirements must be met for granting protection to a cultivar: the cultivar must be distinct, homogeneous and stable (Viana, 2013). According to Nogueira et al. (2008), the 38 descriptors, including the additionals descriptors used to differentiate cultivars of soybean are insufficient, which points out to the need of broadening the list of these descriptors.

According to Nogueira et al. (2008), there is genetic variability for the lengths of hypocotyl and epicotyl in the germplasm of soybean. Studies subsequent to this were carried out in order to estimate the genetic variability of the lengths of hypocotyl and epicotyl and for a better understanding of these characteristics (Matsuo et al., 2012). These authors used a digital caliper to measure the lengths of hypocotyl and epicotyl in seedlings of soybean. This device has mobile parts and the reading is presented digitally. With this, the use of this device demands a training and precaution in its handling, besides to require a source of electric power such as batteries.

In this context, it is important to study the possibility of using instruments alternative to calipers in the measurement of soybean seedlings, however, with efficiency similar to that offered by digital calipers. The millimeter ruler is a tool of easy handling that does not require a source of energy and has a cost much inferior as compared to calipers. Thus, the aim of this study was to evaluate the performance of millimeter ruler and caliper in the measurement of the lengths of hypocotyl and epicotyl in plants of soybean and to compare the two methods.

MATERIALS AND METHODS

The study was conducted in a greenhouse at the Federal University of Viçosa, Campus of Rio Paranaíba, in 2014 and 2015. The seeds used had random sizes and came from different cultivars of soybean. The seeds were sown at a standardized depth of 3.0 cm in a soil previously prepared and were kept in pots of 3 dm^3. The cultural practices were carried out according to the recommendations.

The plants were evaluated as to the lengths of hypocotyl and epicotyl, by digital caliper and millimeter ruler at the developmental stages V2 and V3 (Fehr and Caviness, 1977). A total of 1.084,0 plants were evaluated at the V2 stage and 1.252,0 at V3 stage, distributed over five planting seasons (June/2014, November/2014, January/2015, March/2015 and April/2015).

The two methods of evaluation were compared in order to assess their performance and agreement. This comparison was performed using the software GENES (Cruz, 2013). For this, the following reliability measures were adopted:

(i) Angular coefficient (β) and coefficient of determination (r^2) of the simple linear regression (model: Y = βX).

where Y is the value obtained with a caliper, β is the angular coefficient and X corresponds to the value obtained with a millimeter ruler. The value of β was tested using the t-test at α≤0.05, considering the hypotheses $H_0: \beta = 1$ and $H_a: \beta \neq 1$.

(ii) Bias (bias $= \beta - 1$), when β was significant by the t-test (α≤0.05).

(iii) Concordance index (d) proposed by Willmott et al. (1985).

where $d = 1 - \frac{\sum_{i=1}^{n}(X_i - Y_i)^2}{\sum_{i=1}^{n}(|Y_i - \overline{X}| + |X_i - \overline{X}|)^2}$ and X_i is the value obtained with a millimeter ruler, Y_i is the value obtained with a caliper and $\overline{X}$ corresponds to the average of values obtained by measuring with a ruler.

(iv) Performance index (c) proposed by Camargo and Sentelhas (1997), where $c = \sqrt{r^2} * d$; and

(v) Average, sample standard deviation and confidence interval (with 95% of confidence), separately for digital caliper and millimeter ruler, for the lengths of hypocotyl and epicotyl.

RESULTS AND DISCUSSION

Table 1 shows the coefficient of angular regression and the coefficient of determination, the bias, the index of concordance and the index of performance, obtained from the comparison between the results from caliper and ruler.

The estimates of the angular coefficient of regression for the length of hypocotyl at stages V2 (January/2014) and V3 (January/2014) and for the length of epicotyl at stages V2 (June/2014 and November/2014) and V3 (November/2014 and January/2015) had a β non-significant by the t-test (α>0.05). This indicates that the trend line of the ruler follows the values obtained from caliper in a ratio 1:1. β was significant (α≤0.05) for the other planting seasons indicating that the value of the estimate is different from 1. The estimates of determination coefficient were higher than 0.97, which indicates that the models estimated explained well the variables.

The bias for the length of hypocotyl was positive in the evaluations performed at V2 and V3 stages in four planting seasons (June/2014, November/2014, March/2015, and April/2015). This indicates that the millimeter ruler overestimated the values as compared to the measures obtained from the caliper. The maximum overestimation (0.0508) was observed in March/2015 in stage V2. A positive value of bias was observed for the length of epicotyl in stages V2 (January/2015 and March/2015) and V3 (June/2014 and March/2015). The millimeter also overestimated the values as compared to the digital caliper. However, the highest overestimation found had a low magnitude (0.0147 mm), corresponding to the planting season in March/2015 and to the V3 stage. The negative bias (-0.0074) observed in the stage V2 (April/2015) indicates that the millimeter ruler is underestimating the measure as compared to the digital

Table 1. Values of the regression angular coefficient[1] (β), determination coefficient (r^2), concordance index (d) and performance index[2] (c), for the comparisons between measures of hypocotyl and epicotyl from seedlings of soybean obtained with caliper and millimeter ruler in different stages of development and seasons in a greenhouse.

Season	*β*	*r^2*	*Bies*	*d*	*c*
			Hypocotyl length		
Developmental stage V2					
Jun./2014	1.0250**	0.99	0.0250	0.9997	0.95 - Optmal
Nov./2014	1.0170*	0.98	0.0170	0.9994	0.93 - Optmal
Jan./2015	0.9893^{ns}	0.98	-	0.9993	0.90 - Optmal
Mar./2015	1.0508**	0.99	0.0508	0.9991	0.96 - Optmal
Apr./2015	1.0151**	0.99	0.0151	0.9998	0.95 - Optmal
Developmental stage V3					
Jun./2014	1.0283**	0.98	0.0283	0.9994	0.91 - Optmal
Nov./2014	1.0326**	0.99	0.0326	0.9996	0.95 - Optmal
Jan./2015	1.0017^{ns}	0.99	-	0.9991	0.94 - Optmal
Mar./2015	1.0363**	0.99	0.0363	0.9996	0.97 - Optmal
Apr./2015	1.0113**	0.99	0.0113	0.9999	0.98 - Optmal
			Epicotyl length		
Developmental stage V2					
Jun./2014	1.0063^{ns}	0.99	-	0.9999	0.98 - Optmal
Nov./2014	1.0043^{ns}	0.99	-	0.9999	0.99 - Optmal
Jan./2015	1.0063**	0.99	0.0063	0.9999	0.99 - Optmal
Mar./2015	1.0091**	0.99	0.0091	0.9998	0.99 - Optmal
Apr./2015	0.9926*	0.99	-0.0074	0.9999	0.98 - Optmal
Developmental stage V3					
Jun./2014	1.0111*	0.99	0.0111	0.9999	0.98 - Optmal
Nov./2014	1.0011^{ns}	0.99	-	0.9999	0.99 - Optmal
Jan./2015	0.9921^{ns}	0.99	-	0.9990	0.95 - Optmal
Mar./2015	1.0147**	0.99	0.0147	0.9999	0.99 - Optmal
Apr./2015	1.0028^{ns}	0.99	-	0.9999	0.98 - Optmal

[1]$H_0: \beta = 1$ and $H_a: \beta \neq 1$; [1] **, *Significant at 1 and 5% probability and [ns]non-significant by the t-test, respectively; [2]Performance classification according to Camargo and Sentelhas (1997).

caliper.

The estimates of the concordance index were higher than 0.9999. According to Willmott et al. (1985), this index has a variation from 0, when there is no concordance to 1, when there is complete concordance. The estimates for this index indicate a high concordance for the length of the two characteristics in the two stages and the five growing seasons.

The values of the index of performance were higher than or equal to 0.90 and according to Camargo and Sentelhas (1997), it could be classified as optimal. Thus, the values obtained with a millimeter ruler agreed with those obtained from the digital caliper demonstrating that the millimeter ruler is recommended as an alternative to the digital caliper.

The estimates of the sample standard deviation for the lengths of hypocotyl and epicotyl are shown in Table 2. The greater difference between the estimate obtained for the millimeter ruler and the digital caliper was 0.26 mm and corresponded to the length of epicotyl at stages V2 (January/2015) and V3 (March/2015). According to Gomes (2009), the larger the value of a sample standard deviation, the greater is its variation. With this, it can be observed that the magnitudes of the sample standard deviation were practically similar to the peer-to-peer analysis for the values obtained from caliper and ruler in the different developmental stages and planting seasons.

As shown in Table 2, the confidence interval for the caliper comprises the averages obtained from the ruler and vice versa. This was also observed for other intervals estimated and suggests a similar precision between the two methods. According to Montgomery and Runger

Table 2. Estimates of average, standard deviation (SD) and confidence interval (CI) for the lengths of hypocotyl and epicotyl of seedlings of soybean obtained with a caliper and a millimeter ruler in different stages of development and seasons in a greenhouse.

Season	Average	ED	CI (95%)		Average	ED	CI (95%)	
			IL	SL			IL	SL
				Hypocotyl length				
Developmental stage V2								
Jun./2014	16.95	5.16	16.08	17.78	16.48	4.98	15.64	17.28
Nov./2014	22.08	6.48	21.12	22.99	21.51	6.57	20.54	22.43
Jan./2015	22.14	6.03	21.26	22.99	22.16	6.28	21.24	23.04
Mar./2015	21.94	6.32	21.24	22.60	20.75	6.23	20.06	21.40
Abr./2015	22.76	4.95	21.93	23.54	22.37	4.88	21.56	23.14
Developmental stage V3								
Jun./2014	16.93	4.98	16.09	17.73	16.35	4.82	15.53	17.12
Nov./2014	22.87	6.46	21.92	23.78	22.00	6.48	21.05	22.91
Jan./2015	22.46	6.36	21.77	23.12	22.33	6.31	21.64	22.98
Mar./2015	21.57	5.39	20.97	22.14	20.75	5.29	20.17	21.31
Abr./2015	22.28	4.64	21.51	23.02	22.02	4.58	21.26	22.74
				Epicotyl length				
Developmental stage V2								
Jun./2014	22.56	6.16	21.51	23.54	22.42	6.01	21.40	23.38
Nov./2014	33.06	9.07	31.72	34.33	32.87	9.10	31.54	34.15
Jan./2015	48.79	14.44	46.66	50.81	48.50	14.18	46.42	50.49
Mar./2015	38.01	11.53	36.73	39.22	37.59	11.50	36.32	38.80
Abr./2015	32.44	5.91	31.45	33.37	32.67	5.85	31.69	33.59
Developmental stage V3								
Jun./2014	22.86	6.11	21.82	23.83	22.53	6.16	21.49	23.52
Nov./2014	32.81	9.02	31.48	34.07	32.72	9.07	31.39	33.99
Jan./2015	42.70	14.04	41.17	44.15	42.93	13.80	41.43	44.36
Mar./2015	37.56	11.24	36.31	38.74	36.86	11.50	35.59	38.08
Abr./2015	31.44	5.28	30.56	32.27	31.29	5.45	30.39	32.16

(2012), the confidence interval involves the true value of μ with a confidence of $100(1-\alpha)$ being a precise measure of the estimation of average. According to Gomes (2009), it can affirmed that 100 (1-α) is the fiducial probability that the true value is within a given interval of confidence.

A series of studies report the utilization of the statistics used in this work for comparing the different estimates of reference evapotranspiration (Tagliaferre et al., 2012; Oliveira et al., 2008). In soybean, these statistics were used to validate models of simulation for this crop in Santiago, RG, Brazil (Gomes et al., 2014) and to compare the estimate of leaf area of old and modern varieties by a non-destructive method (Richter et al., 2014).

The statistical indicators showed excellent relation and concordance between the values obtained with a millimeter ruler and those obtained with a digital caliper. The decision of what tool might be used in the evaluation of experiments must consider factors such as the experience, ability and visual acuity of the evaluator, brightness in the place of evaluations as well as the availability of financial resources to acquire the types of equipment.

The results obtained in this work indicate that the lengths of hypocotyl and epicotyl might be used in the tests of distinctiveness, homogeneity and stability of cultivars of soybean. Besides these alternative descriptors, the implementation of computer algorithms based on non-linear approaches represents a modern alternative in the area of genetics and breeding and has provided results with higher precision as compared to traditional statistical approaches (Arqub and Abo-Hammour, 2014; Arqub et al., 2017).

Conclusion

The millimeter ruler had optimum performance in the measurement of the lengths of hypocotyl and epicotyl in plants of soybean in stages V2 and V3. The millimeter ruler consisted of an alternative tool to caliper in most of the planting seasons.

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African Journal of Agricultural Research

Full Length Research Paper

Screening for resistance to *Striga gesnerioides* and estimation of yield loss among Cowpea (*Vigna unguiculata* (L.) Walp.) progenies in the Upper East Region of Ghana

Leandre, S. P.[1,4*], Francis, K.[2], Richard, A.[1], Joseph, B.[4], Jean Baptiste T[4], Jeremy T. Ouedraogo[4], Patrick A.[1], Timothy J. Close[3] and Philip A. Roberbs[5]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[2]CSIR-Savannah Agricultural Research Institute, Tamale, Ghana.
[3]Department of Botany and Plant Sciences, University of California, Riverside, CA, USA.
[4]Institut de l'Environnement et de Recherche Agricole, Kamboinse, Burkina Faso.
[5]Department of Nematology, University of California, Riverside, CA 92521, USA.

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Parasitic weed *Striga gesnerioides* (Willd.) is one of the major constraints of cowpea production. Host-plant resistance seems to be efficient and economical in controlling the pest. The objectives of this study were to evaluate recombinant inbred lines developed between IT97K 499-35 (*Striga* resistant parent,) and Sanzi (*susceptible* parent), by Single Seed Descent (SSD), for *Striga* resistance in Northern Ghana. The study also evaluated the promising *Striga gesnerioides* resistant lines and susceptible checks for yield loss due to *Striga* infestation. The studies involved a field and pot screening under artificial inoculation. Twenty-seven (27) recombinant inbred lines (RILs) out of the 251 RILs screened were resistant to *Striga gesnerioides*. The percentage reduction in the grain yield and dry biomass were lower in the resistant RILs (0.55 to 3.08% and 1.11 to 7.7%, respectively) than the susceptible ones (28.45 to 58.88% and 47.29 to 61.71%, respectively). The negative effect of *Striga* infestation on cowpea grain yield and dry biomass can then be reduced when resistant genotypes are used.

Key words: Cowpea, *Striga gesnerioides,* recombinant inbred lines, yield loss.

