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Full Length Research Paper

Gene action studies on yield and quality traits in okra (*Abelmoschus esculentus* (L.) Moench)

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Selection of suitable breeding methodologies in bringing desirable improvement in crop plant require the complete knowledge about the nature of gene action involved in the inheritance of quantitative and quality traits. Gene action of fruit yield and quality traits in okra (*Abelmoschus esculentus* (L.) Moench) were studied through half diallel analysis of 28 F_1 hybrids derived by crossing 8 parental lines. The present study indicated the preponderance of non-additive gene action for days to 50% flowering, nodes per plant, fruit length, fruit diameter, plant height, fruits per plant and mucilage and a preponderance of additive gene action for days to first picking, first fruit producing node, internodal length, average fruit weight and harvest duration. For fruit yield per plant and dry matter, only dominant component of variance was observed which revealed the presence of non-additive gene action, hence, heterosis breeding is required to be followed for exploitation of these traits.

Key words: Gene action, okra, variance, diallel, fruit yield.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is a warm season vegetable in the tropical and subtropical countries of the world. It is native of Ethiopia (Vavilov, 1951). The immature young seed pods are the edible part of this plant, which are consumed as cooked vegetable, mostly fresh but sometimes sun-dried. Okra is gaining importance with regard to its nutritional, medicinal, and industrial value. Apart from nutritional and health importance, okra plays an important role in income generation and subsistence among rural farmers in developing countries like India. It has a vast potential as one of the foreign exchange earner crops and accounts for 70% of the export of fresh vegetables excluding potato, onion and garlic, the destinations being the Middle East, Western Europe and USA. It is commercially grown in the Indian states of Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. The prominent position of okra among Indian vegetables can be due to its easy cultivation, dependable and regular yield, wider adaptability and year round cultivation. In spite of its importance, no major breakthrough has been made in this crop and the farmers are still growing their own local varieties or open pollinated varieties. Hence, there is a need for restructuring this vegetable crop for increasing the productivity.

Knowledge on the genetic system controlling the quantitative and quality traits is important for formulating an efficient selection program through the use of a suitable mating design. The information about the relative contribution of components of variation *viz*., additive and

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non-additive, is essential for effective crop improvement program (Azhar and Ajmal, 1999). In order to apply an optimum breeding strategy for targeted quantitative and quality traits, a genetic analysis of those traits needs to be performed. Diallel mating design has been used extensively by several researchers to measure gene action for yield and yield components in okra (Jindal et al., 2009; Singh et al., 2009). Several workers studied gene action of the yield and yield attributes and determined that additive and non-additive variance components are important in the genetic control of yield and its associated traits in okra (Jaiprakashnarayan et al., 2008; Singh et al., 2009). The present investigation was, therefore, undertaken with a set of half-diallel crosses to elicit information about the nature and magnitude of gene action for yield and its components in okra so as to formulate suitable breeding strategy.

MATERIALS AND METHODS

Eight okra genotypes viz., P-20, 9801, VRO-4, Parbhani Kranti (PK), P-8, Hisar Unnat (HU), Tulsi-I and SKBS-11 were chosen in this study to represent substantial amount of genetic diversity for different quantitative and quality traits and were maintained through selfing during 2011. These eight genotypes were involved in 8 × 8 half-diallel combinations to develop 28 F_1 hybrids during 2012. All the F_1's along with their parents were evaluated in a Randomized Block Design with three replications during summer-rainy season of 2013 at Experimental Farm of the Department of Vegetable Science and Floriculture, CSKHPKV, Palampur. The crop was raised in three rows of 2.5 m length with inter and intra row spacing of 45 and 15 cm, respectively. Standard agronomic practices were followed and plant protection measures were taken as and when required. The observations were recorded on five competitive plants in each entry and replication for the parameters *viz*., days to 50% flowering, days to first picking, first fruit producing node, nodes per plant, internodal length (cm), average fruit weight (g), plant height (cm), harvest duration (days), fruits per plant, fruit yield per plant (g), dry matter (%) and mucilage (%). For the parameters *viz*., fruit length (cm) and fruit diameter (cm), a random sample of five fruits/entry/replication was drawn from fourth and eighth pickings. The data recorded on five plants per treatment was averaged for use in statistical analysis. Data were analyzed according to ANOVA techniques, as outlined by Panse and Sukhatme (1985), to determine the significant differences among genotypes for all the characters. Components of genetic variance were estimated from the data obtained on the diallel crosses by the method given by Griffing's Method-II and Model-I (Griffing, 1956) as outlined by Singh and Chaudhary (1979).

RESULTS AND DISCUSSION

An important step in a breeding programme is to adopt a suitable breeding strategy for the purposeful management of generated variability which largely depends upon type of gene action in the population for the traits under genetic improvement (Sprague, 1966). A knowledge of gene action helps to set an appropriate breeding strategy to accumulate fixable genes through selection. Genetic improvement in pod yield has always been a top priority of okra breeders. Pod yield and its related parameters are quantitative traits, which are controlled by several genes thus showing a range of values in segregating generation. Genetic analysis helps in identifying traits for improvement of yield potential. Dependable biometrical techniques dealing with the genetic analysis of important characters have greatly helped plant breeder in ascertain the nature of gene action. Among various techniques, genetic analysis formulated by Griffing (1956), provides a workable approach to assess the gene action involved in various attributes, so as to design an efficient breeding plan, for further genetic upgrading of the existing material.

Analysis of variance

The analysis of variance carried out for different traits of okra, *viz.,* days to 50% flowering, days to first picking, first fruit producing node, nodes per plant, internodal length, fruit length, fruit diameter, average fruit weight, plant height, harvest duration, fruits per plant, fruit yield per plant, dry matter and mucilage are presented in Table 1. Analysis of variance reported significant differences for all the traits studied except dry matter and revealed that sufficient genetic variability was generated for yield and related traits after crossing eight diverse genotypes of okra in a diallel mating design (excluding reciprocals).

Estimates of genetic components of variance

The nature of gene action has been inferred from the estimates of GCA and SCA variances. The estimates of combining ability variances (σ^2gca and σ^2sca) and the ratio of σ^2A/σ^2D have been presented in Table 2. The values of σ^2sca ranged from 0.016 (mucilage) to 507.988 (fruit yield per plant), while σ^2gca ranged from -5.483 (fruit yield per plant) to 68.226 (plant height). The estimates of σ^2sca were higher in magnitude as compared to σ^2gca for all the characters except for first fruit producing node and harvest duration. The preponderance of σ^2sca revealed the predominant role of non-additive gene action governing these traits.

In the present investigation, the comparative estimates of σ^2sca, σ^2gca, σ^2A, σ^2D and σ^2A/σ^2D revealed that non-additive gene action is controlling the expression of days to 50% flowering, nodes per plant, fruit length, fruit diameter, plant height, fruits per plant and mucilage. For fruit yield per plant and dry matter, only dominant component of variance (σ^2D) was observed which indicated the presence of non-additive gene action, hence, heterosis breeding is required to be followed for exploitation of these traits. The traits *viz*., days to first picking, first fruit producing node, internodal length, average fruit weight and harvest duration are controlled by additive gene action, as the ratio σ^2A/σ^2D is greater

Table 1. Analysis of variance for quantitative and quality traits in okra.

Traits	Mean squares			
	Sources	Replication	Treatment	Error
	df	2	35	70
Quantitative traits				
Days to 50 % flowering		1.676	10.651*	2.676
Days to first picking		8.951	12.519*	3.939
First fruit producing node		0.073	0.262*	0.062
Nodes per plant		0.100	2.950*	1.023
Internodal length (cm)		0.259	3.927*	0.344
Fruit length (cm)		0.209	0.945*	0.331
Fruit diameter (cm)		1.201	3.713*	0.901
Average fruit weight (g)		1.542	2.126*	1.067
Plant height (cm)		108.193	1123.172*	188.028
Harvest duration (days)		14.074	17.986*	9.459
Fruits per plant		1.715	4.441*	1.159
Fruit yield per plant (g)		117.264	1723.012*	536.737
Quality traits				
Dry matter (%)		0.623	0.815	0.539
Mucilage (%)		0.009	0.049*	0.010

*Significant at 5% level.

Table 2. Variance due to general and specific combining ability and their ratio for different quantitative and qualitative traits in okra.

Components / Traits	σ^2 gca	σ^2sca	σ^2A	σ^2D	σ^2A /σ^2D
Quantitative traits					
Days to 50% flowering	0.593	1.840	1.187	1.840	0.645
Days to first picking	0.816	1.535	1.632	1.535	1.063
First fruit producing node	0.026	0.019	0.052	0.019	2.737
Nodes per plant	0.053	0.670	0.107	0.670	0.160
Internodal length (cm)	0.338	0.647	0.677	0.647	1.046
Fruit length (cm)	0.040	0.156	0.080	0.156	0.513
Fruit diameter (cm)	0.235	0.583	0.471	0.583	0.808
Average fruit weight (g)	0.110	0.167	0.220	0.167	1.317
Plant height (cm)	68.226	219.078	136.452	219.078	0.623
Harvest duration (days)	1.127	0.736	2.254	0.736	3.063
Fruits per plant	0.025	1.306	0.049	1.306	0.038
Fruit yield per plant (g)	-5.483	507.988	-10.965	507.988	-0.022
Quality traits					
Dry matter (%)	-0.001	0.118	-0.003	0.118	-0.025
Mucilage (%)	0.000	0.016	0.001	0.016	0.063

σ^2gca = general combining ability variance; σ^2sca = specific combining ability variance; σ^2A = additive component of variance and σ^2D = dominant component of variance.

than unity. Hence, pedigree selection could be exploited for these traits. For the traits *viz*., days to first picking, internodal length and average fruit weight, where σ^2sca is higher than σ^2gca but, σ^2D is less than σ^2A. It suggests that the estimates of GCA variance include additive variance and also a portion of additive and higher order

epistatic interactions. Under such conditions, recurrent selections shall prove effective. These results of the present investigation are in conformity to the findings of Kachhadia et al. (2011), Parmar et al. (2012) and Kumar et al. (2014). However, contradictory reports are also available in literature with respect to gene action studies which can be due to different genetic material used in the present study. Since both additive and non-additive variances were found to be important in the genetic control of all quantitative and quality traits in the present study, the use of a population improvement method in the form of diallel selective mating or mass selection with concurrent random mating might lead to release of new varieties with higher yield in okra.

Conclusion

Sufficient genetic variability was generated for yield and related traits after crossing eight diverse genotypes of okra in a diallel mating design (excluding reciprocals). The presence of non-additive gene action revealed that heterosis breeding is required to be followed for further improvement of okra.

Conflict of Interests

The authors have not declared any conflict of interests.

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African Journal of Agricultural Research

Full Length Research Paper

Optimizing the nitrogen rate in the rice crop in relation to soil mineralized nitrogen with anaerobic incubation without shaking to different times and temperatures

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Nitrogen fertilization in rice crop are based on crop needs and the capacity of the soil to supply it. In this study six rice soils of central Chile were fertilized with 0, 80 and 160 kg N ha^{-1} and incubated in anaerobic conditions for time periods from 0 to 28 days at 20 and 40°C. Field experiments were conducted in the same soils with equal N rates. Nitrogen mineralization showed a quadratic response that was directly proportional to incubation time, N rate used, and increase in incubation temperature. At the same time, mineralized N exhibited patterns of different magnitude, including in soils of the same order; therefore, this N supply capacity indicator is soil-dependent. The yield was maximized with 80 kg N ha^{-1} and the N uptake was highly correlated with the N mineralized for both 21 days at 20°C and 7 days at 40ºC. The optimum N rate to apply was represented using a lineal model that associates the yield with the crop N needs, the N soil supply through mineralization and the supplement that must be supplied by the N fertilization.

Key words: Paddy rice soils, anaerobic incubation method, nitrogen, mineralization, nitrogen optimization.

INTRODUCTION

Rice, *Oryza sativa* L., is very important in the diet of the world's population because of its nutritional value (Juliano, 1993) and low price. In the year 2008, the world area cultivated with rice was approximately 155 million ha with yields of 661 million tons (Kögel-Knabner et al., 2010), with the greatest cultivated area being in Asia (Bouman et al., 2007; Jing et al., 2008; Kögel-Knabner et al., 2010). Worldwide, cultivated paddy rice soils belong to five taxonomic orders: Entisols, Inceptisols, Alfisols, Vertisols, and Ultisols (López, 1991; Soil Survey Staff, 1999).

Rice production depends on several factors such as climate, soil physical condition, soil chemical fertility, water management, sowing date, cultivar, seed rate, weed control, and fertilization (Angus et al., 1994), of which fertilized Nitrogen (N) is the main nutrient associated with yield (Angus et al., 1994; Bouman et al., 2007; De-Xi et al., 2007; Jing et al., 2008; Sahrawat,

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2006; Wilson et al., 1994a), showed that the use of deficient or excessive N rates generate alterations in the crop cycle which affects the length of the vegetative and reproductive cycles, with negative effects on crop productivity (Hirzel et al., 2011a; Ortega, 2007).

To optimize N fertilizer supply to rice crop, it is important to know of the amount of N supplied by the soil mineralization (Angus et al., 1994; Sainz et al., 2008) since crop N uptake derives mainly from soil reserves (organic matter (OM) mineralization, microbial biomass turnover, and N-NH_4^+ fixed in clay) (Jokela and Randall, 1997; Jensen et al., 2000; Sainz et al., 2004; Sahrawat, 2006), and N fertilization (Wienhold, 2007). A small fraction is derived from irrigation water and other environmental and biotic sources. Soil N supply or the quantity of available mineral N for plant uptake is variable and difficult to estimate, and represents only a very small fraction of total soil N (Scott et al., 2005; Wienhold, 2007). Since mineralization can substantially contribute to plant available N, it is necessary to have adequate methods for its quantification (Angus et al., 1994; Bushong et al., 2007; Soon et al., 2007). Several authors have proven that anaerobic incubation is a good method for assessing potentially mineralized N because the initially available soil N is scarce, and a continuous N supply to the rice crop depends on mineralized ammonium from labile organic N in flooded soil (Angus et al., 1994; Bushong et al., 2007; Rodriguez et al., 2008; Soon et al., 2007; Waring and Bremner, 1964; Wilson et al., 1994a). Additionally, Angus et al. (1994) suggested that mineralized N that is measured in flooded soil during rice growth is a very good indicator of potential N uptake by the crop.

Previous investigations have studied the effects of incubation time and temperature on N mineralization (Angus et al., 1994; Bushong et al., 2007; Wilson et al., 1994a). Shorter incubation time (7 d at 40°C) generally measures the contribution of microbial biomass and soluble N sources, whereas long incubations can measure the whole active fraction of OM (Scott et al., 2005), and the choice of a given method should be based on the correlation between the N uptake and the N mineralized (Wilson et al., 1994b). Moreover, field experiments carried out in Chile indicated that soil N supply in paddy rice soil is higher than that indicated using a short time of anaerobic incubation (Hirzel et al., 2011a), so both time and temperature of soil incubation will be selected in relationship with the N uptake obtained in field experiments.

Sahrawat and Narteh (2001, 2003) indicated that mineralizable N under anaerobic incubation is controlled by the contents of OM and reducible iron (Fe). In a previous study, soils of different orders (Alfisols, Entisols, Inceptisols, Mollisols, Ultisols and Vertisols) had diverse rates of mineralized N-NH_4^+ using anaerobic incubation at 40ºC for 14 days or acid oxidation (Bushong et al., 2007), which could suggest that the N mineralized in the soils may depend on the soil order and the associated clay type, as well as the fraction of residual N that is fixed on to the clays (Jensen et al., 2000). Since OM is an important source for available N in paddy rice soil, the quantity of OM is very important for potential crop yields (Olk et al., 1999). Reichardt et al. (1999) also state that the formation and mineralization of soil OM depend on C and N biogeochemical pathways that are governed by soil enzymes, and the size of the pool of microbial biomass (normally only 2 to 4% of total C), which is the most labile of soil OM. Moreover, the chemical environment of flooded soils affects OM quality, especially the proportion of organic N fractions and dominant chemical structures of specific soil OM pools that contribute to N mineralization (Sahrawat, 2006). Olk and Senesi (1999) point out that soil OM seems to play a significant role in crop nutrient uptake in intensively cropped lowland rice soils, but more research is needed to learn how submerged conditions of this unique cropping system affect soil OM and potential nutrient cycling and uptake by plants. Functions of soil biota related to N immobilization and mineralization are influenced by organic C content and fertilizer inputs, as well as soil redox potential that is influenced by irrigation (Kögel-Knabner et al., 2010; Reichardt et al., 1999).

The supply of N in soil plays an important role in the overall N nutrition of wetland rice because one half to two-thirds of total N uptake, even in paddies with N fertilization, comes from the soil N pool (Sahrawat, 1983). Based on this information, the soil type (taxonomical order and chemical or physical properties) and soil incubation method (time and temperature) could affect the amount of mineralized N. Additionally, applying N to soil could also affect the amount of mineralized N and the subsequent relationship between N uptake and mineralized N, because N fertilization affects microbial activity in soil (Jensen et al., 2000).

Chile has 23,900 ha of soils cultivated with rice (ODEPA, 2007) under the taxonomic orders of Inceptisols, Alfisols, and Vertisols (CIREN, 1997), which are located between 34º and 36º S (Alvarado and Hernaiz, 2007). Those soils were formed in sedimentary deposits of fluvial glacial and volcanic origin, principally from the Andes Mountains (Stolpe, 2006).

Considering that Chile has a variety of rice paddy soils with different N supply capacity and potential for yields, and in order to find out the optimum N rate in the rice crop associate to a method of soil incubation as predictor of the N supply, the objectives of this investigation were; (i) determinate the N mineralized in laboratory incubations in anaerobic conditions for different times and temperatures in six Chilean paddy soils and determine its relationship with the N uptake by the rice crop in field conditions, and (ii) adjust mathematically the N rate to use in the rice crop that will optimize the yield in different soil conditions in relation to its N need and the N soil supply through of the mineralization.

Table 1. Physical and chemical properties of the Inceptisols, Alfisols and Vertisols soils (0 to 20 cm depth) before crop establishment for both seasons.

Parameter	Rice paddy soil					
	Inceptisols		Alfisols		Vertisols	
	1	2	1	2	1	2
	Season1	Season2	Season1	Season2	Season1	Season2
Clay (%)	34.4	26.3	34.1	32.6	36.1	39.9
Silt (%)	41.3	39.4	38.8	36.4	32.6	30.4
Sand (%)	24.3	34.3	27.1	31.0	31.2	29.7
Bulk density (g cm^{-3})	1.72	1.64	2.09	2.01	1.89	1.82
Total porosity (%)	35.1	38.1	22.3	24.2	28.7	31.3
pH (soil:water 1:5)	5.8	6.7	5.2	5.5	5.7	5.9
Organic matter (g kg^{-1})	29.0	12.8	48.0	49.5	26.0	24.5
EC (dS m^{-1})	0.04	0.03	0.10	0.12	0.08	0.07
Ratio C:N	12.0	11.7	12.0	11.8	11.4	11.5
P Olsen (mg kg^{-1})	4.3	2.5	12.0	13.5	7.0	9.8
Exchangeable K ($cmol_c$ kg^{-1})	0.24	0.18	0.26	0.28	0.21	0.28
Exchangeable Ca ($cmol_c$ kg^{-1})	7.30	6.77	6.30	6.92	7.00	8.57
Exchangeable Mg ($cmol_c$ kg^{-1})	2.70	3.20	2.30	2.61	3.60	3.59
Exchangeable Na ($cmol_c$ kg^{-1})	0.22	0.25	0.30	0.29	0.22	0.24
Exchangeable Al ($cmol_c$ kg^{-1})	0.05	0.01	0.12	0.04	0.02	0.03
Available Fe (mg kg^{-1})	173.0	95.0	266.0	203.0	137.0	95.1
Available Mn (mg kg^{-1})	159.0	64.0	189.0	295.0	198.0	123.0
Available Zn (mg kg^{-1})	0.9	0.7	2.3	1.4	1.8	0.9
Available Cu (mg kg^{-1})	4.3	1.8	5.1	5.2	3.9	3.8
Available B (mg kg^{-1})	0.11	0.14	0.19	0.31	0.15	0.17
Available S (mg kg^{-1})	15.0	4.6	32.0	22.1	1.0	1.1

MATERIALS AND METHODS

The experiment was conducted in the 2011-2012 and 2012-2013 growing season in three paddy rice soils in central Chile, including the orders Inceptisol, Alfisol and Vertisol with the soil types Achibueno loam (fine, mixed, superactive, thermic Fluventic Xerochrepts), Parral clay loam (fine, mixed, active, thermic Aquic Haploxeralfs), and Quella clay loam (fine, smectitic, thermic Aquic Durixererts) (CIREN, 1997). The investigation was conducted under both controlled laboratory conditions for soil incubations (soil without plants) to measure N mineralization, and field conditions (crop in the field) to measure the amount of N extracted from soil by rice in both seasons. Soil samples were collected in cores of 0 to 20 cm depth before crop establishment in both seasons; which were air-dried and subsequently characterized for physical and chemical properties (Table 1). Soils were fertilized in both experimental conditions (lab and field) with three N rates of 0, 80, and 160 kg ha^{-1} as urea, 60 kg P_2O_5, and 60 kg K_2O as triple super phosphate and potassium chloride, respectively. Before initiating the laboratory incubation procedure, the N, P, and K rates were adjusted accordingly with regard to bulk density of the soils (Table 1). The N rates were chosen because in previous experiments in the same study area they had been verified to produce a range of crop responses (Hirzel et al., 2011a, b; Ortega, 2007). The analytical procedures to characterize the soils were carried out in the laboratory of the Instituto de Investigaciones Agropecuarias (INIA), Chile using standard methodologies (Sadzawka et al., 2006). All samples were air-dried and ground to pass a 2 mm sieve. Soil pH was determined in 1:2.5 soil:water extracts using a pH meter; electrical conductivity was measured using a conductivity cell (soil:water ratio 1:5); organic C and total N by a total elemental analyzer (Vario MAX CNS, Elementar, Hanau, Germany); soil-extractable P was determined in 0.5 M $NaHCO_3$ (Olsen-P) using the molybdate-ascorbic acid method; soil available K, Ca, Mg, and Na were determined by 1 M NH_4OAc extraction followed by flame emission spectrometry (K and Na) and atomic absorption (Ca and Mg). Soil-extractable S-SO_4 was determinated with 0.01 M calcium phosphate and turbidimeter; soil micronutrient and trace element concentrations were determined in a DTPA (diethylentriamine pentaacetic acid) extract (Lindsay and Norvell, 1978) by atomic absorption spectrometry. The concentration of B was determined by extraction in hot water with azometine-H.