INTRODUCTION

Cowpea *Vigna unguiculata* (L.) Walp.) is an important crop in the semi-arid tropics including parts of Asia, Africa, Southern Europe, Southern United States, Central and South America (Singh, 2005; Timko et al., 2007a). It

*Corresponding author. E-mail: leandre26@yahoo.fr.

is an affordable source of quality protein for rural and urban dwellers in Africa (Ajeigbe et al., 2012; Dube and Fanadzo, 2013). The dry grain protein concentration oscillates from 21 to 33% (Abudulai et al., 2016). It is well adapted to hectic environments where several crops fail to grow well (Bisikwa et al., 2014; Ddamulira et al., 2015). According to FAO, cowpea was cultivated on about 12.08 million hectare in Africa in 2016 with a total production of 5.83 million hectare in West Africa, predominantly in Nigeria, Niger, Burkina Faso, Mali and Senegal (FAOSTAT, 2018). Currently, cowpea yields are estimated around 300 to 500 kg ha^{-1} on farmer's field in Sub Saharan Africa (SSA) while its yield potential is up to 3000 kg ha^{-1} in optimum growing conditions (Tanzubil et al., 2008).

Cowpea production is mostly affected by major constraints. Parasitic plants are a major constraint to today's agriculture with most crop species being potential hosts (Westwood et al., 2010). *Striga gesnerioides*, is a key threat to cowpea production throughout West and Central Africa (Omoigui et al., 2017). It is one of the greatest devastating parasitic weed in most parts of the world. It is an obligate root parasitic flowering weed that belongs to the Orobanchaceae family (Parker, 2012). Out of about 30 *Striga* species which have been identified, *Striga gesnerioides* is the only *Striga* species that is virulent to dicots (Mohamed and Musselman, 2008). *Striga gesnerioides* is a major limitation to cowpea production in Africa (Timko et al., 2007b), causing considerable yield losses (Aggarwal and Ouédraogo, 1989). The extent of the damage in cowpea due to *S. gesnerioides* could be up to 70% depending on the extent of damage and level of infestation (Alonge et al., 2004). On susceptible cultivars, yield losses can reach up to 100% when *S. gesnerioides* population is over 10 emerged shoots per plant (Kamara et al., 2008). Omoigui et al. (2009) reported that yield losses caused by *Striga* in dry savannah of SSA are estimated in millions of tons annually and the prevalence of *Striga* infested soils is steadily increasing. Methods including improved cultural practices and the use of chemicals to control *S. gesnerioides* are available but most of them are ineffective whilst others are not affordable for small-scale farmers of Sub Saharan Africa (Singh et al., 1997; Timko et al., 2007b). The long viability of the seeds and the subterranean nature of the initial stages of parasitism make the control of the parasite by conventional approaches challenging (Berhane, 2016). In general, *S. gesnerioides* control is difficult to achieve due to the close association with its host (Lane et al., 1997). The identification of sources of *S. gesnerioides* resistance and their incorporation into breeding schemes would be a useful approach to combat the damage caused by the parasite in cowpea fields (Omoigui et al., 2017).

The objective of this study was to evaluate the field performance of 251 recombinant inbred lines (RILs) under *S. gesnerioides* infestation in Northern Ghana and to assess yield loss due to *Striga* infestation.

MATERIALS AND METHODS

The experiments were conducted from July 2015 to April 2016 at the Manga Station of Council for Scientific and Industrial Research-Savannah Agricultural Research Institute (CSIR-SARI). Manga near Bawku in the Upper East Region is geographically located within latitude 11.02° and longitude 0.27°, with an altitude of 224 m above sea level. The area is situated in the Sudan Savanna agro-ecological zone of Ghana. The mean rainfall of the area during the period of the experiment was approximately 44.33 mm. The average annual temperature was about 29.44°C, the highest being observed from March to April 2016. The relative humidity (RH) of the location fluctuated significantly, dropping in the dry season and rising during the rainy season with an average humidity of 55.4%. The study was conducted in two stages. The first stage was carried out in the field and the second stage in pots experiment.

Planting materials

Two hundred and fifty one recombinants inbred lines (RILs) at F_8 generation (F_8) (Table 1) derived from a cross between two cowpea lines, 'Sanzi' (susceptible to *Striga*) and 'IT97K-499-35' (resistant to *Striga*) (Omoigui et al., 2009), were used in the study.

Field experiment

The field study was carried out under rain fed conditions (between July and September) and under irrigation during the dry season. In a preliminary screening, each of the RILs and the parents (Sanzi and IT97K-499-35) were planted in a 2-meter single row plot without any replication on a field known to be a *Striga* hot spot. During this preliminary screening, data collected included days to 50% flowering, presence or absence of *Striga* plants, number of *Striga* per plants, total number of *Striga* per plot and *Striga* height. The presence or absence of *Striga* was recorded by visual observation on the different plots from thirty five (35) days after planting (DAP) up to maturity.

Pot experiment

Pot experiment was carried out to confirm the resistance or otherwise of the sixty-nine (69) the RILs that were identified as *Striga* resistant in the field experiment (Table 2). The pots were filled with top soil and then artificially infested with 5 g of *Striga* seeds. The top most, (1/3) portion of the soil per pot was mixed with the 5 g of *Striga* seeds. The infested pots were watered and allowed to condition before planting of the cowpea seeds. The pots were arranged in a randomized complete block designs with three replications. Thirty five days after planting (DAP), the pots were monitored on daily basis to check for *Striga* emergence. At maturity, the early pods were harvested on single plant basis to get some seeds from each plant. This was followed by gently washing the soil off the roots of the plants to confirm or otherwise that there were no *Striga* attachment to the roots of those that did not record *Striga* emergence.

Yield loss assessment due to *Striga* infestation

Twelve RIL's were selected based on their good agronomic traits on the field (white seed coat, big size and early maturity). The 12

Table 1. List of recombinant inbred lines (RILs) used in field experiment.

Genotype				
3	97	181	244	12B
9	98	182	245	12C
11	100	183	249	130A
14	106	184	252	131B
15	108	186	255	134B
21	110	187	257	141A
23	113	188	260	141B
25	119	190	261	144A
28	121	191	262	144B
29	124	195	263	150A2
35	125	199	265	150B
37	126	200	266	154 A
44	128	202	268	155A1
45	129	205	270	155A2
46	135	208	275	155B*
47	136	209	276	158A
48	143	210	277	158B
49	145	212	278	160B
54	148	213	279	165A
55	149	214	280	165B*
56	151	215	104A	166A1
62	152	217	104B	166B
64	153	220	105A	168A
68	157	221	105B	168B1
73	161	223	107A	168B2
74	162	224	107B	16A
79	164	225	109A	170A
80	167	226	109B	170B
81	169	227	112A	171A
84	175	230	112B	171B
90	176	235	114A	174A
93	177	238	114B	174B
94	180	240	12A	178A
178B1	229B	248B	40A1	70B
178B2	22A	251A	40A2	72A
179A	22B	251B	40B	72B
179B	232A	254A	40C1	7B
179C	232B	254B	40C2	7C
192A	234A	256A	42A	85A
192B	234B	256B	42B	86B
197A	234C	258A	43A	89B
197B	237A	258B	43B	8A
19B	237B	259*	51A	92C
1A	239C	269B	51B	95A
1B	242A	273A	59A	95B
201A	242B	273B	59B	96A
201B	242C	282A	63A	96B
211A	246B	282B	63B	Apagbaala
216A	247A	30A	65A	IT 97k-499-35
216B	247B	33A	65B	Sanzi
229A	248A	33B	70A	

Table 2. List of recombinant inbred lines (RILs) used in pots experiment.

Genotype				
23	162	270	197A	33B
25	184	275	197B	40A1
28	188	279	19B	40A2
35	191	280	1A	40B
46	195	104A	1B	40C1
47	200	12C	201A	72A
73	210	155A1	201B	72B
80	212	155A2	211A	89B
97	213	155B*	22A	96A
100	214	165A	22B	96B
151	249	16A	251B	Apagbaala
152	257	178A	256A	IT97K-499-35
153	260	178B1	259*	Sanzi
157	265	179B	30A	

Table 3. Characteristics of germplasm used to determine yield losses by *Striga gesnerioides* infestation.

Genotype	Days to maturity	Growth habit	Seed color	Seed texture
Parent				
IT97k-499-35	69	Erect	White	Smooth
Sanzi	67	Spreading	Brown	Rough
Resistant RILs				
16A	60	Erect	White	Rough
19B	65	Erect	White	Rough
35	68	Erect	White	Rough
155A2	61	Erect	White	Rough
191	68	Erect	White	Rough
Susceptible RILs				
12B	61	Erect	White	Rough
22A	55	Erect	White	Rough
25	62	Erect	White	Rough
112A	62	Erect	White	Rough
211A	57	Erect	White	Rough

consisted of five *Striga* resistant lines, five *Striga* susceptible lines and the two parents (IT97K-499-35 and Sanzi) as checks (Table 3).

The experiment was designed as a split plot with four replications. The *Striga* treatments (infested and no infestation) were the main plots while the 12 lines were the sub plots. The soil used to fill the pots were steam sterilised at 100°C to get rid of all *Striga* seeds. A metallic barrel was used for the sterilization of the soil. A wire mesh was fitted at 1/3 of the length of the barrel from the bottom. This served as a separator between the soil and the water. The setup was placed on fire. Water was poured in the barrel to fill up to the level where the wire mesh is fitted, jute sack was then laid over the wire mesh before filling the remaining two thirds with soil. The soil was covered with jute sack. The steam generated from the boiling water was allowed to pass through the soil for an hour and half to heat up the soil up to 100°C. The fire was put off upon attaining the 100°C to allow the soil to cool down. The soil was then scooped and spread on a plastic sheet to allow it to further cool down under shade before filling the plastic pots.

Forty-eight pots were infested with 5 g with *S. gesnerioides.* The other forty-eight pots were not infested with *Striga* seeds. All the pots were watered to field capacity and allowed to drain for 24 h before planting. The pots were irrigated as when it is needed and kept weed-free through hand pulling. Monitored spray was done against insects. From thirty-five days after planting, *Striga* emergence was recorded on daily based on visual observation. The other agronomic data collected included first day of flowering, days to 50% flowering, plant height, number of peduncles per plant and days to maturity.

The post-harvest data collected included dry pod weight, grain weight, number of seeds per pod, hundred seed weight as well as fresh and dried biomass weight. The dried biomass was obtained after drying in an oven for 24 h to a constant weight.

Yield loss assessment due to *Striga* infestation was estimated using the formula:

$$YL = \frac{yield\ in\ uninfested\ pot - yield\ in\ infested\ pots}{yield\ in\ uninfestded\ pot} \times \mathbf{100}$$

YL: Yield losses.

Statistical analysis

All field data collected were subjected to analysis of variance (ANOVA) using the GenStat analytical software (version 12.1.0.3338). Varietal means were compared using least significant difference at 5% level of probability (LSD 5%).

RESULTS

Cowpea RILs reaction to natural *Striga gesnerioides* in the field screening

The result showed that sixty-six (66) RILs out of the 251 (26.29%) used for this trial were resistant. (Table 4). *Striga* plants emerged from the soil of the plots containing susceptible RILS.

Reaction of cowpea RILs to artificial *Striga gesnerioides* in pot experiments

The results of artificial inoculation showed that 27 RILs were found to be resistant (no *Striga* emergence or *Striga* attachment) whiles 39 were susceptible (having *Striga* emergence or *Striga* attachment at the roots level) (Table 5). The number of days to flowering and maturity varied from 35 to 55 and 60 to 86, respectively.

Evaluation of *Striga* promising lines in yield loss

Genotypes varied significantly in terms of days to 50% flowering and maturity under both infested and not infested (Table 6). Days to flowering and maturity varied from 41 to 55 and 63 to 73 days after planting. Under *Striga* infestation, the genotype 191 was the earliest to flower at 44 days after planting (Table 6). Under no *Striga* infestation, the genotypes 25 and 112A flowered earlier than the rest of the genotypes (41 and 43 days). 155A2 flowered 50 days after planting. The remaining genotypes were considered as medium maturity cultivars based on the days to flowering (43 to 49 days).

Under *Striga* infestation, all the resistant lines significantly ($P<0.001$) flowered and matured almost at the same time as in no *Striga* infested pots whiles the susceptible lines delayed in flowering and maturity (Table 6).

The resistant genotype 19B for instance flowered at 48 DAPS and matured at 65 and 66 days DAP in the non-infested and *Striga* infested pots, respectively.

The susceptible genotype 12B flowered at 48 days after planting under no *Striga* infestation and 55 DAP under *Striga* infestation. The days to maturity were 65 and 73 days non-infested and *Striga* infested pots respectively ($P< 0.001$).

Seed yield and dry biomass per hectare

The analysis of variance revealed significant differences between the progenies under *Striga* infestation and no *Striga* infestation (Table 9)

Among the RILs, 16A, under no infestation produced the highest grain yield (754.2 kg ha^{-1}) followed by 25 (473.3 kg ha^{-1}) and 19B (470.1 kg ha^{-1}) (Table 7). The genotype, 155A2, a resistant cultivar recorded the lowest grain yield (320.1 kg ha^{-1}). The cultivar 16A which recorded the highest yield under no infestation (754.2 kg ha^{-1}) also recorded the significant yield under *Striga* infestation. (750 kg ha^{-1}). Ironically, the susceptible cultivar 25, one of the highest grain producers under no *Striga* infestation (436.1 kg ha^{-1}) also had one of the lowest grain yield under the infestation (338.6 kg ha^{-1}). In general, the reduction in grain yield was higher in the susceptible progenies than the resistant ones.

Dry biomass yield showed significant differences among the *Striga* infested ($P < 0.001$) and non-infested conditions. The mean values of dry fodder yield were 1507 kg ha^{-1} under no *Striga* condition and 1126 kg ha^{-1} in the infested conditions. The progenies with the highest dry biomass under no infestation conditions were 155A2 and 12 B with 2234 and 1901 kg ha^{-1}, respectively. The lowest fodder yield was recorded for the cultivar 25 with yield of 876 kg ha^{-1}. The dry biomass yields for the other genotypes ranged from 1076 to 1812 kg/ha.

Under the *Striga* infestation condition, the genotype 155A2 still recorded the highest fodder likewise in the no infestation. The dry fodder yield of genotype 12B drastically dropped from 1901 kg ha^{-1} in the non-infested condition to 1002 kg ha^{-1} under the infested condition. The genotype 16A also recorded good production of fodder in both infested (1813 kg ha^{-1}) and no infested condition (1898 kg ha^{-1}).