For the laboratory experiment, soil samples were incubated in controlled conditions with anaerobic incubation without shaking for 7, 14, 21, and 28 days at 20 and 40ºC using a CARBOLITE model PIC 200 incubator. As previously stated, shorter incubation times generally measure microbial biomass and soluble N sources, whereas longer incubation times can measure potentially mineralized N from the whole active fraction of OM (Scott et al., 2005; Hirzel et al., 2011a) and indicate more precisely the soil N supply capacity. The effects of the incubations at different times and temperatures were compared to N mineralization in field conditions of both seasons (N uptake by the control without N).

The field experiments also included N uptake as determined in whole-plant analysis and grain yield adjusted to 14.5% of moisture content.

The anaerobic incubation methods to estimate N mineralization as N-NH_4^+ were carried out as follows: Five gram of soil and 12.5

mL of distilled water were placed in a test tube, sealed with a stopper, and incubated anaerobically for 7, 14, 21 and 28 days at 20 and 40ºC without shaking. In addition, the initial N-NH_4^+, without incubation (0 days), was determined. To measure the mineralized N, extracts of ammonium from soil were obtained by adding 12.5 mL 2M KCl to the soil-tubes, and the mixture shaken for 1 h (Mulnavey, 1996), filtered, and N-$NH_4^+$was measured with a Skalar auto-analyzer.

For the field experiments, all plots were cultivated under traditional management to optimize crop growth in accordance with standard agronomic practices for rice crops in central Chile. Nitrogen (urea) was applied three times: 33% the day prior to sowing, 33% at tillering, and 34% at initial panicle (Hirzel et al., 2011b). The seed dose was 160 kg ha^{-1} in all experimental locations, and the cultivar used in the experiment was Zafiro-INIA (the second main variety used in Chile). The seed had been pre-germinated two days before sowing. After emergence, weed control consisted of a combination of herbicides of Penoxsulam (Ricer 240 g L^{-1}), MCPA (MCPA 750 SL 750 g L^{-1}), and Bentazon (Basagran 480 g L^{-1}) at rates of 0.03, 0.19, and 0.72 kg a.i. ha^{-1}, respectively. The crop was harvested at grain maturity (at 20% moisture content of grain), and grain moisture content was measured with a Satake model SS-5 moisture meter. Whole-plant dry matter and N concentration were determined with tissue samples collected at harvest time. In addition, the grain yield at 14.5% moisture content was calculated for the crop. Dried subsamples (2 g) were ground in a mill, passed through a 2 mm sieve, and analyzed for total N as determined by the macro-Kjeldahl procedure (Sadzawka et al., 2006). The total N extraction for the crop was calculated by multiplying the total dry matter by its N concentration.

Experimental design

The experimental design for soil incubation at two temperatures (20 and 40ºC) and three N rates (0, 80, and 160 kg ha^{-1}), was a split-split-split-split plot where the main plot was the season, the split plot was the temperature, the split-split plot the soils (three orders), the split-split-split plot incubation time (0, 7, 14, 21, and 28 days), and the split-split-split-split plot the N-rates. Experiments consisted of four replicates per treatment.

For the field experiment the experimental design was split-split plot where the main plot was the season (2), the split plot was the soil (three orders), and the split-split plot was the N rates. Experiments consisted of four replicates per treatment.

Results were analyzed with ANOVA and the Tukey test (P = 0.05) by the SAS general model procedure (SAS Institute, 1989). When there was interaction between sources of variations, its effects were compared by orthogonal contrasts.

The relationships between mineralized N in incubations without shaking for 7, 14, 21, and 28 days at two temperatures and N uptake by the crop was determined for each soil and evaluated with a linear mathematical model (Wilson et al., 1994b; Sahrawat, 2006; Hirzel et al., 2011b), using the SAS procedure for simple regression. At the same time, the relation between the grain yield of rice and the mineralized N in incubations for the times previously indicated was determined with a linear mathematical model.

Model of optimizing of the nitrogen rate

The model to be used is a linear equation where N uptake is associated with a range of grain yield per unit area, which is obtained during both experimental seasons and is the simple sum of N uptake from soil supply and N uptake as complementary fertilization expressed in Equation (1).

$$\underset{(kg\ ha^{-1})}{\text{N uptake}} = \underset{(kg\ ha^{-1})}{\text{N uptake from soil supply}} + \underset{(kg\ ha^{-1})}{\text{N uptake as complementary fertilization}} \quad (1)$$

Grain yields (GY) in the present study were associated with crop N uptake (N_{upt}), which was associated with mineralized N in each soil ($N_{soil\ mineralized}$) and the complementary effect of N applied as fertilizer (N rate). In this way, applied N was calculated as the difference between N_{upt} and $N_{soil\ mineralized}$ with specific values for each soil where $N_{soil\ mineralized}$ was determined by a linear regression model that associates N uptake with $N_{soil\ mineralized}$. Given that GY varies within an agronomic range for each study condition (Artacho et al., 2009; Hirzel et al., 2011a, b; Ortega, 2007), the need for N uptake was related to GY by using the maximum yield value obtained at each study site and expressed as an N uptake index in the rice crop (NUI-Rice) (kg N Mg $grain^{-1}$). The effect of N fertilization increases crop N uptake that is lower than the applied quantity because of the dynamic processes affecting N applied as fertilizer (Jensen et al., 2000; Scott et al., 2005; Wienhold, 2007). Therefore, the need to apply N to increase N uptake was expressed as the Index of the relationship between N applied and N uptake by the rice crop (IRNN) (kg N applied to kg N $uptake^{-1}$). The simple model that allows optimizing the N rate in the rice crop with regard to $N_{soil\ mineralized}$ is shown in Equation 2.

$$\underset{(kg\ ha^{-1})}{\text{N rate}} = [\underset{(Mg\ grain\ ha^{-1})}{GY} * \underset{(kg\ N\ Mg\ grain^{-1})}{\text{NUI-Rice}} - \underset{(kg\ ha^{-1})}{N_{soilmineralized}}] * \underset{(kg\ N\ applied\ to\ kg\ N\ uptake^{-1})}{\text{IRNN}}$$

Then, N rate, N to applied through of the fertilization, corresponding to the N uptake as complementary fertilization (kg ha^{-1}); GY, grain yield (Mg grain ha^{-1}); NUI-Rice, N uptake index in the rice crop (kg N Mg $grain^{-1}$); $N_{soil\ mineralized}$ (kg ha^{-1}), soil supply of N through mineralization in incubation conditions without shaking at 20ºC for 21-days or 40ºC for 7-days

IRNN, Index of the relationship between N applied and N uptake by the rice crop (kg N applied to kg N $uptake^{-1}$) as effect of the N fertilization (determinate as the difference of N uptake between the treatment fertilized and the control without N fertilization)

RESULTS AND DISCUSSION

Soil physical and chemical properties (Table 1) mainly indicated that no limitations exist for rice crop production, with the exception of P concentration in the Inceptisol in both of the evaluated seasons and B concentration in all the soils and both evaluated seasons in accordance with the critical levels that have been established by the Instituto de Investigaciones Agropecuarias for the rice zone in Chile. Although the exchangeable K concentration can be a limiting factor in the Inceptisol for the second season and in the Vertisol for the first season that was evaluated (Table 1), applying this element in field experiments allowed the correction of these limitations in accordance with the reference rate used in the zone under study (Ortega, 2007).

The statistical analysis of mineralized N in incubated soils (Table 2) indicated there were differences between seasons ($p<0.01$), incubation temperatures ($p<0.01$), soils ($p<0.01$), incubation times ($p<0.01$), and interactions between most of the combinations of the sources of variation ($p<0.01$); there was no interaction between

Table 2. Contrast and statistical analysis of sources of variation for incubations without shaking at different temperatures, for different soils, incubations times, and N rates.

Contrast	Significant
Year (Y)	
Season 1 - Season 2	**
Temperature (ºC) (T)	
20 - 40	**
Soil order (S)	
Inceptisols - Alfisols	**
Inceptisols - Vertisols	**
Alfisols - Vertisols	**
Y * T	**
Y * S	**
T * S	**
Y * T * S	**
Incubation times (days) (t)	
7 - 14	**
7 - 21	**
7 - 28	**
14 - 21	**
14 - 28	NS
21 - 28	**
Y * t	**
T * t	**
S * t	**
Y * T * S * t	**
N-rates (kg ha^{-1}) (N)	
0 - 80	**
0 - 160	**
80 - 160	**
Y * N	**
T * N	NS
S * N	NS
t * N	**
Y * T * S * t * N	**

*, ** Significant at the 0.05 and 0.01 probability levels. NS, not significant.

temperature and N rate ($p>0.05$) and between soils and N rate ($p>0.05$). The contrasts of different incubation times indicated a difference for most of the times (Table 2), with the exception of the comparison between 14 and 28 d since mineralized N exhibited a quadratic behavior with a maximum on day 21 and a generalized decrease in mineralized N when time was increased from 21 to 28 days, independently of temperature and N rate (Figure 1a to f). The contrasts between N rates (Table 2) indicated differences in mineralized N for the three N rates being used, which showed that mineralized N tends to be directly proportional to the N rate being used (Figure 1a to f). The differences in mineralized N between both evaluated seasons are mainly related to the differences in chemical properties of each soil (Table 1) since mineralized N is associated with OM content and reducible Fe (Sahrawat and Narteh, 2001, 2003). Meanwhile, differences in mineralized N obtained at both incubation temperatures (Table 2, Figure 1a to f) suggest that the correlation to be performed between N uptake and mineralized N must be adjusted for each incubation temperature and also take into account differences between soils and incubation times given the interactions that were obtained between these sources of variation and the incubation temperature ($p<0.01$). In addition, for future soil incubations with the objective of standarized the N supply capacity to rice crop by soil type, would selected the incubation temperature between both evaluated in this experiment (20 and 40°C).

Differences in mineralized N between different soil orders

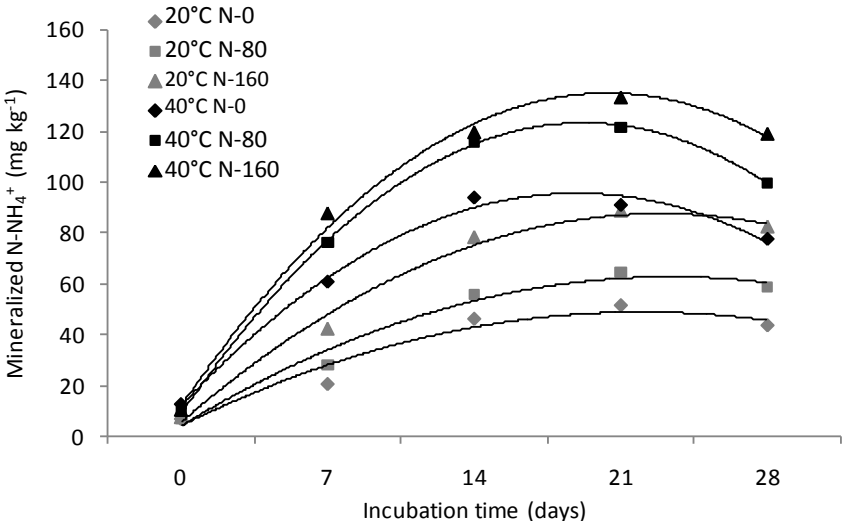

Figure 1a. Mineralized $N\text{-}NH_4^+$ in anaerobic conditions in the Inceptisol1 soil incubated to two temperatures (20 and 40ºC) and three N rates (0, 80 and 160 kg ha^{-1}) during a period of 28 days. The quadratic model for each temperature and N rates were; $Y = 28.74 + 37.25*X - 4.485*X^2$, $R^2 = 0.94$ for 20ºC and N-0 kg ha^{-1}; $Y = 35.96 + 45.33*X - 5.214*X^2$, $R^2 = 0.98$ for 20ºC and N-80 kg ha^{-1}; $Y = 52.35 + 65.39*X - 7.646*X^2$, $R^2 = 0.99$ for 20ºC and N-160 kg ha^{-1}; $Y = 60.55 + 84.17*X - 11.35*X^2$, $R^2 = 0.99$ for 40ºC and N-0 kg ha^{-1}; $Y = 87.90 + 112.60*X - 15.03*X^2$, $R^2 = 0.99$ for 40ºC and N-80 kg ha^{-1}; $Y = 86.04 + 113.00*X - 14.43*X^2$, $R^2 = 0.99$ for 40ºC and N-160 kg ha^{-1}.

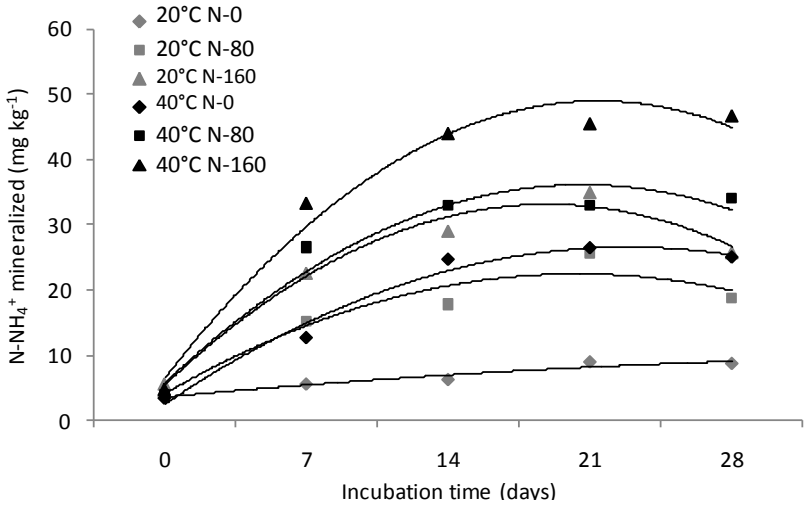

Figure 1b. Mineralized $N\text{-}NH_4^+$ in anaerobic conditions in the Inceptisol2 soil incubated to two temperatures (20 and 40ºC) and three N rates (0, 80 and 160 kg ha^{-1}) during a period of 28 days. The quadratic model for each temperature and N rates were; $Y = 1.35 + 2.296*X - 0.158*X^2$, $R^2 = 0.94$ for 20ºC and N-0 kg ha^{-1}; $Y = -10.46 + 16.75*X - 2.143*X^2$, $R^2 = 0.92$ for 20ºC and N-80 kg ha^{-1}; $Y = -19.0 + 28.11*X - 3.803*X^2$, $R^2 = 0.98$ for 20ºC and N-160 kg ha^{-1}; $Y = -14.36 + 19.16*X - 2.249*X^2$, $R^2 = 0.98$ for 40ºC and N-0 kg ha^{-1}; $Y = -18.30 + 27.59*X - 3.498*X^2$, $R^2 = 0.95$ for 40ºC and N-80 kg ha^{-1}; $Y = -25.93 + 36.91*X - 4.553*X^2$, $R^2 = 0.98$ for 40ºC and N-160 kg ha^{-1}.

(Table 2) are mainly due to the chemical properties of each soil (Table 1) (Sahrawat and Narteh, 2001, 2003) since mineralized N for the same soil order in both seasons showed differences in magnitude (Figure 1a to f)

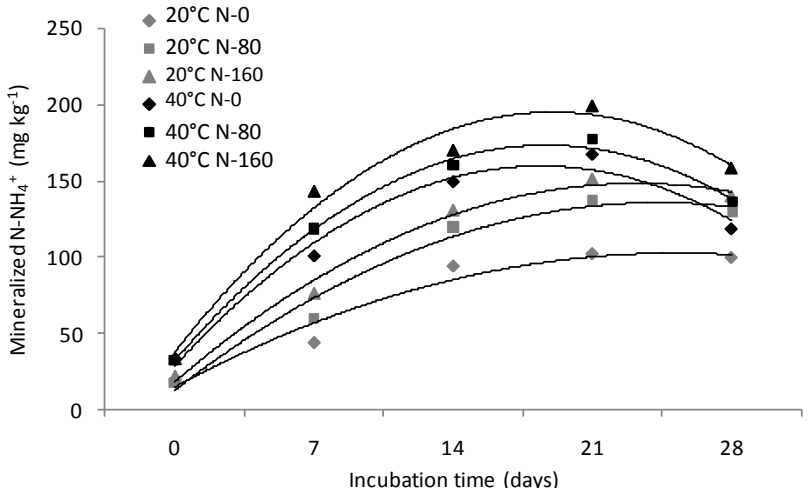

Figure 1c. Mineralized $N\text{-}NH_4^+$ in anaerobic conditions in the Alfisol1 soil incubated to two temperatures (20 and 40ºC) and three N rates (0, 80 and 160 kg ha^{-1}) during a period of 28 days. The quadratic model for each temperature and N rates were; $Y = 40.18 + 62.05*X - 6.739*X^2$, $R^2 = 0.95$ for 20ºC and N-0 kg ha^{-1}; $Y = 67.96 + 90.78*X - 10.14*X^2$; $R^2 = 0.97$ for 20ºC and N-80 kg ha^{-1}; $Y = 72.74 + 102.3*X - 11.85*X^2$, $R^2 = 0.99$ for 20ºC and N-160 kg ha^{-1}; $Y = 89.08 + 136.6*X - 18.82*X^2$, $R^2 = 0.98$ for 40ºC and N-0 kg ha^{-1}; $Y = 93.75 + 145.5*X - 19.83*X^2$, $R^2 = 0.99$ for 40ºC and N-80 kg ha^{-1}; $Y = 100.10 + 158.5*X - 21.33*X^2$, $R^2 = 0.98$ for 40ºC and N-160 kg ha^{-1}.

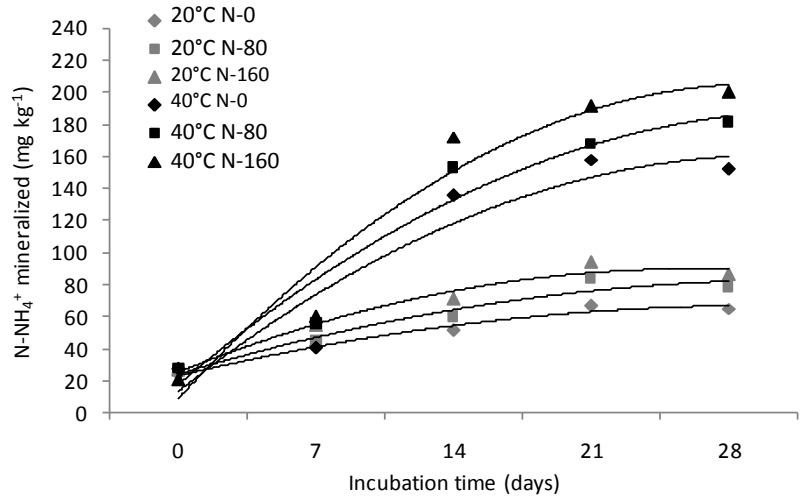

Figure 1d. Mineralized $N\text{-}NH_4^+$ in anaerobic conditions in the Alfisol2 soil incubated to two temperatures (20 and 40ºC) and three N rates (0, 80 and 160 kg ha^{-1}) during a period of 28 days. The quadratic model for each temperature and N rates were; $Y = 0.783 + 24.77*X - 2.332*X^2$, $R^2 = 0.98$ for 20ºC and N-0 kg ha^{-1}; $Y = -3.76 + 30.75*X - 2.738*X^2$, $R^2 = 0.96$ for 20ºC and N-80 kg ha^{-1}; $Y = -13.33 + 43.45*X - 4.60*X^2$, $R^2 = 0.97$ for 20ºC and N-160 kg ha^{-1}; $Y = -63.37 + 84.29*X - 7.931*X^2$, $R^2 = 0.89$ for 40ºC and N-0 kg ha^{-1}; $Y = -65.09 + 89.88*X - 7.967*X^2$, $R^2 = 0.94$ for 40ºC and N-80 kg ha^{-1}; $Y = -96.1 + 115.5*X - 11.07*X^2$, $R^2 = 0.94$ for 40ºC and N-160 kg ha^{-1}.

and interactions with the season being evaluated (Table 2).