Plant height and number of pods per plant

The number of pod per plant was significantly different in both infested ($P < 0.001$) and non-infested environment. The analysis of variance indicated a significant difference for the plant height in both infested and non-infested environments.

The resistant plants were taller than the susceptible RILs under *Striga* infested condition (Table 8). The resistant parent IT97k-499-35 was the tallest plant (35.61 cm) followed by 16A and then 191 with plant heights of 31.63 and 31.53 cm, respectively. The susceptible cultivars 12B recorded the shortest plants height with 15.74 cm, in the no *Striga* infested pots. *Striga* susceptible

Table 4. Reaction of cowpea RILs derived from a cross of IT97K-499-35× Sanzi to *Striga gesnerioides* infestation in field trial (Manga Station, 2016).

RILs	Field trial	RILs	Field trial	RILs	Field trial
1A	R	72B	R	179B	R
1B	R	73	R	184	R
12C	R	80	R	188	R
16A	R	89B	R	191	R
19B	R	96A	R	195	R
22A	R	96B	R	197A	R
22B	R	97	R	197B	R
23	R	100	R	200	R
25	R	104A	R	201A	R
28	R	151	R	201B	R
30A	R	152	R	210	R
33B	R	153	R	211A	R
35	R	155A1	R	212	R
40A1	R	155A2	R	213	R
40A2	R	155B*	R	214	R
40B	R	157	R	249	R
40C1	R	162	R	251B	R
46	R	165A	R	256A	R
47	R	178A	R	257	R
72A	R	178B1	R	259*	R
260	R	55	S	109A	S
265	R	56	S	109B	S
270	R	59A	S	110	S
275	R	59B	S	112A	S
279	R	62	S	112B	S
280	R	63A	S	113	S
IT 97k-499-35	R	63B	S	114A	S
3	S	64	S	114B	S
7B	S	65A	S	119	S
7C	S	65B	S	121	S
8A	S	68	S	124	S
9	S	70A	S	125	S
11	S	70B	S	126	S
12A	S	74	S	128	S
12B	S	79	S	129	S
14	S	81	S	130A	S
15	S	84	S	131B	S
21	S	85A	S	134B	S
29	S	86B	S	135	S
33A	S	90	S	136	S
37	S	92C	S	141A	S
40C2	S	93	S	141B	S
42A	S	94	S	143	S
42B	S	95A	S	144A	S
43A	S	95B	S	144B	S
43B	S	98	S	145	S
44	S	104B	S	148	S
45	S	105A	S	149	S
48	S	105B	S	150A2	S
49	S	106	S	150B	S

Table 4. Contd.

51A	S	107A	S	154 A	S
51B	S	107B	S	158A	S
54	S	108	S	158B	S
160B	S	202	S	245	S
161	S	205	S	246B	S
164	S	208	S	247A	S
165B*	S	209	S	247B	S
166A1	S	215	S	248A	S
166B	S	216A	S	248B	S
167	S	216B	S	251A	S
168A	S	217	S	252	S
168B1	S	220	S	254A	S
168B2	S	221	S	254B	S
169	S	223	S	255	S
170A	S	224	S	256B	S
170B	S	225	S	258A	S
171A	S	226	S	258B	S
171B	S	227	S	261	S
174A	S	229A	S	262	S
174B	S	229B	S	263	S
175	S	230	S	266	S
176	S	232A	S	268	S
177	S	232B	S	269B	S
178B2	S	234A	S	273A	S
179A	S	234B	S	273B	S
179C	S	234C	S	276	S
180	S	235	S	277	S
181	S	237A	S	278	S
182	S	237B	S	282A	S
183	S	238	S	282B	S
186	S	239C	S	Sanzi	S
187	S	240	S	Apagbaala	S
190	S	242A	S		
192A	S	242B	S		
192B	S	242C	S		
199	S	244	S		

R, Resistant; S: Susceptible; flow: Flowering, mat: Maturity.

genotypes were shorter compared to the resistant RILs.

Among the progenies, the highest mean number of pods per plant (10 pods) was recorded in the susceptible genotype, 22A, under no *Striga* infestation, but produced 7 pods under *Striga* infested condition. However, for the resistant RIL 19B, the mean number of pods was not affected when grown on Striga infested soils.

Grain yield and dry biomass loss due to *Striga gesnerioides*

In general the grain and biomass yield loss were higher in the susceptible lines compared to the resistant RILs. For the resistant RILs the dry grain yield losses ranged from 4.5 kg ha^{-1} (0.55%) to 14.5 kg ha^{-1} (3.08%) (Table 9). In the susceptible genotypes, grain yield losses oscillated from 134.7 kg ha^{-1} (28.45%) to 262.5 kg ha^{-1} (58.88%).

The highest grain yield loss (58.88%) was recorded for the susceptible RIL 12B followed by the susceptible line 22A which registered 37.9% grain yield loss. Grain yield losses for the resistant progenies were found to be between 0.55% for the cultivar 16 A to 3.08% for the genotype 19B. The resistant line 16A also showed a lower yield loss (0.55%) than the resistant parent (1.51%).

Table 5. Reaction of cowpea RILs derived from a cross of IT97K-499-35× Sanzi to *Striga gesnerioides* infestation in field trial and pot experiment (Manga station, 2016).

SN	Genotype	Field trial status	Pot trial status	50% flow. (days)	50% mat. (days)
1	23	R	R	38	63
2	35	R	R	43	67
3	46	R	R	53	66
4	151	R	R	37	65
5	162	R	R	62	80
6	184	R	R	56	78
7	191	R	R	38.5	73
8	249	R	R	60	81
9	257	R	R	56	70
10	279	R	R	37	66
11	280	R	R	58	70
12	12C	R	R	56	72
13	155A1	R	R	53	65
14	155A2	R	R	36	60
15	16A	R	R	35	60
16	178A	R	R	71	86
17	19B	R	R	44	64
18	1A	R	R	69	72
19	201A	R	R	73	86
20	22B	R	R	50	63
21	251B	R	R	58	66
22	40A1	R	R	58	72
23	40A2	R	R	41	66
24	40B	R	R	43	62
25	40C1	R	R	63	76
26	89B	R	R	65	81
27	96B	R	R	61	85
28	IT97K-499-35	R	R	46	68
29	25	R	S	49	66
30	28	R	S	65	85
31	47	R	S	60	79
32	73	R	S	66	75
33	80	R	S	53	78
34	97	R	S	58	79
35	100	R	S	51	63
36	152	R	S	66	74
37	153	R	S	57	73
38	157	R	S	55	72
39	188	R	S	51	65
40	195	R	S	57	71
41	200	R	S	69	84
42	210	R	S	58	70
43	212	R	S	67	80
44	213	R	S	54	69
45	214	R	S	50	62
46	260	R	S	55	76
47	265	R	S	64	70
48	270	R	S	60	78
49	275	R	S	52	62
50	104A	R	S	63	86

Table 5. Contd.

51	155B*	R	S	58	69
52	165A	R	S	44	62
53	178B1	R	S	53	68
54	179B	R	S	71	88
55	197A	R	S	63	78
56	197B	R	S	42	60
57	1B	R	S	62	80
58	201B	R	S	41	68
59	211A	R	S	54	68
60	22A	R	S	42	60
61	256A	R	S	58	74
62	259*	R	S	62	67
63	30A	R	S	55	68
64	33B	R	S	49	68
65	72A	R	S	51	67
66	72B	R	S	53	70
67	96A	R	S	51	73
68	Apagbaala	S	S	49	71
69	sanzi	S	S	39	66

R: Resistant; **S**: Susceptible; **flow**: Flowering; **mat**: Maturity.

Table 6. Mean days to flowering and maturity of cowpea RILs on no infestation and *S. gesnerioides* infested plots (Manga Station, 2016).

Genotype	Days to flowering		Days to maturity	
	No infestation	Infestation	No infestation	Infestation
Parents				
IT97k-499-35	49	48	68	69
Sanzi	46	49	66	73
R. progenies				
16A	47	48	68	70
19B	48	48	65	66
35	47	46	65	66
155A2	50	51	67	67
191	44	44	62	63
S. progenies				
12B	48	55	65	73
22A	43	47	69	73
25	41	45	62	67
112A	43	48	63	68
211A	46	51	67	73
Mean	45.81	48.38	65.56	68.75
LSD (5%)	3.641	2.089	3.75	2.833
CV (%)	5.5	3	4	2.9

Values represent means of four replications.

Dry biomass losses for the susceptible progenies ranged from 889 kg ha^{-1} (47.29%) to 664 kg ha^{-1} (61.71%) for the cultivars 12B and 112A, respectively.

Similarly for the dry grain yield, the resistant RILs did

Table 7. Mean grain weight and dry biomass of cowpea RILs under no infestation and *Striga gesnerioides* infested plots.

Genotype	Grain yield (kg/ha)		Dry biomass (kg/ha)	
	Uninfested	Infested	Uninfested	Infested
Parent				
IT97k-499-35	554.2	545.8	1812	1779
Sanzi	488.8	302.8	1251	557
R. progenies				
16A	754.2	750	1898	1813
19B	470.1	455.6	1627	1548
35	397.4	389.7	1099	1036
155A2	320.1	313.2	2234	2209
191	390.3	385.4	1424	1314
S. progenies				
12B	445.8	183.3	1901	1002
22A	436.1	270.8	1567	810
25	473.3	338.6	876	379
112A	389.3	259	1076	412
211A	481.1	340.3	1317	656
Mean	466.7	377.9	1507	1126
LSD (5%)	95.79	116.1	336	170.1
CV (%)	14.3	21.4	15.5	10.5

Values represent means of four replications

Table 8. Mean plant height, number of pods per plant of cowpea RILs under no infestation and *S. gesnerioides* infested plots.

Genotype	Plant height (cm)		Mean Number of pods /plant	
	Uninfested	Infested	Uninfested	Infested
Parent				
IT97k-499-35	35.61	36.04	11.19	11
Sanzi	21.83	16.63	12	9
R. progenies				
16A	31.63	31.16	7.5	7.02
19B	29.32	29.11	8.42	8.37
35	26.1	25.87	7.9	8
155A2	24.76	24.34	7.55	7.4
191	31.53	30.35	8.32	8.25
S. progenies				
12B	15.74	12.83	7.55	4.05
22A	28.38	23.03	10.28	7.37
25	24.85	20.6	9	6.42
112A	17.79	13.8	8.47	5.92
211A	28.16	22.9	9.38	6.1
Mean	26.31	23.89	8.96	7.41
LSD (5%)	1.74	2.11	1.756	2.15
CV (%)	4.6	6.1	13.6	20.1

Table 9. Percentage dry grain and biomass loss per hectare under to *S. gesnerioides* infestation.

Genotype	Grain yield (kg/ha)			Dry biomass (kg/ha)		
	No *Striga*	*Striga*	Yield losses (%)	No *Striga*	*Striga*	Biomass losses (%)
Parents						
IT97k-499-35	554.2	545.8	1.51	1812	1779	1.82
Sanzi	488.8	302.8	38.05	1251	557	55.47
R. progenies						
16A	754.2	750	0.55	1898	1813	4.47
19B	470.1	455.6	3.08	1627	1548	4.85
35	397.4	389.7	1.93	1099	1036	5.73
155A2	320.1	313.2	2.15	2234	2209	1.11
191	390.3	385.4	1.25	1424	1314	7.72
S. progenies						
12B	445.8	183.3	58.88	1901	1002	47.29
22A	436.1	270.8	37.9	1567	810	48.3
25	473.3	338.6	28.45	876	379	56.73
112A	389.3	259	33.47	1076	412	61.71
211A	481.1	340.3	29.26	1317	656	50.18
Mean	466.7	377.9		1507	1126	
LSD (5%)	95.79	116.1		336	170.1	
CV (%)	14.3	21.4		15.5	10.5	

Values represent the means of four replications.

not show any significant biomass losses. With regard to the biomass losses, the cultivar 155A2 performed better in both *Striga* infested (2209 kg ha^{-1}) and non-infested (2234 kg ha^{-1}) then to the resistant parent IT97K-499-35, and also recorded the least biomass loss (1.1%) (Table 9).

DISCUSSION

Field screening for cowpea genotypes resistant to *Striga gesnerioides*

The field study recorded high emergence of *Striga gesnerioides* per plot (243 shoots) of the susceptible lines and this is similar to observations in other studies (Carsky et al., 2003; Kamara et al., 2008). The high *Striga* emergence observed on the Striga Susceptible lines was an indication that the site was really a hot spot for *S. gesnerioides* and the field had been infested with high concentration of *Striga* seeds over the years. However, a rigorous screening of the 66 genotypes in artificially infested soils in pot experiments revealed that only 10.75% were truly resistant to *Striga*. A susceptible genotype could be heavily infested underground without any *Striga* emergence as a results of several factors. According to Kim et al. (2002), one of the major limitations of screening under natural infestation is the variability in *Striga* seeds dissemination and cultivars escaping infestation. *Striga* sp. seeds need warm stratification for a certain time at a right temperature (approximately 30°C) before the seeds start responding to germination stimulants (Matusova et al., 2004). The high interference such as soil and climatic factors observed in the field makes the field screening less accurate (Baptiste et al., 2013).

Pot screenings

Field screening under artificial infestation is not always practical due to the fact that it can cause *Striga* seeds spreading to novel regions and it is moreover not consistent because breeders do not have any control of the parasite density and distribution (Haussmann et al., 2000). Pot screening has been operative as an alternative technique to confirm uniform infestation of *Striga* seeds.

After the pot experiment, the number of resistant lines was reduced from sixty-six (66) RILs to twenty seven (27) RILs after the pots experiment. This is essentially due to the high level of infestation (five grams of *Striga* seed per pot), the uniformity and a better control of the environment. A previous study done by Baptiste et al. (2013), confirmed the reliability of the pot screening compared to field screening.

The increased number of susceptible recombinant inbred lines found among the 66 could also be implied that these genotypes though showed no emerged seedlings of *Striga* had *Striga* attached to their roots. According to Ba (1983), some cowpea genotypes stimulate the *Striga* to germinate and penetrate their root tissues, but the *Striga* fails to grow more.

After both field and pot screening for *Striga* resistance, and taking into consideration farmers preferred traits, the genotypes 16A, 19B, 35, 155A2 and 191 were identified as promising *Striga* resistant lines.

Striga infestation delayed the flowering and maturity of susceptible cowpea genotypes. The susceptible genotypes also experienced huge reduction in grain yield and dry biomass in the *Striga* infested environment compared to when they were grown under no *Striga* environment. The study also confirmed that *Striga* infestation induces stunted growth hence the significant reduction of plant height at 50% flowering recorded for the susceptible genotypes. It also had an effect on the production of number of pods per plant. These data corroborated with previous studies (Press, 1995; Alonge, 1999; Gworgwor et al., 1991), which produced similar results. The stunted growth of genotypes, 12B, 22A, 25, 112A and 211A, could be attributed to the competition between the host and *Striga* for resources. The reduced vegetative growth of the susceptible varieties resulted in reduced leaf area, photosynthetic capacity and therefore affected flowering, podding and seed production (Alonge, 1999). According to Press (1995), the lower biomass accumulation by the susceptible genotypes could be the result of competition among the host and the weed for solutes, as well as carbon, water and minor rate of photosynthesis in the leaves of *Striga* infested plant. The reduced photosynthesis might have resulted in lower number of pods per plant and translocation of photosynthates to the sink.

Graves et al. (1992), showed that the low chlorophyll content which characterizes susceptible genotypes may account for the reduced development of the susceptible cowpea genotypes causing a decrease in both grain and biomass yield. The low biomass yield could also be attributed to the reduced shoot growth of the susceptible genotypes. The same phenomenon has also been reported for cereals infected with *Striga hermonthica* and for some cowpea genotypes infected with *S. gesnerioides* (Graves et al., 1992).

The resistant cultivars showed a relatively good growth compared to the susceptible lines in the infested pots. The relative good growth and the reduced export of assimilate to the weed would have ensured sufficient biomass accumulation and seed development as suggested by Gworgwor et al. (1991) on *S. gesnerioides*. The superior growth of some genotypes like 16A, 19B, 35, 155A2 and 191 indicated the positive relationship between crop vigour and crop performance even in *Striga* infested pots.

Grain and biomass loss due to *S. gesnerioides*

This current study has shown that all the resistant cowpea cultivars (16A, 19B, 35, 155A2 and 191) exhibited lower grain and dry biomass loss compared to the susceptible ones (12B, 22A, 25, 112A and 211A) indicating that these cultivars could play an essential role in controlling *Striga* in the endemic areas.

The susceptible genotypes recorded an average yield loss of 37.66% for dry grain yield which is quite consistent with the yield loss of 31±4% with a range of 26 to 65% observed by Aggarwal and Ouedraogo (1989). According to these authors, the loss could be attributed exclusively to the genotype effect as a consequence of *Striga* direct parasitism of susceptible cowpea lines (Muleba et al., 1996). *S. gesnerioides* diverts the host nutrient into themselves via the haustorium which establishes contact with the host tissues (xylem and phloem) (Okonkwo and Nwoke 1978; Okwonkwo, 1966). Consequently, this competition among host and parasite for water, and essential metabolites could be the explanation for the yield loss (Stewart and Press, 1990). Setty and Nanjapp (1985) and Kuijt (1969), reported that the osmotic pressure of the parasite is higher in both leaf and root than its host making the *Striga* more competitive. The use of high yielding *Striga* resistant varieties coupled with good agronomic practices can therefore help to reduce the yield losses in soil infested with *Striga* in the traditional farming systems.

Conclusion

The study revealed different reactions of cowpea RILs to *S. gesnerioides* during the field and the pot experiments. Out of the 251 RILs used, 27 RILS were found resistant similar to the resistant parent (IT97K-499-35), whiles 224 RILs were susceptible.

Yield loss assessment showed that the *Striga* resistant genotypes suffered less yield loss compared to the susceptible ones and therefore resistant genotypes can be one of the best means to minimize yield loss. These genotypes that expressed complete resistance are potential lines that will serve as resistant genotypes. The latest discovery of new sources of resistance to *Striga* provides an excellent way to supply farmers with new genotypes to replace their susceptible varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Cultivation of common bean with the application of biochar of ouricuri (*Syagrus coronata* (Mart) Becc.) endocarp

Felipe Alexandre Tenório[1*], Abel Washington De Albuquerque[1], Tania Marta Carvalho Dos Santos[1], João Inácio Soletti[2], Ferdnando Mariano Brito Silva[3] and Karoline De Melo Padilha[1]

[1]Center of Agricultural Sciences, Federal University of Alagoas, Rio Largo, AL, Brazil.
[2]Technology Center, Federal University of Alagoas, Maceió, AL, Brazil.
[3]Department of Earth Sciences and Environment, State University of Feira de Santana, BA, Brazil.

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Biochar has attracted the attention of the scientific community due to its promising applicability and contribution to the elevation of soil chemical and biological aspects, directly influencing the microbiota, fertility levels and yield of agricultural crops. The objective of this study is to determine the chemical and biological attributes of an acrisol cultivated with beans and submitted to the application of ouricuri biochar. The design was completely randomized in a 4 × 4 factorial scheme, with 4 replications. The factors were the combination of four granulometric bands: G1 (0.42 mm), G2 (0.84 mm), G3 (1.19 mm), G4 (1.68 mm) and four biochar doses (8, 16, 24 and 32 Mg ha^{-1}). Then, a control treatment without biochar was added. Morphophysiological aspects of bean culture, and chemical and biological soil indicators were evaluated. Ouricuri biochar promoted improvements in some soil quality indicators. The dose of 32 Mg ha^{-1} of biochar positively influenced the vegetative development of the plants. The results of this study showed that there is a direct relationship between the particle size and the amount of biochar in the soil. This had a direct effect on the carbon stock of the soil and the microbial population.

Key words: Biocarbon, pyrogenic carbon, soil quality, *Phaseolus vulgaris,* productivity.

INTRODUCTION

In searching for new solutions to minimize environmental impacts such as degradation of agricultural soils, the use of biochar as a soil conditioner has been one of the most frequent management practices for improving the chemical, physical and biological properties of the soil. Biochar has been evidenced after the discovery of soils called Terra Preta de Índio (TPI) in the Amazon, referring to the soils associated with the former indigenous occupations, in which the natives deposited coal, animal bones and ceramics, among other residues of human activity (Van Zwieten et al., 2010; Mangrich et al., 2011).

Biochar has attracted the attention of the scientific community because of its promising applicability in different areas, both at environmental and economic

*Corresponding author. E-mail: felipetenorio2012@gmail.com.

Table 1. Chemical and physical analysis of a dystrophic Red-Yellow Oxisol, with a medium/clay texture in depth of 0 to 20 cm, collected in a rural area in the municipality of Anadia – AL.

	Chemical attributes										Physical attributes		
pH (H_20)	P	K	Na	Ca	Mg	Al	H + Al	CEC	V	MO	Sand	Silte	Clay
	mg dm^{-3}			cmol$_c$ dm^{-3}					%	g kg^{-1}	g kg^{-1}		
5.7	5.0	56.0	24	1.40	1.0	0.68	6.80	8.30	28	18.60	310	100	410

levels (Qian et al., 2015). The addition of biochar can affect the physical properties of soil via indirect and direct means (Burrell et al., 2016). It can contribute to increase of the pH levels, by correcting the acid soils. It promotes an increase in the cation exchange capacity (CEC) and availability of nutrients to the soil, resulting in improvements in soil fertility (Lima et al., 2015). In the soil biological conditions, biochar acts by influencing the composition, diversity and microbial activity of the soil (Doan et al., 2014; Purakayastha et al., 2015; Wang et al., 2015; Pan et al., 2016).

In the soil, oxidation of the biochar can produce carboxylic groups, which increase its reactivity and its cation exchange capacity, making the biochar more efficient in improving soil quality conditions. Its high porosity and high specific surface area gives favorable conditions for the absorption of soluble organic compounds, which can contribute to increase in the availability of nutrients. When the partial oxidation of the edges of the aromatic structures of the biochar occurs, new electrochemical sites emerge, an effect that may aid in the retention and availability of nutrients for plants (Petter and Madari, 2006).

The mixture of substrates with biochar, aiming to improve soil physicochemical properties, has been studied as a valuable resource that can improve crop yield in tropical infertile and acid soils, and can be used by farmers to increase the productivity of crops of agricultural importance and promote the carbon stock in the soil (Arruda et al., 2007; Maia et al., 2011).

The bean culture has a significant economic and social importance in Brazil, since it is cultivated largely by small farmers. Its importance goes beyond the economic aspect, due to its relevance as a food security factor, and nutritional and cultural relevance in the cuisine of different countries and cultures. Brazil obtained a national average of bean production, in the 2016/2017 harvest, estimated at 3.029.3 thousand tons (CONAB, 2017). Productivity varies by region, as it depends on factors such as the climate, the planting season and the level of technology used.

In this way, it is necessary for the crop to manifest its productive potential, that the fertility of the cultivated soils is in chemical equilibrium, and that the essential elements are available in the soil to be absorbed. The use of alternative inputs in agriculture to raise crop productivity levels, such as biochar as a soil conditioner, appears as an ally to improve the conditions of low fertility soils, such as the Brazilian soils. The objective of the present work was to determine the chemical and biological attributes of an acrisol cultivated with beans, subjected to ouricuri biochar application.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the Center of Agricultural Sciences, Federal University of Alagoas (CECA/UFAL), Delza Gitaí Campus, km 85, Rio Largo - AL, located at 9° and 29'45" south latitude, 35° and 49'54" longitude west and 165 m of altitude. The soil was classified as a dystrophic Red - Yellow Oxisol, with a medium/clay texture in depth of 0 to 20 cm, collected in a rural area in the municipality of Anadia - AL.

Before implementation of the experiment, the chemical and physical characteristics of the soil were determined (Table 1), following the methodology of Embrapa (2009). Based on this, a base mineral fertilization with NPK was carried out at the dosages of 20, 80 and 40 kg ha^{-1}, respectively, according to the recommendation for bean cultivation, consistent with the Recommendation Bulletin of Corrective and Fertilizer for State of Pernambuco (IPA, 2008). After its fertilization, the soil under study had the following characteristics described in Table 1.

The experiment was installed in a completely randomized design, in a 4 x 4 factorial scheme, with 4 replicates. The factors were composed of the combination of four granulometric bands: G1, G2, G3 and G4 (0.42, 0.84, 1.19 and 1.68 mm, respectively) and four biochar doses equivalent to 8, 16, 24 and 32 Mg ha^{-1}. At the time, a control treatment without biochar was added.

The biochar used was produced by carbonization at 500°C, with a heating rate of 20°C min^{-1}, from the endocarp samples of the fruit of ouricuri *Syagrus coronata* (Mart) Becc.), in the Laboratory of Separation and Optimization Systems of Processes, LASSOP, from the Technology Center- CETEC, Federal University of Alagoas, UFAL. The samples composed of the endocarp with the rest of the fruit (almonds) were initially ground in a roller mill, prior to pyrolysis, for cleaning leaving only the endocarp.

The experimental system used to perform the pyrolysis consists of a Jung tubular furnace model LT6 2010, with a time and temperature controller J200. The furnace reaches a maximum temperature of 1000°C, heating the cylindrical reactor that is connected to the condensation system for the collection of bio-oil. The last condenser is connected to a FANEM model 089-Cal compressor/aspirator with a maximum volumetric flow rate of 0.024 m^3/min and a power of 550 W. For cooling of the condensers, a TECNAL thermostatic bath model TE-184 was used. Uncondensable gases resulting from the pyrolysis were released into a vessel containing water, thereby preventing its direct release into the atmosphere and allowing part to be trapped.

The soil and biochar mixtures were placed in polyethylene pots with a diameter of 26 cm and a capacity of 10 dm^{-3}, which had a drainage hole in the bottom covered with polypropylene fabric. The weighing was done with a balance for 20 kg with a resolution of 5 g.

Table 2. Chemical analysis of ouricuri biochar produced at 500°C.

pH (H_2O)	P	Ca	Mg	K	S	Fe	Zn	Cu	Al
	(%)								
7.6	10.01	8.22	-	61.21	3.84	3.89	1.66	2.11	-

[a]Percentage in mass.

The pots were filled with the soil mix with each corresponding treatment of the biochar and kept in a greenhouse for subsequent planting.

Biochar chemical analysis was performed by x-ray dispersive energy (EDX) spectrometry analysis shown in Table 2. The analysis was performed on a *Shimdzu* model EDX 800HS equipment at the electron microscopy scanning laboratory (SEM) of the group of optics and nanoscopy of the Federal University of Alagoas.

At the time of the study, four seeds per pot of common bean (*P. vulgaris* L.) cv. *BRS Agreste* were seeded, and at 10 days after sowing (DAS), the thinning of the less vigorous seedlings was performed, leaving only one plant per pot. Preventive phytosanitary control of pests was carried out using 2% (v/v) neem extract (*Azadirachta indica*) in three applications before flowering (R6) with a 7-day interval.

During the development of the bean crop, soil moisture was maintained at around 70% of the field capacity (FC), irrigating daily according to the water requirement of the crop. The soil field capacity was determined using a graduated beaker, a glass funnel with previously moistened filter paper, and 100 g of soil. The soil sample was initially weighed (dried at 60°C to constant weight) and transferred to the glass funnel. Then, 200 mL of distilled water was added to the soil contained in the funnel until saturation, noting the volume of percolated water. The FC was given by the difference between the added water and the percolated water.

At eighty DAS, in full harvest maturation (R9), all portions of the experiment were collected for further analysis. After that, the soil samples were collected and sieved, passing through a 2.0 mm mesh sieve, removing the visible roots and residues of plants and soil organisms. The soil samples were conditioned in paper bags and kept in a forced circulation hothouse at 105°C for 48 h until their total drying.

Soil chemical analyzes were carried out in the soil fertility and plant nutrition laboratory (CECA/UFAL), where the pH in 0.01M $CaCl_2$ solution was determined by potentiometry (pH meter) determined in the soil suspension after agitation and decantation. After reading, 5 mL of the SMP solution were added to determine the SMP pH in potentiometry (pHmeter) determined in the soil suspension after agitation and decanting. With the pH SMP readings, the value of the potential acidity (H + Al) was obtained. To obtain P content in the soil, the method of Mehlich^{-1} HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}, was used by spectrophotometry, and the K by flame photometry. The extraction of Ca^{2+}, Ca^{2+}+ Mg^{2+} and Al^{3+} was carried out with the extracting solution of KCl 1 N, titling with EDTA solution 0.0125 Mol L^{-1}. Finally, for Al^{3+}, 5 drops of the Blue Bromothymol indicator were added and titrated with the standard NaOH 0.025 N solution.

The number of leaves per plant (NL), the stem diameter (SD), expressed in mm, was determined. The plants were cut close to the soil and the dry weight of the aerial part (DWA) and dry weight of roots (DWR), expressed in grams, were obtained in air hothouse with forced circulation, at a temperature of 60°C up to constant weight, and total dry matter (TDM), expressed in grams. The seeds provided the final yield after manual threshing (correcting their humidity to 13% and transforming the data to kg ha^{-1}). The average number of pods per plant (NPP), obtained by the ratio between the total number of pods and the number of plants in the plot, were determined; the weight of pods per plant (WPP), and weight of grains per plant (WGP) expressed in grams; mass ratio of 100 plant grains (r100), were determined taking the seed samples randomly.