Mineralized N in the Inceptisol1 soil (first evaluated season) with three applied N rates had values fluctuating between 7 and 88 mg $N\text{-}NH_4^+$ kg^{-1} and between 10 and 134 $N\text{-}NH_4^+$ kg^{-1} for temperatures of 20 and 40°C,

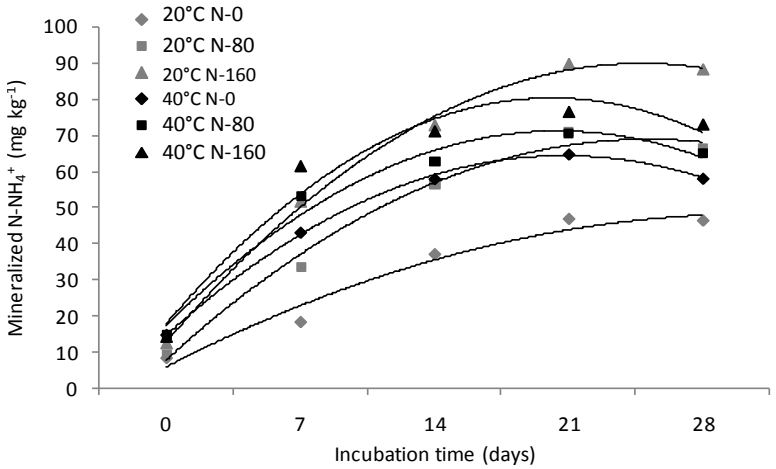

Figure 1e. Mineralized $N\text{-}NH_4^+$ in anaerobic conditions in the Vertisol1 soil incubated to two temperatures (20 and 40ºC) and three N rates (0, 80 and 160 kg ha^{-1}) during a period of 28 days. The quadratic model for each temperature and N rates were; $Y = 14.99 + 23.16*X - 2.123*X^2$, $R^2 = 0.97$ for 20ºC and N-0 kg ha^{-1}; $Y = 31.29 + 43.56*X - 4.736*X^2$, $R^2 = 0.99$ for 20ºC and N-80 kg ha^{-1}; $Y = 36.75 + 55.61*X - 6.109*X^2$, $R^2 = 0.99$ for 20ºC and N-160 kg ha^{-1}; $Y = 24.13 + 44.47*X - 5.601*X^2$, $R^2 = 0.99$ for 40ºC and N-0 kg ha^{-1}; $Y = 26.16 + 49.67*X - 6.330*X^2$, $R^2 = 0.98$ for 40ºC and N-80 kg ha^{-1}; $Y = 33.04 + 58.40*X - 7.529*X^2$, $R^2 = 0.96$ for 40ºC and N-160 kg ha^{-1}.

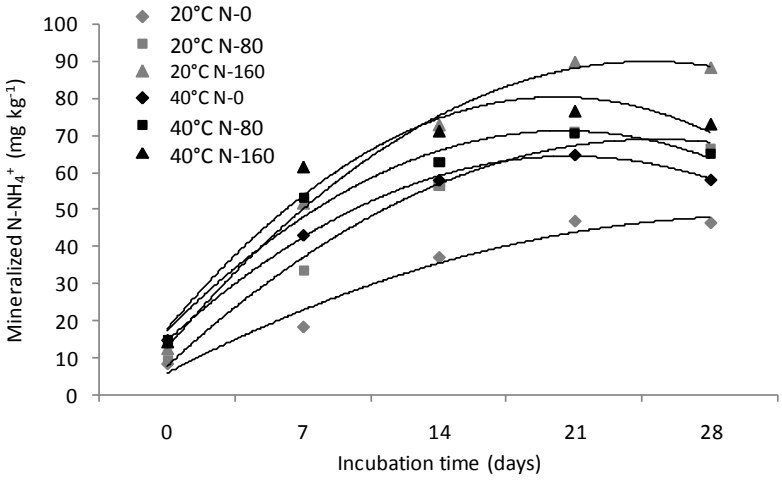

Figure 1f. Mineralized $N\text{-}NH_4^+$ in anaerobic conditions in the Vertisol2 soil incubated to two temperatures (20 and 40ºC) and three N rates (0, 80 and 160 kg ha^{-1}) during a period of 28 days. The quadratic model for each temperature and N rates were: $Y = -14.61 + 22.05*X - 2.641*X^2$, $R^2 = 0.98$ for 20ºC and N-0 kg ha^{-1}; $Y = -21.30 + 31.66*X - 3.823*X^2$, $R^2 = 0.98$ for 20ºC and N-80 kg ha^{-1}; $Y = -30.72 + 43.05*X - 5.496*X^2$, $R^2 = 0.99$ for 20ºC and N-160 kg ha^{-1}; $Y = -42.61 + 60.60*X - 7.178*X^2$, $R^2 = 0.98$ for 40ºC and N-0 kg ha^{-1}; $Y = -49.47 + 71.89*X - 9.041*X^2$, $R^2 = 0.96$ for 40ºC and N-80 kg ha^{-1}; $Y = -60.37 + 87.57*X - 11.20*X^2$, $R^2 = 0.95$ for 40ºC and N-160 kg ha^{-1}.

respectively (Figure 1a). For the Inceptisol2 soil (second evaluated season), mineralized N with three applied N rates fluctuated between 4 and 35 mg $N\text{-}NH_4^+$ kg^{-1} and between 4 and 46 mg $N\text{-}NH_4^+$ kg^{-1} for temperatures of 20 and 40°C, respectively (Figure 1b). Differential mean values in mineralized N for the 80 and 160 kg N ha^{-1} rates

compared with the control in the Inceptisol1 soil were 9 and 26 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 20°C), and 18 and 27 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 40°C) (Figure 1a), whereas the Inceptisol2 soil, showed different mean values compared with the control of 10 and 17 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 20°C) and 8 and 16 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 40°C) (Figure 1b) for the 80 and 160 kg N ha^{-1} rates compared with the control, respectively. The differences in magnitude that were obtained in mineralized N for the Inceptisol order and at the same incubation temperature (Figure 1a and b) suggest that it is not possible to find a general correlation between N uptake in the rice crop and mineralized N for this soil order; work with must be done with soil-specific correlations, which is also indicated by the interaction obtained between soil and incubation temperature (Table 2).

For soils of the Alfisol order, mineralized N in the Alfisol1 soil exhibited values fluctuating between 18 and 152 mg $N\text{-}NH_4^+$ kg^{-1} and between 33 and 199 $N\text{-}NH_4^+$ kg^{-1} at temperatures of 20 and 40°C, respectively (Figure 1c). Mineralized N in the Inceptisol2 soil (second evaluated season) fluctuated between 24 and 94 mg $N\text{-}NH_4^+$ kg^{-1} and between 20 and 200 mg $N\text{-}NH_4^+$ kg^{-1} at temperatures of 20 and 40ºC, respectively (Figure 1d). Different mineralized N mean values for rates of 80 and 160 kg N ha^{-1} compared with the control for the Alfisol1 soil (Figure 1c) were 21 and 32 and 11 and 27 mg $N\text{-}NH_4^+$ kg^{-1} at temperatures of 20 and 40°C, respectively. For the Alfisol2 soil, different mineralized N mean values for the 80 and 160 kg N ha^{-1} rates compared with the control were 9 and 17 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 20°C) and 15 and 27 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 40°C), respectively (Figure 1d). Just as it was found for the Inceptisol order, variations in magnitude of mineralized N for the Alfisol order soils at the same incubation temperature (Figure 1c and d) suggest that correlations between N uptake for the rice crop and mineralized N for soils used in the present study are soil-specific and cannot be generalized for this soil order.

For soils of the Vertisol order, mineralized N exhibited values fluctuating between 8 and 90 mg $N\text{-}NH_4^+$ kg^{-1} and between 14 and 77 $N\text{-}NH_4^+$ kg^{-1} at temperatures of 20 and 40°C, respectively, in the Vertisol1 soil (Figure 1e). In Vertisol2, mineralized N values fluctuated between 5 and 55 mg $N\text{-}NH_4^+$ kg^{-1} and between 8 and 107 $N\text{-}NH_4^+$ kg^{-1} at temperatures of 20 and 40°C, respectively (Figure 1f). Different mineralized N mean values in for the 80 and 160 kg N ha^{-1} rates compared with the control for Vertisol1 (Figure 1e) were 16 and 32, and 6 and 12 mg $N\text{-}NH_4^+$ kg^{-1} at temperatures of 20 and 40°C, respectively. For the Vertisol2 soil, the different mineralized N means for the 80 and 160 kg N ha^{-1} rates compared with the control were 9 and 15 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 20°C) and 7 and 19 mg $N\text{-}NH_4^+$ kg^{-1} (incubation at 40°C) (Figure 1d), respectively. Likewise for the Inceptisol and Alfisol soils, variations of magnitude obtained in mineralized N for soils of the Vertisol order with the same incubation temperature (Figure 1e and f) suggest that correlations between N uptake for the rice crop and mineralized N in the soils of the Vertisol order used in the present study are soil-specific and cannot be generalized for this soil order. For the three soils orders evaluates in the two seasons, both the N mineralization and the correlations soil-specific with N uptake, could response to the chemical properties as reducible Fe and OM content (Sahrawat and Narteh, 2001, 2003), and the variations of yield between seasons (Figure 2a and b).

Grain yield for the field experiment for the Inceptisols fluctuated between 5.1 and 7.7 Mg ha^{-1} (season 1) and between 7.8 and 11.8 Mg ha^{-1} (season 2) (Figure 2a and b), while that for the Alfisols fluctuated between 6.4 and 9.1 Mg ha^{-1} (season 1) and between 8.7 and 11.3 Mg ha^{-1} (season 2) (Figure 2a and b), and in the Vertisols fluctuated between 6.1 and 11.5 Mg ha^{-1} (season 1) and between 6.4 and 11.4 Mg ha^{-1} (Figure 2a and b). In general the highest yield in the three soils was achieved with 80 kg N ha^{-1} (Figure 2a and b), but too were observed differences between seasons for the same order soil which could be associate to its different climatic conditions (data not shown). Whole-plant dry matter production varied between 12.2 and 21.6 Mg ha^{-1} (season 1) and between 17.0 and 25.1 Mg ha^{-1} (season 2) in the Inceptisols; 16.8 and 24.6 Mg ha^{-1} (season 1) and between 13.3 and 21.7 Mg ha^{-1} (season 2) in the Alfisols; and 12.4 and 23.8 Mg ha^{-1}(season 1) and between 10.3 and 18.5 Mg ha^{-1} (season 2) in the Vertisols, and the highest DM production in the three soils was achieved with the 160 kg N ha^{-1} rate (data not shown). Whole-plant N concentration ranged from 5.8 to 6.6 g kg^{-1} (season 1) and from 8.5 to 8.7 g kg^{-1} (season 2) in the Inceptisols; 6.2 to 6.9 g kg^{-1} (season 1) and from 7.6 to 7.7 g kg^{-1} (season 2) in the Alfisols; and 7.1 to 7.6 g kg^{-1} (season 1) and from 7.2 to 7.9 g kg^{-1} (season 2) in the Vertisols (data not shown), and as in DM production the highest whole-plant N concentration was achieved with the 160 kg N ha^{-1} rate (data not shown). Differences between the DM production between seasons for the same soil order could response to its different climatic conditions (data not shown). The N uptake fluctuated between 71 and 142 kg ha^{-1} (season 1) and between 148 and 210 kg ha^{-1} (season 2) in the Inceptisols; 103 and 169 kg ha^{-1} (season 1) and between 102 and 168 kg ha^{-1} (season 2) in the Alfisols; and 88 and 181 Mg ha^{-1} (season 1) and between 81 and 134 kg ha^{-1} (season 2) in the Vertisols (Figure 3a and b). The highest N uptake in the six soils was achieved with 160 kg N ha^{-1}.