The soil samples were conditioned in plastic bags and kept under refrigeration at 4°C for the determination of total organic carbon (TOC), carbon of microbial biomass (Cmic) and soil basal respiration (SBR), determined in the laboratory of general microbiology of CECA/UFAL. For the determination of TOC, the modified Walkley-Black method was used (Embrapa, 2009). Microbial carbon (Cmic) was determined by the irradiation-extraction process, described by Mendonça and Matos (2005) and quantified according to Bartlett and Ross (1988). The Cmic contents were expressed based on the mass of oven dried soil at 105°C for 24 h.

The results of the evaluations were subjected to analysis of variance with application of the F test ($p < 0.05$), and the means were compared by the Tukey test ($p < 0.05$). For the quantitative variables, regression equations were adjusted using ASSISTAT software version 7.7.

RESULTS AND DISCUSSION

The results of the soil chemical analysis for the obtained data, showed that there was statistical difference for pH, phosphorus, potassium, hydrogen and aluminum (H + Al), calcium, cation exchange capacity and basal saturation (V%), with the addition of ouricuri biochar in the soil at 80 days after application (DAA). However, for the levels of Al, base sum (SB), calcium and magnesium (Ca + Mg) and Mg, no statistical difference was observed with the use of biochar.

For SB and Mg values, non-significant results probably occurred due to the absence of this element in the chemical composition of ouricuri biochar (Table 2), which influenced the non-significant SB values observed in this study. In other works, authors such as Van Zwieten et al. (2010), using biochar from other raw materials such as sewage sludge and paper mill residue, observed an increase in the levels of elements such as Ca and Mg, in Ferralsol in Australia with the addition of the equivalent of 10 Mg ha^{-1}.

There was influence of the biochar doses on the increase of P level on the soil, ranging from 18.81 mg dm^{-3} at the dose of 8 Mg ha^{-1}, to 40.5 mg dm^{-3} with the application of 32 Mg ha^{-1} of ouricuri biochar. Results were significantly higher ($p<0.05$) than that of the control treatment (18.10 mg dm^{-3}) (Figure 1A). Another researcher (Castro et al., 2018) showed the application of biochar of branches and pruned logs of *Gliricidia sepium* resulted in an increase of the content of macronutrients such as phosphorus and potassium.

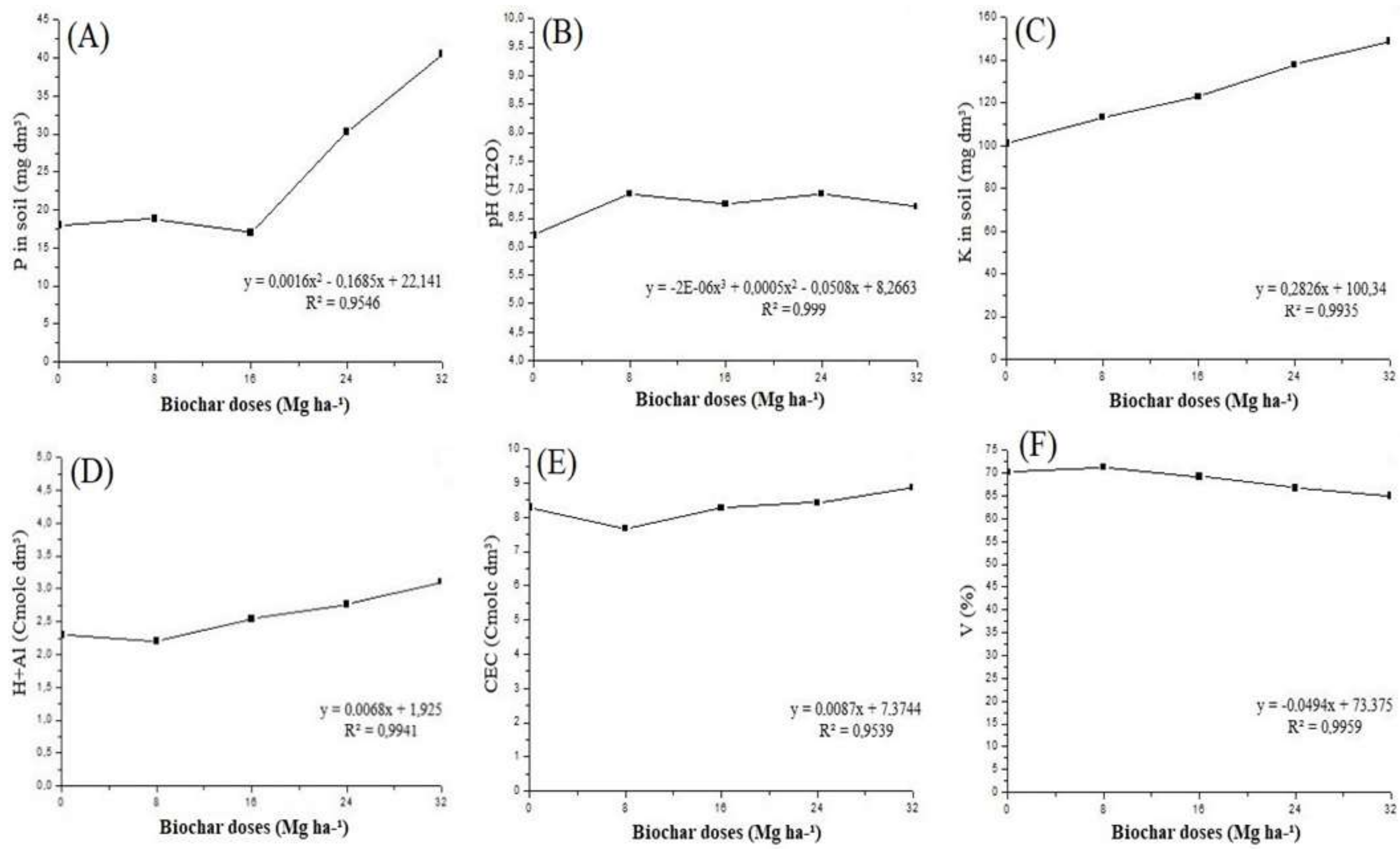

Figure 1. Chemical characteristics of soil after application of different doses of biochar. A- Phosphorus (P); B- Hydrogen ion potential (pH); C- potassium (K); D - hydrogen and aluminum - (H + Al); E - cation exchange capacity (CEC); F - base saturation - (V%)

Phosphorus can be connected to the biochar by means of physical adsorption (Van Der Waals), being a force of low magnitude, allows the easy exchange of P with the solution of the soil and for this reason, the adsorption on the biochar does not cause fixation process, differently from the kaolinites and oxides (Deluca et al., 2009; Gatiboni et al., 2013). The biochar has the capacity to strongly adsorb the orthophosphate ions (Lehmann, 2007), corroborating with the levels of P found in the soil of this study. Cui et al. (2011) also observed that the presence of the biochar reduced the adsorption of P in the Fe and Al oxides, increasing their residual power. In the plant, the P plays the role of storing and transferring energy through the phosphate molecules (ADP and ATP). In addition, it plays a key role in photosynthesis, respiration and cell division and is also a component of several proteins and nucleic acids (Dechen and Nachtigall, 2007).

The alkalinity of the biochar increased the pH values in the soil, with a significant interaction ($p<0.01$) between the factors, that remained above 6.5 and the control that presented 6.25 in its pH value, demonstrating that the addition of biochar, regardless of the dose applied, makes it possible to raise soil pH levels (Figure 1B), which probably maintained influence on Al adsorption, and absence of toxicity in the soil. After the pyrolysis, the biochar may have the potential to neutralize the soil acidity, as it presents high levels of calcium carbonate and magnesium (Van Zwieten et al., 2010).

Soil pH influences the rate of nutrient release, the solubility of all soil materials and the amount of ions stored at the exchange sites. The high reactivity of biochar caused by the dissociation of the functional groups present in the peripheries of its structures, can adsorb H^+ ions of the soil raising the pH (Madari et al., 2009). Smebye et al. (2016) showed that the application of a carbon dose at 10% (m/m) was able to alter the soil pH from 4.9 to 8.7. According to Si et al. (2018), soil pH was significantly increased by approximately 0.1 unit on average when the rice straw-derived biochar was applied.

Promoting a 15-fold increase in pH with the addition of ouricuri Biochar, Dai et al. (2014) have inferred that the application of biochar to the soil in addition to altering the pH value, is able to increase the soil buffering range. Castro et al. (2018) in his studies, using the biochar of *Gliricidium sepium* managed to raise the soil pH to 5.9, being statistically superior as compared to the pH control of 4.8. Castellini et al. (2015) pointed out that obtaining these benefits is dependent on soil and biochar type, as well as its rate of application.

Biochar also contributed to the increase in available K levels in the soil, ranging from 101 (control) to 148.75 mg dm^{-3} at the maximum dose of 32 Mg ha^{-1} (Figure 1C). Yao et al. (2010), Madari et al. (2006) and Oguntunde et al. (2004) also observed increases in K levels in the soil with the application of biochar. Steiner et al. (2004) stated that much of the biochar ashes is rich in potassium in its constitution, depending on which part of the plant material was charred. The treatments with biochar were characterized by larger K content, supporting the data by Martinsen et al. (2014) that biochar is rich in K.

According to Petter and Madari (2012), biochar contributes to a higher absorption of nutrients, mainly as a function of the reactive surfaces at the edges of the aromatic structures of the biochar pores. This characteristic of the biochar raises the concentrations of bases and consequently reduces the acidity in the substrate. However, it is believed that this increase of K levels in the soil is due to the presence of this nutrient in the biochar (Table 2), which shows that this increase occurred in doses equal to or greater than 8 Mg ha^{-1}.

However, an increase in potential acidity (H + Al) was observed as the dosage of biochar in the soil increased (Figure 1D). This increase in the potential acidity values possibly occurred due to a nutrient dissolution effect in the soil solution, where greater nutrient retention by coal surface structures would lead to an increase in nutrient uptake and greater availability of reactive sites in the surface of the clays to bind to H and Al (Rondon et al., 2006). According to Gao et al. (2016), over time, the leaching of the alkaline components of the biochar takes place as the water percolates the soil, therefore the pH can decrease and the acidity increase.

The application of increasing doses of biochar, allowed an increase in CEC values in the soil, presenting levels of 8.3 cmolc dm^3 for the control, up to 9.0 at the dose of 32 Mg ha^{-1} (Figure 1E). This could be attributed to a combination of high availability of cations in exchangeable form in this treatment, possibly related to the presence of biochar (Novotny et al., 2015). Liang et al. (2006) quoted two reasons for a high biochar efficiency in retaining nutrients. The first one is attributed to the pyrogenic coal presenting higher density of negative charge per unit of surface area and consequently a higher charge density. The second one is that in which the nutrients are trapped through physical forces in the fine pores of the carbonized material or that the slow biological oxidation of the aromatic structures at the edges contribute to the elevation of the cation exchange capacity (CEC) (Glaser et al., 2002).

In the case of biochar, reactive sites are formed over the years, whereas the particles are attacked by microorganisms in the soil, changing the chemical and physical characteristics of the surface (Cohen-Ofri et al., 2006). This also corroborates the statistically significant increase in effective CEC of the soil in the biochar, inoculant and fertilizer plots over the duration of the study by Castro et al. (2018).

In adding doses of biochar to the soil, one of the fertility indicators represented by the base saturation index (V%), increased from 62.25 (control) to 71.25% at the dose of 8 Mg ha^{-1} (Figure 1F), showing significant improvements in soil chemical aspects. However, as an increase from the 8 Mg ha^{-1} dose of bio-carbon was provided, base saturation values below 70% were obtained, still, providing conditions that allow the development of most plants in acidic soils such as the Brazilian Cerrado.

The addition of biochar also contributed to the increase of Ca in the soil. It was verified in the study that, with the increase of the biochar granulometry, there was a significant increase ($p<0.05$) from 2.32 (G1) to 2.73 cmolc dm^{-3} (G4) in the contents of Ca in soil. The results of the application of ouricuri biochar were superior to the control (2.23 cmolc dm^{-3}) (Figure 2), demonstrating positive results in the increase of Ca in the soil, with the application of ouricuri biochar. There were no significant differences between the doses × granulometry factors used. The increase of Ca in the soil is due to the high content of the element in the ouricuri biochar (Table 2). Van Zwieten et al. (2010) also evaluated two biochar obtained from paper mill residue in an experiment during two months in a greenhouse and reported that the use in acid soil raised the level of available Ca in the soil from 1.23 to 8.87 cmolc kg^{-1}, as compared to the control soil.

For the biological results of the soil, with the increase in the biochar dosages, there was a significant increase ($p<0.05$) from 175.72 (control) to 256.35 mg kg^{-1} (32 Mg ha^{-1}) on microbial biomass carbon (Cmic) (Figure 3B). Nevertheless, it was found that when the dose 0 was compared with the 8 Mg ha^{-1} dose, the Cmic kept the level of 107.2 mg kg^{-1}, which shows that after the application of the biochar in the soil, there was immediate consumption of readily available C as compared to the Cmic values obtained with the addition of biochar. The biochar granulometries used did not influence the Cmic levels in the soil, with the reaction period equivalent to 80 days. However, during the pyrolysis process, there is also the formation of more labile forms of carbon, which is readily available to the microorganisms in the soil, causing a part of C labile and another part of C stable in the material, where the labile part presents an aliphatic fraction that is more rapidly mineralizable and exists in less abundance in the biochar produced at high temperatures. The stable part presents an aromatic portion that is more slowly oxidized, creating functional groups, such as the carboxylic acid (Lehmann and Stephen, 2009).

For the total organic carbon (TOC), the contents presented a significant increase ($p<0.01$) of 11.35 (dose 0) to 15.40 g kg^{-1} (8 Mg ha^{-1}), about 27% increase in soil

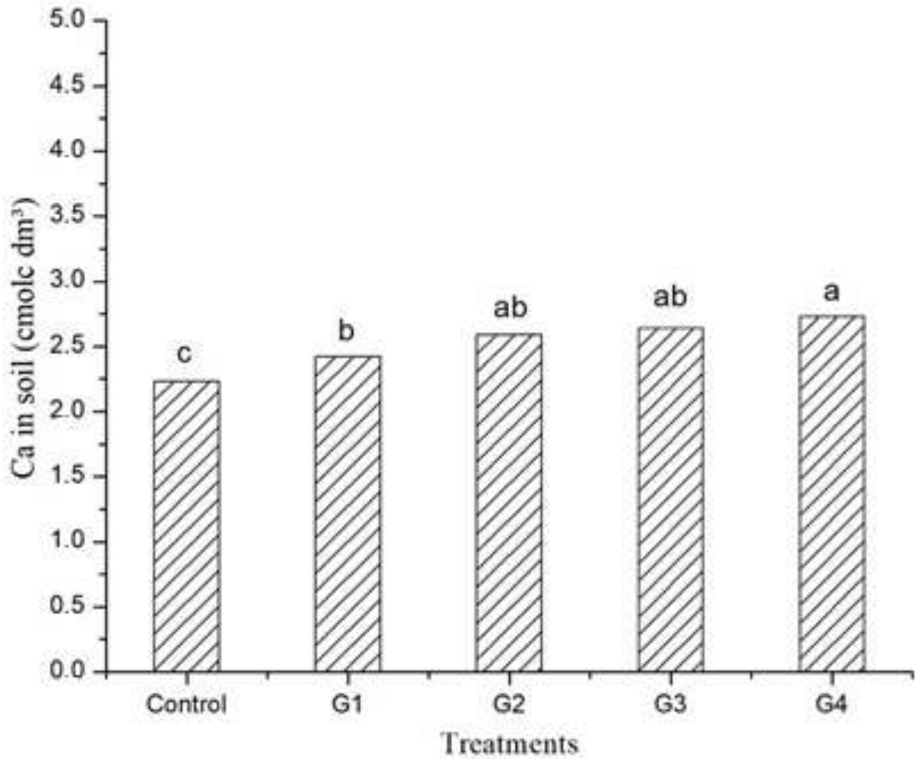

Figure 2. Availability of calcium in the soil after application of different biochar granulometric ranges. Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level.

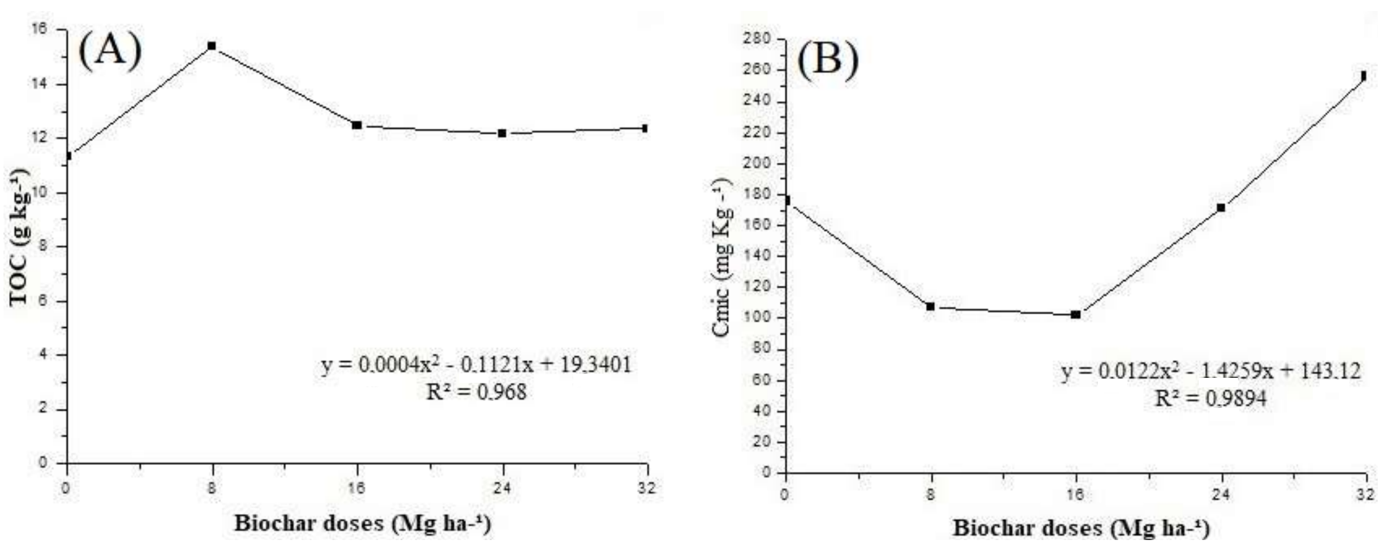

Figure 3. (A) Total organic carbon and (B) availability of microbial carbon of soil subjected to different doses of biochar

TOC stock. However, as doses were increased to a maximum of 32 Mg ha^{-1}, the TOC content was reduced to 12.36 g kg^{-1} (Figure 3A). For the granulometries used, the highest TOC value was obtained with the G1 treatment in the particle size, obtaining an average of 14.35 g kg^{-1}, as compared to the control treatment (11.35 g kg^{-1}) (Figure 4).

Increases in organic carbon additions improved the

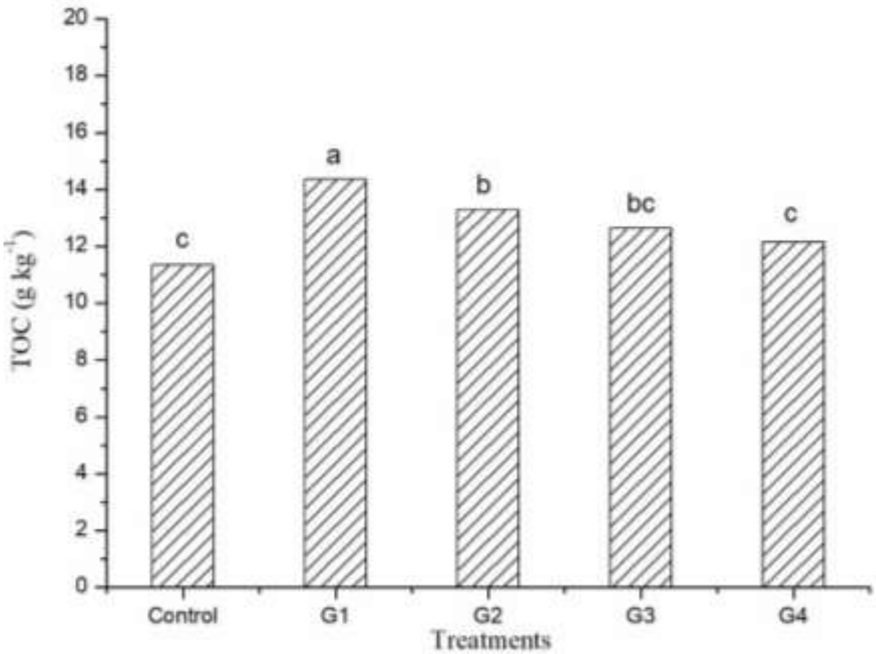

Figure 4. Total organic carbon (TOC) of soil after application of different granulometric bands of biochar

retention of nutrients that become accessible to microorganisms on the particle surface (Lehmann et al., 2011). Chen et al. (2013), in a long-term field experiment in sandy soil with 0, 20 and 40 Mg ha^{-1} of wheat straw biochar, verified that communities of bacteria increased by 28 and 64% in soils conditioned with 20 and 40 Mg ha^{-1} of biochar.

Graber et al. (2010), studying the use of biochar in the soil, suggest that the changes observed in the growth of the microbiological composition were stimulated by the organic tars that are residual of the biochar. In general, the specific soil surface influences all essential functions for soil fertility, including water, air and nutrient cycling and microbiological activity (Bailey et al., 2011).

The agronomic data from the obtained from common bean cultivation obtained were significantly ($p <0.05$) influenced by the application of biochar, in relation to the number of pods per plant (NPP), weight of pods per plant (WPP), grain weight per plant (WGP), dry weight of roots (DWR), dry weight of the aerial part matter (DWA) and mass ratio of 100 grains (r100).

For the variable NPP, the values increased from 17 to 18 pods per plant, with the addition of the biochar in the dose of 32 Mg ha^{-1} as compared to the dose 0 (control), being also superior to the other dosages used (8, 16 and 24 Mg ha^{-1}) (Figure 5A). Avila et al. (2010), in their studies using common bean cultivation with and without irrigation for the same cultivar, obtained NPPs of 13 and 21, respectively. For WPP (Figure 5B), WGP (Figure 5C), TDM (Figure 5E) and r100 (Figure 5F) variables, increases in yield were respectively 22.76, 16.17, 12.18 and 3.70%, with the addition of biochar in the dose of 32 Mg ha^{-1} as compared to the control. Castro et al. (2018) also had the physiological parameters of the bean influenced by the application of biochar with fertilizer.

However, the highest agronomic development of bean plants was verified for dry weight of root (DWR), which reached a mean of 35.24% in weight gain with the addition of biochar at the dosage of 32 Mg ha^{-1}, as compared to the control treatment (Figure 5D). Smider and Singh (2014) showed that the dry mass of the maize crop increased in response to application of biochar, and Vaccari et al. (2011) verified a 30% increase in the biomass of wheat with application of 30 Mg ha^{-1} biomass of wood. Some authors such as Graber et al. (2010) and Jones et al. (2012), also highlighted indirect changes in microbial activity in soil with biochar and suggested that biochar stimulates plant growth by inducing effects on the rhizosphere, with effects on quality and quantity of root exudates, thus influencing good root development. Results from a field trail across multiple years and in multiple locations across the USA supported the hypothesis that crop yield in different locations responds differently to complicated interactions of soil, biochar and climate (Laird et al., 2017).

According to Ramos Junior et al. (2005), number of grains and r100 are considered the main components that influence productivity, which, in the same way, responded in a significant way. Mete et al. (2015) also tested the joint application of biochar with the addition of

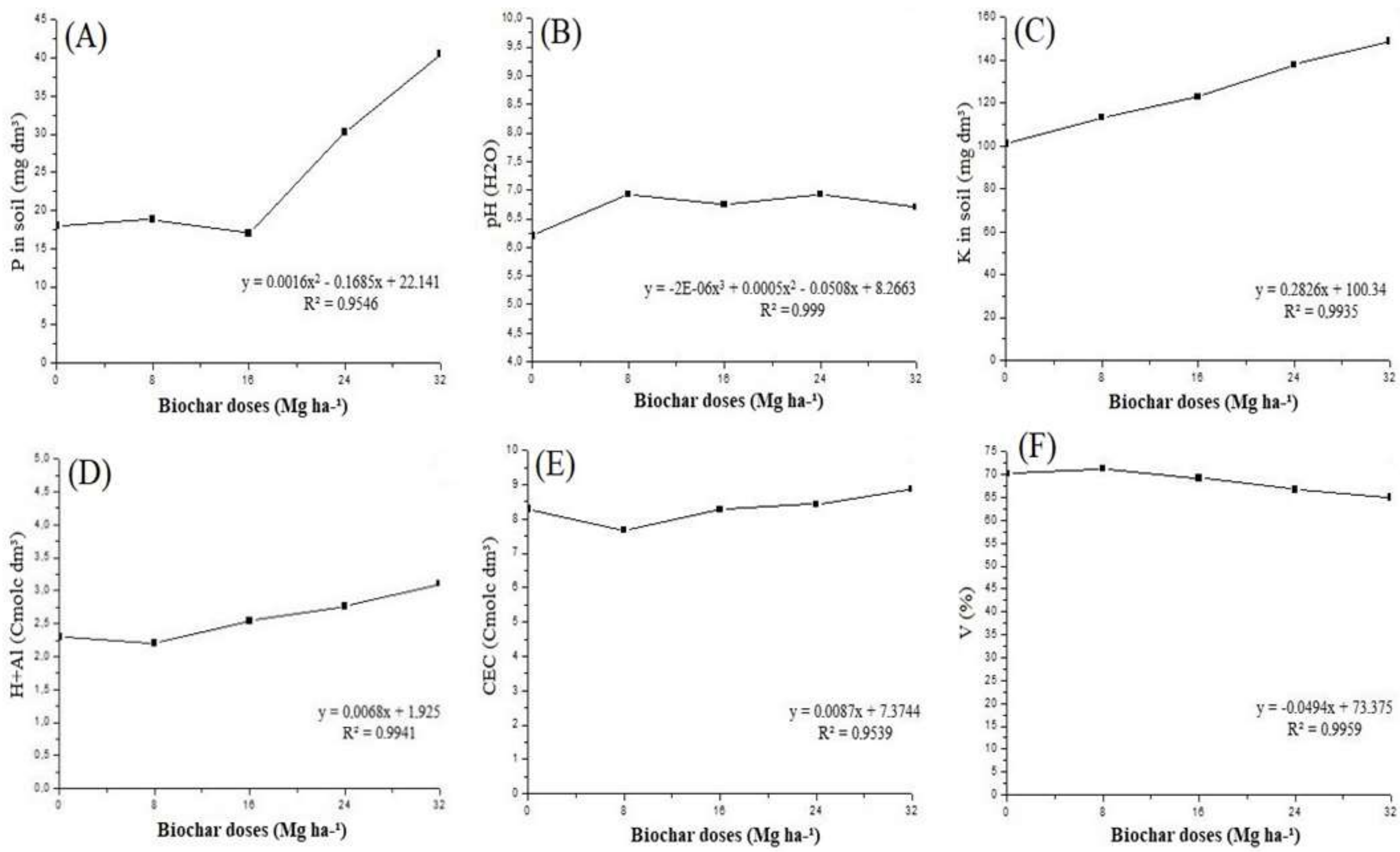

Figure 5. Number of pods per plant (A), weight of pods per plant (B), weight of grains per plant (C), weight of dry root matter (D), weight of total dry matter (E), mass ratio of 100 grains (F) of common bean "*Phaseolus vulgaris*" grown under different granulometries and doses of biochar

NPK fertilizer in an alkaline soil in soybean cultivation. Results showed that the simultaneous application of both products increased on average, the yield in the production of biomass and seeds by 361 and 391%, respectively.

Güereña et al. (2015) showed promising results on the use of biochar in common bean cultivation. Thus, when compared with the control, the application of biochar changed on average, 262% biomass of the aerial part, 164% radicular biomass and 357% biomass of nodules. Other researchers (Schmidt et al., 2015; Glaser et al., 2015) showed that the effect of biochar on crop productivity is a function of a range of factors such as the type of biochar and the amount of biochar added to the soil, where biochar is being applied and how much additional nutrient is added.

Conclusion

Ouricuri biochar caused improvements in the major chemical indicators of soil quality (pH, Ca, P, K and CEC) even reacting to a short time (80 DAA). The development of plant roots, total organic carbon (TOC) and Cmic were positively influenced by ouricuri biochar, mainly at the dose of 32 Mg ha^{-1}. However, it is believed that its effect on plants cannot be explained as a factor dependent on soil fertility results.