Grain yields (Figure 2a and b) were similar to those observed by several authors (Artacho et al*.*, 2009; Hirzel et al*.*, 2011a; Ortega, 2007) for the same cultivation area. Considering the mean of both seasons, the Vertisols had higher yield than the Inceptisol, which may be associated with its physical properties (structure and clay type) and its relationship with protecting organic N in the fine

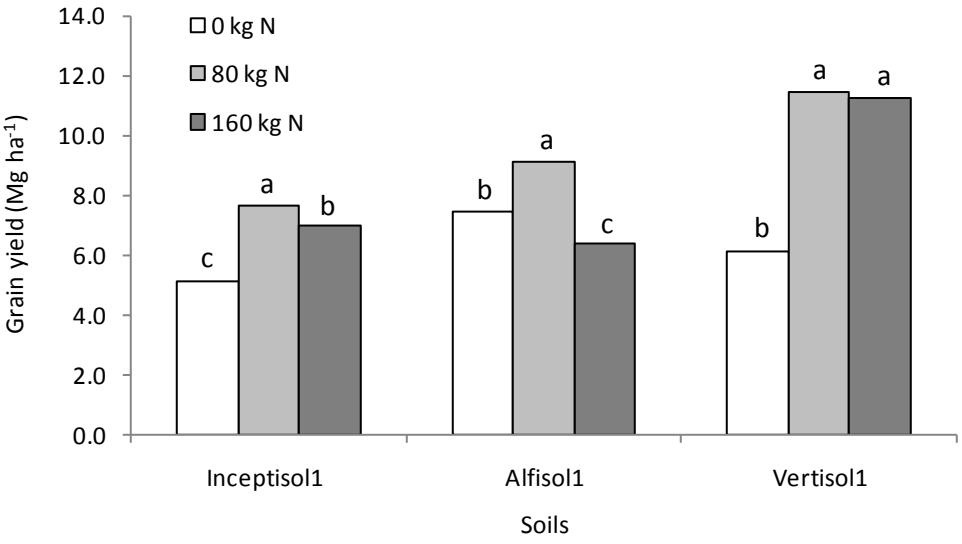

Figure 2a. Grain yield of rice in three paddy rice soils fertilized with three N rates (0, 80 and 160 kg ha^{-1}) for the first season of evaluation. Different letters over the bars of the same soil indicate significant differences according to Tukey's test ($p<0.05$).

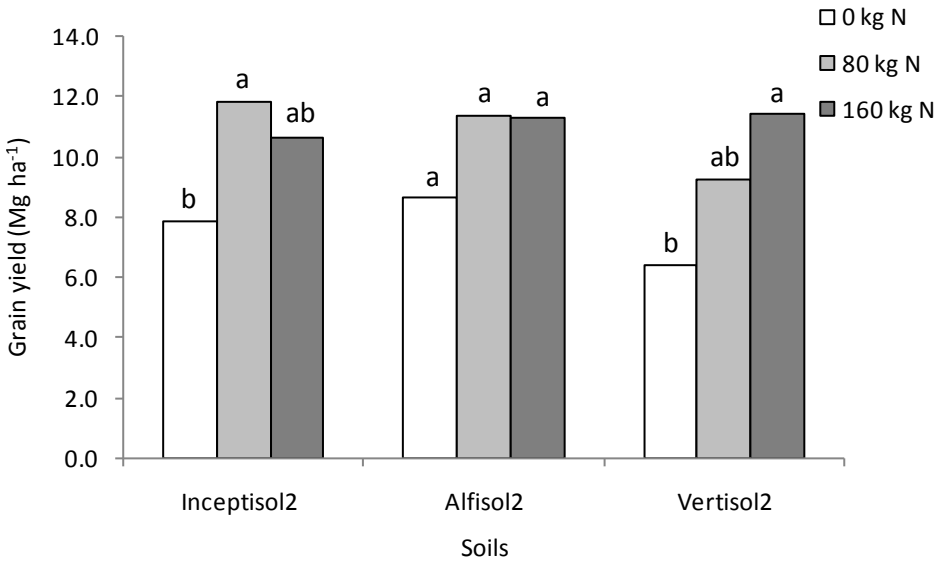

Figure 2b. Grain yield of rice in three paddy rice soils fertilized with three N rates (0, 80 and 160 kg ha^{-1}) for the second season of evaluation. Different letters over the bars of the same soil indicate significant differences according to Tukey's test ($p<0.05$).

fraction of the soil (Elliott, 1986; Greenland, 1965; Videla et al., 2004). The N uptake increased in association with the increase in N rate (Figure 3a and b), however the highest grain production in the six soils occurred with the 80 kg ha^{-1} rate (Figure 2a and b). This indicates that the highest N rate used (160 kg ha^{-1}) produced excessive consumption and no yield response or a negative response (Vanotti and Bundy, 1994), which was pointed out by Hirzel et al. (2011a) and Ortega (2007) in N fertilization studies for the same production zone. Mean differences obtained of the grain yield for Inceptisols and Alfisols between seasons indicate the effect of the interaction between the climate within the same season and the response to the N application, as was indicated by Ortega (2007) for the same production zone.

The simple linear regressions relating the mineralized N in incubations without shaking at 20 and 40ºC and the extracted N in the field crop are presented in the Table 3.

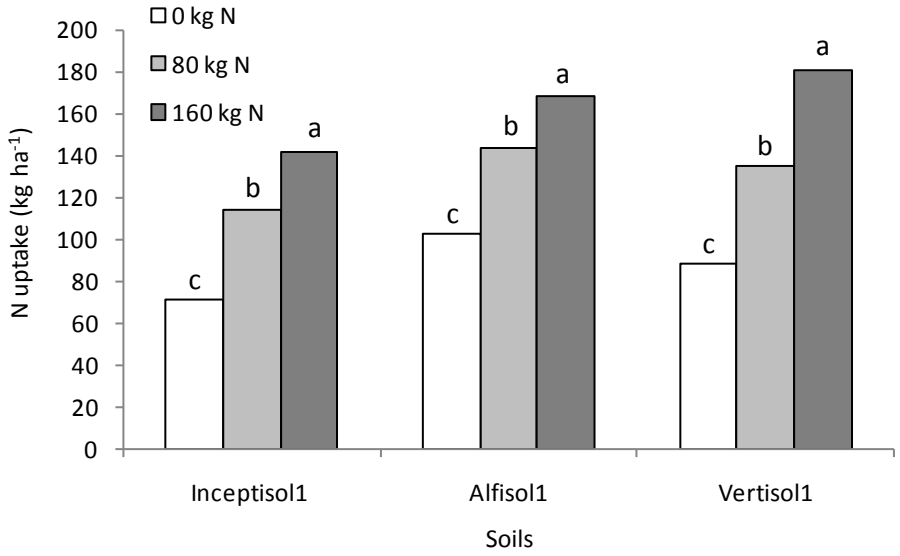

Figure 3a. N uptake in whole plant of rice in three paddy rice soils fertilized with three N rates (0, 80 and 160 kg ha^{-1}) for the first season of evaluation. Different letters over the bars of the same soil indicate significant differences according to Tukey's test ($p<0.05$).

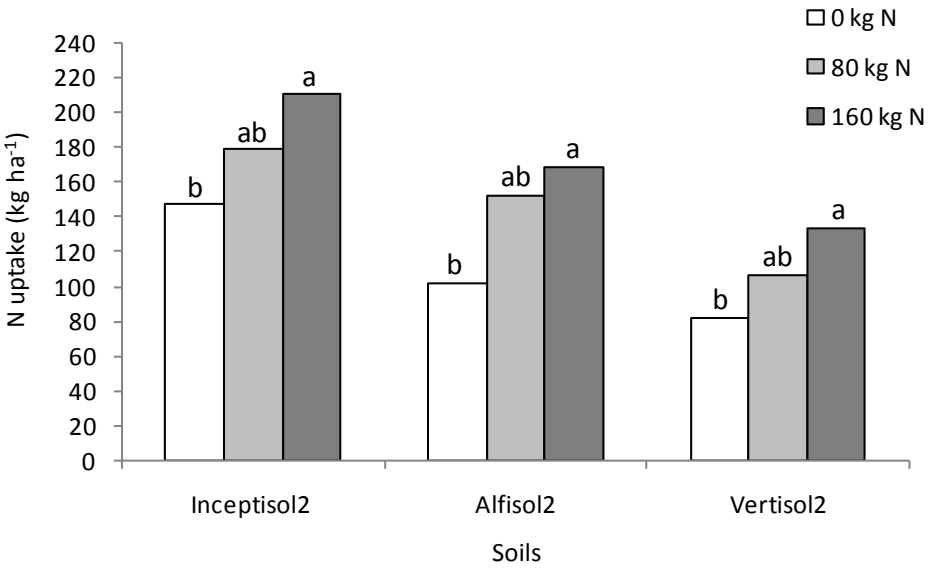

Figure 3b. N uptake in whole plant of rice in three paddy rice soils fertilized with three N rates (0, 80 and 160 kg ha^{-1}) for the second season of evaluation. Different letters over the bars of the same soil indicate significant differences according to Tukey's test ($p<0.05$).

For incubations at 20°C in general for both seasons the highest coefficient of determination (R^2) was in the 21-days, which was highly significant in Inceptisols, Vertisols and in one of the Alfisols soils (Table 3). The highest R^2 in the Alfisols were obtained in the 14-days incubation, which was slightly higher than the 21-days incubation value, but both values were significant (Table 3). For the incubations at 40°C in general the highest R^2 in all the soils was obtained at 7-days (Table 3), which was highly significant. However, the R^2 for the Inceptisol at 28-days in the first season was slightly higher than at 7-days, but both were highly significant (Table 3). A similar situation was presented for the R^2 in the Vertisol at 14-day in the second season (Table 3). The differences in mineralized N for temperatures and incubation times allow for determining an optimal incubation time for each

Table 3. Regression coefficients and lineal equations between total N uptake in rice crop for Inceptisols, Alfisols and Vertisols rice paddy soil in Chile during the season 2011-2012 (season 1) and 2012-2013 (season 2) and mineralized N-ammonium in anaerobic conditions without shaking for different incubation times.

Soil	Incubation time(days)	Season and soil	Regression coefficients and linear mathematical models			
			20°C		40°C	
			R^2	Equation	R^2	Equation
Inceptisol	7	1	0.249^{NS}	Y = 69.5 + 1.24*X	0.780**	Y = -68.0 + 2.36*X
		2	0.644**	Y = 130.6 + 3.36*X	0.791**	Y = 103.6 + 2.99*X
	14	1	0.649**	Y = 21.3 + 1.40*X	0.420*	Y = -61.4 + 1.55*X
		2	0.735**	Y = 130.9 + 2.72*X	0.623**	Y = 81.3 + 2.89*X
	21	1	0.778**	Y = -5.1 + 1.65*X	0.704**	Y = -55.0 + 1.42*X
		2	0.715**	Y = 124.2 + 2.87*X	0.773**	Y = 66.6 + 3.22*X
	28	1	0.756**	Y = 4.9 + 1.70*X	0.815**	Y = -48.5 + 1.59*X
		2	0.641**	Y = 124.6 + 3.32*X	0.510**	Y = 101 + 2.22*X
Alfisol	7	1	0.574**	Y = 38.9 + 1.66*X	0.555**	Y = -17.5 + 1.29*X
		2	0.358*	Y = -12.4 + 3.26*X	0.790**	Y=-128 + 5.51*X
	14	1	0.675**	Y = -49.4 + 1.64*X	0.415*	Y = -103 + 1.51*X
		2	0.527**	Y = -34.9 + 2.90*X	0.729**	Y = -122 + 1.71*X
	21	1	0.600**	Y = -13.8 + 1.17*X	0.369*	Y = -101 + 1.32*X
		2	0.426*	Y = -46.8 + 2.39*X	0.565**	Y = -110 + 1.45*X
	28	1	0.366*	Y = 5.5 + 1.08*X	0.467*	Y = -17.4 + 1.13*X
		2	0.309^{NS}	Y = 10.5 + 1.70*X	0.555**	Y = -52 + 1.08*X
Vertisol	7	1	0.478*	Y = 69.8 + 1.96*X	0.575**	Y = -64.7 + 3.80*X
		2	0.550**	Y = 29.6 + 3.18*X	0.739**	Y = -55.5 + 2.29*X
	14	1	0.569**	Y = -1.7 + 2.78*X	0.526**	Y = -190 + 5.09*X
		2	0.420*	Y = 32.7 + 1.89*X	0.747**	Y = -75.1 + 2.22*X
	21	1	0.707**	Y = 2.5 + 1.87*X	0.403*	Y = -180+4.47*X
		2	0.527**	Y = -20.0 + 2.94*X	0.288NS	Y = -1.22+1.17*X
	28	1	0.566**	Y = 7.4 + 1.93*X	0.444*	Y = -120+3.90*X
		2	0.587**	Y = -5.9 + 2.87*X	0.280NS	Y = -32.1+1.55*X

*, ** Significant at the 0.05 and 0.01 probability levels; NS, not significant; Y, N uptake (kg ha^{-1}); X, N mineralized (mg kg $soil^{-1}$).

temperature (Table 3 and Figure 1a to f) since N mineralization responds to these two factors (Angus et al., 1994; Bushong et al., 2007; Scott et al., 2005; Wilson et al., 1994a). Although soil type interacted with incubation times, using the regression coefficient criterion and the significance level between mineralized ammonium-N and N absorbed by the crop, it is possible to determine the appropriate incubation time for each temperature (Table 3). For both seasons, the incubation times and temperatures suggest were 21-days by 20ºC and 7-days by 40°C, but in general the maximum R^2 values were obtained with incubations to 40ºC (Table 3). On the other hand the gradients of the equations were positive for all the soils and temperatures evaluated, showing the positive relationship between the total N uptake and the $N\text{-}NH_4^+$ mineralized (Table 3), as had been indicated by some authors (Hirzel et al., 2011b; Sahrawat, 2006; Wilson et al., 1994b). In addition, the relationship obtained by the N uptake and N mineralizated in this study for anaerobic incubations in the first season by 21-days to 20ºC were of 1.39, 1.01 and 1.90 kg mg^{-1} in inceptisol, alfisol and vertisol respectively; while that for incubations by 7-days to 40ºC this relationship were of 16.73, 1.53 and 2.59 kg mg^{-1} in inceptisol, alfisol and vertisol respectively (Figures 1a, b, c, and Figure 3a). For the second season the relationship between N uptake and N mineralizated for incubations by 21-days to 20°C this relationship were of 1.17, 1.03 and 2.05 kg mg^{-1} in inceptisol, alfisol and vertisol respectively; while that for incubations by 7-days to 40°C this relationship were of 11.68, 2.54 and 1.45 kg mg^{-1} in inceptisol, alfisol and vertisol respectively (Figure 2a, b, c and Figure 3b). For this index of relationship between the N uptake and N mineralizated, all the values fluctuated between 1.01 and 2.69 kg mg^{-1}, except in the inceptisol used in the second season, which could be associate to the high Mn content (Table 1) that reduce the N mineralization (Chang and Broadbent, 1982).

Table 4. Nitrogen Uptake Index in the rice crop (NUI-Rice) (kg N Mg−grain^{-1}) for Inceptisols, Alfisols and Vertisols rice paddy soil for both season of evaluation.

Nitrogen rate (kg ha^{-1})	Inceptisols		Alfisols		Vertisols	
	1	2	1	2	1	2
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
0	14.1^{Aa}	19.7^{Aa}	13.8^{Ab}	12.0^{Aa}	14.3^{Aab}	12.8^{Aa}
80	15.4^{Aa}	15.4^{Aa}	16.2^{Ab}	14.0^{Aa}	11.8^{Ab}	11.6^{Aa}
160	20.3^{Aa}	20.3^{Aa}	26.9^{Aa}	15.0 ^{Ba}	16.1^{Aa}	11.8^{Aa}

For each soil order different capital letters for the medium values of the treatments with a same N rate (0, 80 and 160 kg ha^{-1}) indicate significant differences between seasons according to Tukey's test ($p<0.05$). For each soil order different lower case letters for the medium values of the treatments in the same season indicate significant differences between the N rates used (0, 80 and 160 kg ha^{-1}) according to Tukey's test ($p<0.05$).

Table 5. Index of relationship between nitrogen applied and nitrogen uptake by the rice crop (kg N applied by kg N uptake^{-1}) (IRNN).

Soils	Season	IRNN(kg N applied by kg N uptake^{-1})
Inceptisol1	1	1.86^{a}
Inceptisol2	2	2.60^{a}
Alfisol1	1	1.97^{a}
Alfisol2	2	1.60^{a}
Vertisol1	1	1.71^{a}
Vertisol2	2	3.14^{a}
CV (%)		51.94

Different letters indicate significant differences according to Tukey's test ($p<0.05$). The IRNN was calculated in base of the N rate that allowed the maximixed grain yield (80 kg ha^{-1}).

Optimizing N applied as fertilizer was determined by equation 2 where GY (Mg grain ha^{-1}) is obtained from yield results from both seasons under study (Figure 2a and b); NUI-Rice (kg Mg grain^{-1}) (Table 4) is the relationship between N uptake (Figure 3a and b) and GY for the N rate that maximizes GY (Figure 2a and b). The $N_{soil\ mineralized}$ (kg ha^{-1}) is soil N supply through mineralization under incubation conditions without shaking at 20°C for 21 days or 40°C for 7 days (Table 3), and IRNN (kg N applied for kg N uptake^{-1}) (Table 5) was calculated on the basis of the N rate that maximizes GY (80 kg ha^{-1}) (Figure 2a and b).

The NUI-Rice values for each soil order were similar between seasons ($p<0.05$) and exhibited differences between N rate in the same soil order in only the first season evaluations in Alfisols and Vertisols ($p<0.05$) (Table 4). In general, NUI-Rice values were directly proportional to the N rate being used, with the exception of the Inceptisol and Vertisol in the first season (Table 4), which was associated with the marked response of the crop faced with low N rates that produce a dilution effect of this nutrient in the plant (Hirzel et al., 2011a) and a decrease in the N need index per Mg grain to be produced. When comparing soils for the N rate that maximized yield (80 kg ha^{-1}) (Figure 2a and b), Inceptisols and Alfisols presented a higher NUI-Rice value than Vertisols (mean values of 15.3 vs.11.7), which is associated with the highest GY (Figure 2a and b) and the lowest N uptake (Figure 3a and b) found in the Vertisols for the first and second season, respectively; this causes a dilution effect of N and a lower need index of this nutrient per yield unit. In this regard, some studies relating N uptake to GY allow obtain NUI-Rice values fluctuating between 8.3 and 43 kg N Mg grain^{-1} (Hossain et al., 2005; Peng et al., 2006; Witt et al., 1999; Ying et al., 1998) and with mean values that were higher than those found in the present study.

Although there was no significant difference associated with the high coefficient of variation (51.94%) in IRNN values (Table 5), quantitative differences were found between orders as well as for the same soil order, which were associated with the differences in N uptake in each season and soil being evaluated (Figure 3a and b). In general, the highest mean values were found in Vertisols followed by Inceptisols and with a lower value and higher stability in Alfisols (Table 5). These variations for the same soil order corroborate the fact that the N optimization model that was applied cannot be generalized for soil order and must be soil-specific; mineralizable N (soil N supply) also shows a greater

Table 6. Simulated N application rates according to the proposed optimization model.