The results of this study demonstrate a direct relationship between the particle size and the amount of biochar in the soil, which influences the effect on the carbon stock of the soil and the microbial population.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Greenhouse gas emissions from an alkaline saline soil amended with urea: A laboratory study

César Valenzuela-Encinas[1], Leslie Elizalde Contreras[2], Rocio J. Alcántara-Hernández[3], Marco Luna-Guido[2], Olivia Franco-Hernández[1], Víctor M. Ruíz-Valdiviezo[2], Luc Dendooven[2] and Rodolfo Marsch[2*]

[1]Department of Chemistry, Unidad Profesional Interdisciplinaria de Biotecnología-IPN (UPIBI), México.
[2]Laboratory of Soil Ecology, Cinvestav, México.
[3]Laboratorio de Ecología Bacteriana y Epigenética Molecular, Instituto de Ecología, UNAM, México.

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Soil of the former lake Texcoco is nitrogen (N) depleted, so any attempt to vegetate the area will require the application of an N fertilizer. Urea is commonly used as fertilizer, but its application to soil might affect emissions of greenhouse gases (GHG), such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), and the high pH and electrolytic conductivity (EC) in the Texcoco soil might inhibit the hydrolysis of urea. Four soils of the former lake bed with EC 3.3, 88.3, 96.9, and 121 dS m^{-1}, were amended with urea while dynamics of mineral N and emissions of GHG were monitored. Urea increased emission of CO_2 in all soils and emission of N_2O in soil with EC≤88.3 dS m^{-1}, but emission of CH_4 was not affected. Hydrolysis of urea occurred in all soils although it was significantly lower in soil with EC≥88.3 dS m^{-1}. Oxidation of NH_4^+ occurred in soil with EC≤96.9 dS m^{-1}, but oxidation of NO_2^- only in soil with EC 3.3 dS m^{-1}. It was found that oxidation of NH_4^+ and NO_2^-, and hydrolysis of urea was inhibited by the high EC in soil of the former lake bed, while emissions of CO_2 and N_2O, but not CH_4 were affected by application of urea.

Key words: Carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), former lake bed, mineral N in soil.

INTRODUCTION

The former lake of Texcoco located in the valley of Mexico City (Mexico) at an altitude of 2240 m above sea level with a mean annual temperature of 16°C and annual precipitation of 705 mm was drained from the 17th century onwards to avoid flooding in Mexico City (O'Hara and Metcalfe, 1997). The drainage of the lake left a soil with a high Ph and salinity and little vegetation (Dendooven et al., 2010). During the dry season, the wind erosion was high. However, during the rainy season, flooding occurred frequently. The groundwater, which is highly alkaline and saline, was just under the soil surface and after heavy rainfall the area flooded. The national water authority (Commission Nacional de Agua, CNA) installed drainage pipes so that the area could be

*Corresponding author. E-mail: rmarschm@me.com. Tel: +52 55 5747 3800/4345. Fax: +52 55 5747 3313.

vegetated to stop wind erosion and dust storms in Mexico City (Luna-Guido et al., 2000). However, previous research showed that the soil of the former lake Texcoco is N depleted (Conde et al., 2005). It can be speculated that the high pH and salinity inhibits N_2 fixing microorganisms thereby limiting the amount of mineral N that enters the soil (Barua et al., 2011; Welsh et al., 2007).

Urea is cheap and often applied to fertilize crops. It could easily be applied to grass, shrubs and trees that might be used to vegetate the former lake bed. Hydrolysis of urea can occur in three ways, biotic (Burton and Prosser, 2001), abiotic in soil with a high pH (Ghandi and Paliwal, 1976) and abiotic through extracellular ureases (Conrad, 1996) generating two NH_3 molecules. As such, concentration of NH_4^+ will increase in urea-amended soil (Burton and Prosser, 2001). However, if most of the process is biological then the high pH and salinity might inhibit the release of NH_4^+. Additionally, application of urea is known to increase emissions of nitrous oxide (N_2O) and might affect emissions of carbon dioxide (CO_2) and methane (CH_4) (Wang et al., 2011). Hydrolysis of urea will generate ammonium ions that can be oxidized by nitrifiers, first to NO_2^- and subsequently to NO_3^-. However, high electrolytic conductivity (EC) is known to inhibit the activity of nitrifiers so emissions of N_2O after application of urea might not increase (Zhu et al., 2011). Therefore, four soils of the former lake Texcoco with different pH and EC were amended with urea, with or without acetylene (C_2H_2), known to inhibit nitrification (Bateman and Baggs, 2005), while emissions of CO_2, CH_4 and N_2O were monitored in an aerobic incubation. Acetylene was applied to half of the soil samples so that the importance of the nitrification process in the emissions of N_2O could be determined. The objective of this study was to investigate the effect of high alkalinity and salinity on hydrolysis of urea, emissions of GHG and dynamics of mineral N (ammonium (NH_4^+), nitrite (NO_2^-) or nitrate (NO_3^-)).

MATERIALS AND METHODS

Soil sampling

Details of the soil of the former lake Texcoco can be found in Dendooven et al. (2010) and mineralogy in Gutiérrez-Castorena et al. (2005). Part of the former lake bed has been drained and irrigated with sewage effluent from a waste water plant to remove excess of salt (Luna-Guido et al., 2000). For instance, the concentration of the sodium ions (Na^+) decreased from 21 g kg^{-1} dry soil to 3 g kg^{-1} dry soil after 8 years of flooding and the chloride ions (Cl^-) from 21 g kg^{-1} dry soil to undetectable amounts (Luna-Guido et al., 2000). The *Distichlis spicata,* an indigenous grass with a high tolerance to salt and Na^+ and tamarix (*Tamarix* species) have been introduced since the early 1970s to control erosion, and they now cover much of the area. More details on the vegetation and the effluents used to drain the plots can be found in Luna-Guido et al. (2000).

At four locations with different EC and pH due to different periods of drainage, five approximately 400 m^2 plots were defined and sampled by augering the 0 to 15 cm layer 30-times with a stony soil auger with diameter 7 cm (Eijkelkamp, Nl) at random on 7 March 2011. The 30 soil samples taken from each site (*n* = 4) and plot (*n* = 5) were pooled, 5 mm sieved and characterized (Table 1). As such, 20 soil samples were obtained. Details of the sampled soils can be found in Table 1. The soil with an EC 3.3 dS m^{-1} was denoted soil A, with EC 88.3 dS m^{-1} soil B, with EC 96.9 dS m^{-1} soil C and with EC 121 dS m^{-1} soil D. This field based replication was maintained in the incubation study.

Treatments and experimental set-up

The experimental design was a completely randomized 2 $\times$ 4 factorial with five replications (maintained from the field site replications for each soil). The factors were four soil types and four soil amendments which were: 1) 200 mg N kg^{-1} applied as urea; 2) 200 mg N kg^{-1} applied as urea plus acetylene (C_2H_2) at 0.1%; 3) unamended soil; and 4) unamended soil plus C_2H_2 at 0.1%.

Sixteen sub-samples of 10 g dry soil from each of the four soils and five sampled plots were added to 120 ml flasks. Eight soil samples were adjusted to 40% water holding capacity (WHC) by adding distilled water and eight by adding an urea solution. The flasks were air-tight sealed with a Suba-seal. Four of the flasks amended with distilled water and four with the urea solution were injected with 0.1 ml acetylene (C_2H_2) to inhibit nitrification (Bateman and Baggs, 2005). Additionally, 15 flasks without soil were air-tight sealed and incubated in the same way to determine the concentration of CO_2, N_2O and CH_4 in the atmosphere. The flasks were incubated in the dark at 25 ± 2°C. After 0, 1, 3 and 7 days, one flask was selected from each soil and treatment at random and the headspace was analyzed for CO_2, CH_4 and N_2O. Additionally, three flasks without soil were selected at the same time and the headspace analyzed. The flasks were opened, the soil removed and extracted for mineral N (ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-)) with 0.5 M K_2SO_4.

Chemical analyses

Details of the techniques used to measure WHC, pH, EC, total N and soil particle size distribution can be found in Ruíz-Valdiviezo et al. (2010). The extracted NH_4^+, NO_3^- and NO_2^- were measured colourimetrically with a San Plus System-SKALAR automatic analyzer (Breda, The Netherlands).

The headspace of the vials was analyzed for CO_2 and N_2O on an Agilent Technologies 4890D gas chromatograph fitted with an electron capture detector (ECD) and CH_4 on an Agilent Technologies 4890D gas chromatograph fitted with a flame ionization detector (FID). Details of the columns used, gas flow, and oven, detector and injector temperatures can be found in Ruíz-Valdiviezo et al. (2010). Concentrations of CO_2, N_2O and CH_4 were calculated by comparing peak areas against a standard curve prepared from known concentrations, 10 ppm N_2O in N_2, 5 ppm CH_4 in N_2 and 2500, 20000 and 40000 ppm CO_2 in N_2, every time samples were analysed.

Statistical analysis

The experimental design was a completely randomized 2 $\times$ 4 factorial with five replications. The factors were four soil types with different EC and four soil treatments, that is, unamended soil, urea-amended soil, C_2H_2 applied soil, and urea + C_2H_2. Emission of CH_4, N_2O and CO_2 was regressed on elapsed time using a linear regression model, which was forced to pass through the origin, but allowed different slopes (production rates) for each treatment. Significant differences for the production of CH_4, N_2O and CO_2

Table 1. Some characteristics of the different soils from the former lake Texcoco.

	EC[a] (dS m^{-1})	pH	Organic C	Total N	WHC[b]	Clay	Silt	Sand	Textural classification
			(g kg^{-1} soil)						
Soil A	3.3	10.3	21.72	1.22	431	167	47	786	Loamy sand
Soil B	88.3	10.3	9.17	0.88	575	234	60	706	Sandy clay loam
Soil C	96.9	10.3	11.63	0.79	530	174	80	746	Loamy sand
Soil D	121.0	10.5	30.84	1.12	467	147	27	826	Loamy sand

[a]EC : Electrolylic conductivity, [b] WHC : Wather holding capacity.

production between treatments, soil and their interactions were determined using PROC MIXED (SAS Institute Inc. 1989).

Concentrations of NH_4^+, NO_2^- and NO_3^- were subjected to an analysis of variance using PROC GLM (SAS Institute Inc. 1989) to test for significant differences between soils, treatments and their interactions with Tukey's Studentized Range test. All data presented were the mean of five replicates, that is, $n = 5$.

RESULTS

Emissions of CO_2, CH_4 and N_2O

In the unamended soil, the emission of CO_2 was largest in soil A and lowest in soil D (Figure 1a). Application of C_2H_2 to the unamended soil had no significant effect on the CO_2 emission rates. The emission of CH_4 was similar in all unamended soils and was not affected by the application of C_2H_2 (Figure 1b). In the unamended soil, the emission of N_2O was significantly larger in soil A than in the other soils (P<0.05) (Figure 1c). Application of C_2H_2 had no significant effect on the N_2O emission rate.

Application of urea increased the emission of CO_2 in soils A, B and D significantly, but not in soil C (P<0.05) (Figure 1d). Application of C_2H_2 to the urea-amended soil decreased the emission of CO_2 significantly in soils A and B, but not in soils C and D (P<0.05). The emission of CO_2 was similar in the C_2H_2-amended soils applied with or without urea. Application of urea did not affect the emission of CH_4 and was similar in the C_2H_2-amended soils applied with or without urea (Figure 1e). Application of urea increased the emission of N_2O significantly in soil A, but not in the other soils (P<0.05) (Figure 1f). Application of C_2H_2 to the urea-amended soil decreased the emission of N_2O in soils A and B, but not in soils C and D.

The emission of CO_2 was significantly affected by soil and the interactions between urea $\times$ C_2H_2 and soil $\times$ urea $\times$ C_2H_2 (P<0.05) (Table 2). The emission of N_2O was significantly affected by the different interactions between urea, soil and C_2H_2, and the emission of CH_4 was affected significantly only by soil (P<0.05).

Dynamics of inorganic N

The concentration of NH_4^+ was similar in the unamended soils and soils applied with C_2H_2 (Figure 2a). Application of C_2H_2 to the unamended soil reduced the concentration of NO_2^- significantly in soils B and C, but not in soils A and D (P<0.05) (Figure 2b). The concentration of NO_3^- was similar in the unamended soils and soils with applied C_2H_2 (Figure 2c).

Application of urea increased the concentration of NH_4^+ significantly in all soils and the increase was most accentuated in soil A (P<0.05) (Figure 2d). Application of C_2H_2 to the urea-amended soil decreased the amount of NH_4^+ significantly in soil A, but not in the other soils (P<0.05). Application of urea increased the concentration of NO_2^- significantly in soil A, but not in the other soils (P<0.05) (Figure 2e). Application of C_2H_2 to the urea-amended soil decreased the amount of NO_2^- significantly in soil A, but not in the other soils (P<0.05). Application of urea increased the concentration of NO_3^- significantly in soil A, but not in the other soils (Figure 2f). Application of C_2H_2 to the urea-amended soil decreased the amount of NO_3^- in soil A, but not in the other soils.

The concentrations of NH_4^+, NO_2^- and NO_3^- were affected significantly by urea, C_2H_2, soil and their interactions, except for the effect of urea and its interaction with C_2H_2 on the concentration of NO_3^- (Table 2).

DISCUSSION

Emissions of CO_2, CH_4 and N_2O

The emission of CO_2 decreased with increased EC in the soils. It is well known that increased salinity reduces the soil microbial biomass and inhibits microbial activity (Setia et al., 2011a, b). However, it has to be remembered that other characteristics, such as soil organic matter content, clay content and pH, are also known to affect microbial activity and thus emissions of CO_2 (Setia et al., 2011a, b).

The application of urea increased the CO_2 emission rate significantly in soils A, B and D compared to the unamended soil. It is well known that application of an N fertilizer to an N depleted soil can increase emission of CO_2 as microbial activity is stimulated (Wang et al., 2011). The high salt content in the Texcoco soils will inhibit N_2 fixation, which will limit the N content of the soil (Barua et al., 2011; Welsh et al., 2007). Additionally,

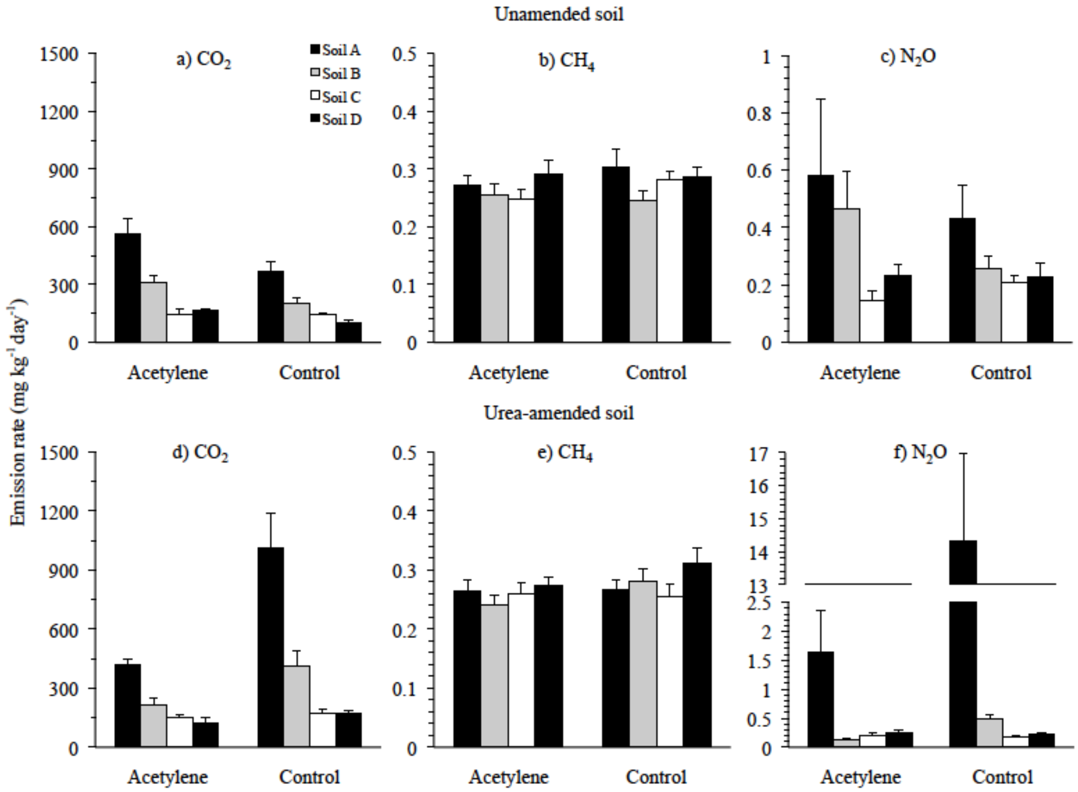

Figure 1. (a) Emission of CO_2, (b) CH_4 and (c) N_2O (mg kg^{-1} soil day^{-1}) from the unamended Texcoco soil and soil amended with urea (d), (e) and (f). Bars are ± one standard deviation.

hydrolysis of urea will release CO_2 (Snyder et al., 2009). The emission of CO_2 will thus increase with 86 mg CO_2 after the application of 200 mg N kg^{-1} soil if all urea was hydrolyzed.

The application of C_2H_2 had no effect on emission of CO_2. Acetylene can be used by certain organisms, e.g. *Rhodococcus opacus*, *Rhodococcus ruber* and *Gordona* species, as C substrate thereby increasing emission of CO_2 (Rosner et al., 1997). Soil characteristics are known to affect C_2H_2 degradation (Brzezinska et al., 2011), although the limited time that the soil microorganisms were exposed to C_2H_2 (7 days) might have reduced the possibility that they use C_2H_2 as C source, that is, they were not yet adapted.

Agricultural soils can be a source or a sink for CH_4, but they are normally a sink and fluxes are normally small (Wang et al., 2011). Large amounts of CH_4 are only emitted from paddy soils or wetlands (Wright et al., 2011). Production of CH_4 occurs under anaerobic and oxidation under aerobic conditions. Although, the soils were incubated aerobically, emission of CH_4 occurred in all soils. Anaerobic micro-sites exist even in a soil at 40% WHC that will stimulate production of CH_4, and oxidation of CH_4 did not match production. The high salt content might have inhibited methanotrophic activity.

Application of urea or C_2H_2 did not affect emissions of CH_4. Application of NH_4^+, released after the hydrolysis of urea, is known to inhibit oxidation of CH_4, but not in the Texcoco soils (Stiehl-Braun et al., 2011; Xu et al., 2011). Aronson and Helliker (2010) reported after a meta-analysis that not only the amount of N applied, but also the history of the soil affected the inhibitory effect. They reported that managed soil and soil with a longer duration of fertilizer application showed greater inhibition of CH_4 uptake with added N. The Texcoco soil was not fertilized and is N depleted so it can be assumed that N fertilizer would not inhibit CH_4 oxidation. Bronson and Mosier (1994) reported a strong inhibitory effect of C_2H_4 on oxidation of CH_4 (76 to 100% inhibition) in two soils. No such inhibitory effect was found in the Texcoco soil, so it can be speculated that little or no CH_4 oxidation occurred as stated before.

Application of urea increased emission of N_2O in soil A compared to the unamended soil, but not in the other soils. It is well known that application of urea to soil increases emission of N_2O (Wang et al., 2011). Emission of N_2O from soil is mainly due to nitrification, that is, the oxidation of NH_4^+ to NO_2^- and NO_2^- to NO_3^- under aerobic conditions and denitrification, that is, the reduction of NO_3^- to NO_2^-, N_2O and N_2 under anaerobic conditions (Wrage et al., 2001). As the soil was incubated under aerobic conditions and the concentration of NH_4^+ sharply

Table 2. Effect of urea, acetylene, soil and their interaction on the emissions of CO_2, CH_4 (mg C kg^{-1} day^{-1}), and N_2O (mg N kg^{-1} day^{-1}), and concentrations of mineral N (NH_4^+, NO_2^- or NO_3^-) (mg N kg^{-1} dry soil).

	Concentration of						Emission of					
	NH_4^+		NO_2^-		NO_3^-		CO_2		N_2O		CH_4	
Variable	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**
Urea	14.97	0.0001	14.87	0.0002	1.19	0.2769	0.01	0.9091	1.83	0.1779	0.01	0.9258
Acetylene (C_2H_2)	4.77	0.0302	13.36	0.0003	13.13	0.0004	0.32	0.5715	1.92	0.1669	0.06	0.8085
Soil	9.49	<0.0001	12.96	<0.0001	42.27	<0.0001	16.13	<0.0001	1.96	0.1217	5.12	0.0020
Urea C_2H_2	4.88	0.0283	10.77	0.0012	1.81	0.1807	21.26	<0.0001	5.46	0.0205	0.35	0.5526
Urea Soil	7.82	<0.0001	15.83	<0.0001	4.79	0.0031	2.02	0.1138	6.39	0.0004	0.18	0.9096
Soil C_2H_2	4.43	0.0049	8.01	<0.0001	5.14	0.0019	1.35	0.2616	3.87	0.0101	0.07	0.9780
Urea Soil C_2H_2	4.51	0-0044	9.12	<0.0001	5.78	0.0008	4.91	0.0029	4.51	0.0044	0.22	0.8816

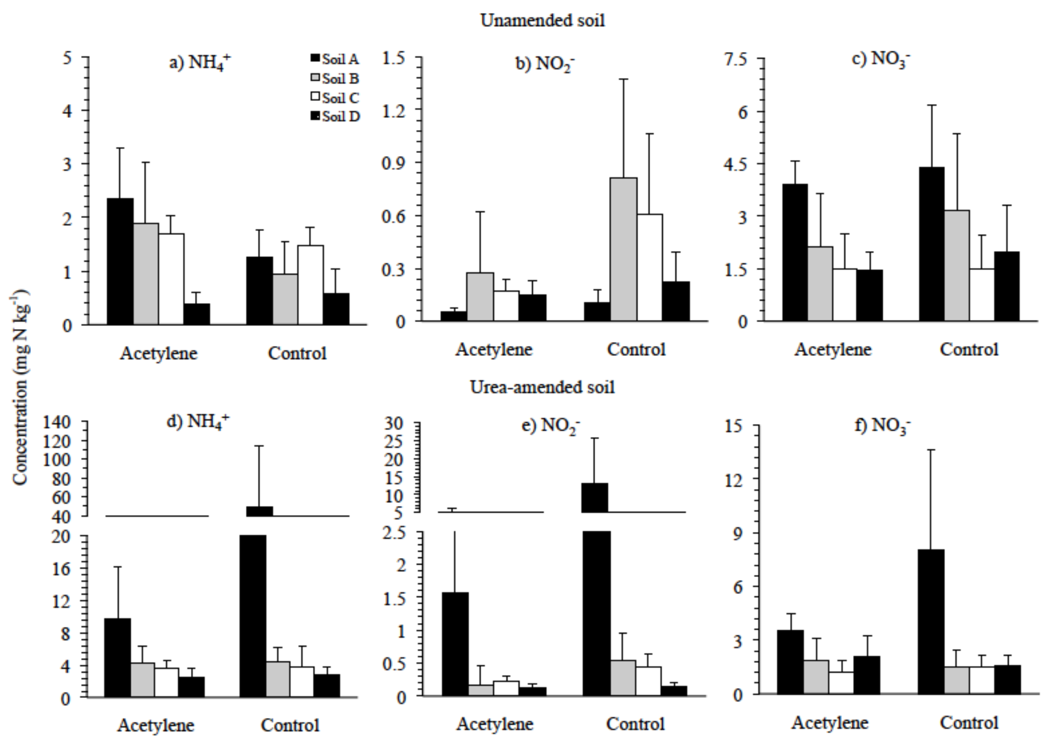

Figure 2. (a) Concentration of NH_4^+, (b) NO_2^- and (c) NO_3^- (mg N kg^{-1} soil) in the unamended Texcoco soil and soil amended with urea (d), (e) and (f). Bars are ± one standard deviation.

increased after application of urea and its subsequent hydrolysis, emission of N_2O was most likely due to oxidation of NH_4^+. Application of C_2H_2 (as an inhibitor of the oxidation of NH_4^+) to soil A sharply reduced the emission of N_2O confirming that oxidation of NH_4^+ was the main source of N_2O emission. The emission of N_2O in soil A amended with urea plus C_2H_2, however, was still higher than in the unamended control soil. As such, although the soil was incubated aerobically, it is likely that some anaerobic microsites were formed in soil stimulating denitrification and thus emission of N_2O.

Emission of N_2O also increased when urea was added to soil B, and C_2H_2 decreased it. As such, nitrification contributed to the emission of N_2O in soil B. No increase in emission of N_2O occurred in soils C and D amended with urea. As such, the high salt content inhibited the nitrification process in soils C and D. Application of urea might be used as N fertilizer for a pioneering vegetation to minimise N_2O emission in the alkaline saline Texcoco soil. However, it would have to be injected into the soil as the high pH will favour NH_3 volatilization.

The soils were incubated at a constant water content in this experiment. In the field, water content will fluctuate continuously thereby changing soil conditions constantly. These constantly changing conditions will put a further strain on the microbial population. The soil micro-organisms will have to adapt strategies to survive a dried out or flooded environment, to altering anaerobic and aerobic conditions, and salt concentrations that decrease in the rainy season but increase in the dry season when evaporation will concentrate the salt ions mostly in the upper soil layer.

Dynamics of mineral N

Concentration of NH_4^+ increased in all urea-amended soils. Hydrolysis of urea can occur in three ways, biotic (Burton and Prosser, 2001), abiotic in soil with a high pH (Ghandi and Paliwal, 1976) and abiotic through extracellular ureases (Conrad, 1996) generating two NH_3 molecules. As such, concentration of NH_4^+ will increase in urea-amended soil (Burton and Prosser, 2001) as found in this study. The increase in the concentration of NH_4^+ was lower in soil with EC ≥88.3 dS m^{-1} than in soil with EC 3.3 dS m^{-1}, so the high EC had an inhibitory effect on the hydrolysis of urea (Wilson et al., 1999).

Application of C_2H_2 reduced the concentration of NO_2^- in the unamended or urea-amended soils A, B and C, but not in soil D. Consequently, oxidation of NH_4^+ occurred in soil with EC ≤96.9 dS m^{-1}. Ammonium oxidizing organisms have been found in extreme environments (Sorokin and Kuenen, 2005) so it would come as no surprise that oxidation of NH_4^+ occurred in soil with EC≤96.9 dS m^{-1}. Oxidation of NO_2^- only occurred in the soil with the lowest EC. Although NO_2^- oxidizing bacteria have been isolated from alkaline environments (Sorokin et al., 1998), it might well be that the extreme high EC inhibited NO_2^- oxidation as the energetic gain from this process is low (Oren, 2011).

Under the experimental conditions, NO_2^- oxidation seems to be absent in soils with EC ≥88.3 dS m^{-1}. However, NH_4^+ oxidation occurred even at 96.9 dS m^{-1} suggesting that NH_4^+ oxidation is less sensitive to salinity than NO_2^- oxidation. From a biological point of view, there are several possible explanations for this phenomenon. First, aerobic ammonium oxidation yields more energy for growth than nitrite oxidation (Bock and Wagner, 2006). Second, aerobic NH_4^+ oxidation is done not only by a restricted group of Bacteria, but also by Archaea belonging to the phylum Thaumarchaeota (Leininger et al., 2006). These Archaea have a different physiology than NH_4^+ oxidizing Bacteria. In some environments, these Archaea can even be the major NH_4^+ oxidizers (Prosser and Nicol, 2008). Furthermore, even when aerobic NH_4^+ and NO_2^- oxidizers form tight associations, that is, the NO_2^- produced by NH_4^+ oxidizers is consumed by NO_2^- oxidizers, there seems to be ecophysiological differences between both groups that might be in part the consequence of the evolution of their metabolic life styles. Nitrite oxidizers can be heterotrophic/mixotrophic or strict chemotrophics (Bock and Wagner, 2006), and even between them there are differences since *Nitrospira* species can be K-strategists with high substrate affinity and low growth rate, while *Nitrobacter* species might be r-strategists (Schramm et al., 1999). However, more studies need to be done as new nitrite oxidizers groups are emerging and their physiological must still be studied.

In this study, the microbial population was not investigated. It would be interesting to study the microbial population in each of the treatments and investigate which organisms were involved in each of the processes discussed, e.g. nitrifiers, methanogens and methanotrophs. A transcriptomics analysis would surely reveal genes that are relevant in these extreme environments, but absent in more normal soil conditions.

It was found that urea increased emission of CO_2 in all soils and emission of N_2O in soil with EC ≤88.3 dS m^{-1}, but emission of CH_4 was not affected. Hydrolysis of urea occurred in all soils although it was significantly slower in soil with EC ≥88.3 dS m^{-1}. Oxidation of NH_4^+ occurred in soil with EC≤96.9 dS m^{-1}, but oxidation of NO_2^- only in soil with EC 3.3 dS m^{-1}.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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