Component of the optimization model	Soils					
	Inceptisol1	Inceptisol2	Alfisol1	Alfisol2	Vertisol1	Vertisol2
GY (Mg ha^{-1})	7	10	8	10	10	9
NUI-Rice (kg N Mg grain^{-1})	15.4	15.4	16.2	14.0	11.8	11.6
N uptake (kg ha^{-1})	107.8	154.0	129.6	140.0	118.0	104.4
IRNN (kg N applied by kg N uptake^{-1})	1.86	2.60	1.97	1.60	1.71	3.14
N mineralized in 21-d to 20ºC(mg kg^{-1})	51	9	102	67	47	30
N soil supply by incubations to 21-d to 20ºC(kg ha^{-1})	79	150	105	112	90	69
N mineralized in 7-d to 40ºC(mg kg^{-1})	61	13	101	40	43	56
N soil supply by incubations to 7-d to 40ºC(kg ha^{-1})	75	141	112	92	99	73
N rate in according to incubations to 21-d to 20ºC (kg ha^{-1})	53	12	48	44	49	112
N rate in according to incubations to 7-d to 40ºC (kg ha^{-1})	61	33	34	77	33	100

GY, Grain yield (Mg grain ha^{-1}); NUI-Rice, N uptake index in the rice crop (kg N Mg grain^{-1}); IRNN, Index of relationship between N applied and N uptake by the rice crop (kg N applied by kg N uptake^{-1}) as effect of the N fertilization (the supply of N soil without N is discounted).

association with chemical soil properties, such as reducible Fe and OM content (Sahrawat and Narteh, 2001, 2003).

Finally, simulations that were performed with the optimization model generated in the present study (Table 6) for the real maximum GY conditions (Figure 2a and b), and mineralized N without adding N at different temperatures (Figure 1a to f) showed differences in magnitude of the N rate to be used that fluctuated between 8 and 33 kg N ha^{-1} for the six evaluated soils; this is agronomically acceptable for the productive conditions of rice soils in Chile where total N rates do not surpass 112 kg N ha^{-1} (Table 6). These values are similar to those pointed out in previous studies by Hirzel et al. (2011a, b) and Ortega (2007) and lower than those found by Artacho et al. (2009) to maximize GY in the same study area.

Conclusions

Nitrogen mineralization in anaerobic incubation conditions at different times and temperatures showed a quadratic response that was directly proportional to incubation time, N rate used, and increase in incubation temperature. At the same time, mineralized N exhibited patterns of different magnitude, including in soils of the same order; therefore, this N supply capacity indicator is soil-dependent. Nitrogen uptake for the rice crop in field conditions was highly correlated in a linear way with mineralized N for both 21-days at 20ºC and 7-day at 40ºC. For almost all the soils studied the index of relationship between the N uptake and N mineralizated fluctuated between 1.01 and 2.69 kg mg^{-1}. Finally, the determination of the need for N to be applied in the rice crop for the evaluated soils can be represented by a linear optimization model that associates grain yield potential, N need per yield unit, natural soil supply through mineralization, and the N rate to increase uptake to meet the crop need that is not covered by the natural soil supply.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

The relationship between succulence and shoot biomass differences according to nutritional status in *Jatropha curcas* L.

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Diverse types of studies have examined the application of *Jatropha curcas* L. as a source of biofuel. Mineral nutrition levels modify the patterns of crops' growth and productivity, as well as the expression of morphophysiological traits of adaptive value. This study investigated the effects of nutrient solution concentration on biomass partition patterns and morphological attributes linked to water content in organs of *J. curcas*. Selected seedlings of accession 842 were cultivated in full experimental nutritive solution, and in solutions diluted to half concentration and a quarter concentration, all adjusted to pH 6.0. After 28 days under controlled conditions, plants were harvested and measured for height, leaf area and fresh and dry mass of leaves, petioles, stems, roots and total mass. From these data, specific leaf mass, leaf succulence and stem water content levels were calculated. The results indicated that according to increased nutritive solution concentration, plant shoots had up to a two-fold increase in height, and that a decrease in these concentrations caused drastic root and total dry mass reduction. At full concentration, there was a tendency towards dry mass allocation in roots. Comparatively, leaf traits were very sensitive to nutritional level without affecting leaf succulence. Contrastingly, relative to stems, these values significantly increased according to increased nutritive concentration. It could be concluded that beyond its productive importance, the nutritional level available to these plants exerts a positive influence on tissue water contents of succulent stems, whose ecophysiological importance demand additional studies.

key words: Leaf area, relative concentration of nutritive solution, specific leaf dry mass, stem succulence.

INTRODUCTION

Among the oleaginous plants grown with bioenergy purposes, *Jatropha curcas* L., a shrubby species of the Euphorbiaceae family, has received considerable attention as a possible raw material for biodiesel

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Figure 1. Partial view of *J. curcas* plants in the growth room: seedlings of access 852 (from Petrolina, Pernambuco State, Brazil) grown in plastic pots containing Hoagland and Arnon nutritive solution (1950), at three levels of dilution, with five repetitions.

production (Jongschaap et al., 2009). This use is based on a high potential of oil accumulation in the seeds (27-40%) and for being a perennial non-edible species, thus not competing with food crops for agricultural lands (Achten et al., 2008). Originating in Central America, and widely distributed in tropical and subtropical regions, the species has been celebrated for its rusticity, evidenced by its adaptation to diverse edapho-climatic conditions (Makkar and Becker, 2009; Divakara et al., 2010). However, *J. curcas* can still be considered a semi-wild species, in process of domestication and without defined cultivars (King et al., 2009), where many physiological and agronomic aspects remain open to investigation (Achten et al., 2010).

A structural property of *J. curcas* is the succulence of its stem tissues (Maes et al., 2009), a characteristic present in many species of the Euphorbiaceae family (Lüttge, 2008; Mwine and Van Damme, 2011). Generally, tissue succulence is associated with the occurrence of Crassulacean acid photosynthetic metabolism (CAM) or intermediate C_3/CAM in leaves or stems (Virzo de Santo et al., 1983; Martin et al., 1990; Hastilestari et al., 2013). In leaves, the succulence is related to the capacity for water storage by leaf area unit (Mantovani, 1999), while in stems, it can be approached by its water content, according to Maes et al. (2009).

Among the determinants of plant productivity, knowledge of nutritional requirements is essential for the proper formulation of crop fertilization practices. In this respect, various aspects of mineral nutrition of *J. curcas* have been focused in recent years (Laviola and Dias, 2008; Fernandes et al., 2013; Freiberger et al., 2014). Beyond its direct contribution to the formation of total biomass and its distribution between organs during the growth cycle of the plant, mineral nutrition level influences the expression of important morphological attributes for the adaptation of the species to diverse environmental conditions (McDonald et al., 1996; Illenseer and Paulilo, 2002). However, in respect to mineral nutrition of *J. curcas*, available information relative to both types of influences is quite limited.

For these reasons, our working hypothesis was that the nutritional level available for the initial vegetative growth of *J. curcas* exerts combined influences on biomass production and distribution as well as on morphological traits linked to tissues' water content.

MATERIALS AND METHODS

The experiment was conducted in the Department of Soils of the Federal Rural University of Rio de Janeiro (UFRRJ), Brazil (22°45'48"S; 43°41'23"W), in controlled environmental conditions. Mature seeds of the accession 842, from the UFRRJ *Jatropha* Germoplasm Bank, originally collected in Petrolina, Pernambuco State, Brazil (09°23'55"S; 40°30'03"W) were superficially disinfected (NaClO, 2%, 2 min) and submitted to germination in a greenhouse, using plastic trays filled with autoclaved sand (101.3 kPa; 120°C; 60 min) as substrate.

Initially, the seeds were embedded with distilled water and, after 10 days, were supplied with Hoagland and Arnon solution (1950) diluted to 10%. After complete germination and emergence, seedlings of uniform size, visually free of diseases, were transferred to pots of 3.0 dm^3 (two plants pot^{-1}), containing the experimental solutions (treatments): Hoagland and Arnon full solution, equivalent to Relative Concentration (RC) =1.0; solution diluted to half concentration (RC = 0.5); or solution diluted to a quarter of original concentration (RC = 0.25), with the pH adjusted to 6.0 ± 0.1. The pots remained under pre-programmed environmental conditions (photosynthetic irradiance: 450 µmol m^{-2} s^{-1}; photoperiod: 12 h; day/night air temperature: 28/24°C), placed according to an entirely randomized experimental design with five replications (Figure 1).

The solutions were changed at 7-day intervals and, during this period, the volume of water lost by seedling transpiration was

restored with an equal volume of nutritive solution, according to each treatment. During the experimental period, stem height (taken as the distance between the base of the stem and the extremity of the terminal bud in the main branch) and principal root length were measured repeatedly. After 28 days, plants were harvested and fractionated into leaf (L), petiole (P), stem (S) and root (R). Subsequently, these fractions were weighed to obtain the respective fresh masses (LFM, PFM, SFM and RFM), and as soon as possible placed for drying in a forced air oven at 65°C until constant mass, to obtain the corresponding dry masses (LDM, PDM, SDM and RDM). Leaf area (LA) was determined through digital analysis of images obtained with an HP scanner at 200 dpi resolution, and processed with the software SIARCS® 3.0 (Integrated System for Roots and Soil Coverage Analysis developed by Embrapa - Agricultural Instrumentation, Brazil). From these data, the following traits were calculated: Root and stem elongation rate (ER, mm day^{-1}) and specific leaf mass (DML LA^{-1}), leaf succulence from the expression LS = (LFM - LDM) LA^{-1} (Evans, 1972; Moreira et al., 2009), and stem water content (SWC, %), calculated as: 100* [1- (DMS FMS^{-1})]. The results obtained were submitted to variance analysis, treatments being discriminated by test F ($P \leq 0.05$), and the means compared by Tukey test ($P = 0.05$).

RESULTS AND DISCUSSION

At the end of the growth period, plants differed significantly in height ($F= 15.13$; $P = 0.0005$), since stem elongation rate of plants grown in the full solution was 2.2 fold higher than those of plants grown in the more diluted solutions (3.82; 2.8 and 1.73 mm day^{-1} for RC = 1.0, 0.5 and 0.25, respectively). In roots, the pattern was slightly different since there was no significant difference in the root elongation rate (RER) of plants grown in RC = 1.0 or 0.5 (6.28 vs. 6.14 mm day^{-1}). However, in RC = 0.25, the RER was drastically reduced (2.89 mm day^{-1}).

In relation to total dry mass production (TDM), the data indicate that *J. curcas* plants significantly reduced their production (36.2%) in RC = 0.25 (Figure 2A). This result confirms recent information indicating that although *J. curcas* can adapt its growth to nutrient-poor soils (Divakara et al., 2010), it responds positively to fertilization practices in terms of production of dry mass and seeds (Yong et al., 2010; Lima et al., 2011; Prates et al., 2012; Freiberger et al., 2014). In terms of TDM distribution, there was a tendency towards greater allocation in roots at RC = 1, since the root $shoot^{-1}$ relationship varied from 0.145 to 0.132 g g^{-1} when the RC of the solution passed from 1.0 to 0.25 without, however, reaching statistical significance ($P > 0.05$).

In comparison to dry mass accumulation, leaf area (LA) production was more sensitive to nutritional level once, when RC = 0.25 the LA per plant was just 32.5% of that obtained from RC = 1 (Figure 2B). Experiments with *J. curcas* plants of diverse origins, cultivated in nutritive solution or solid substrate, in controlled environments or greenhouses, have shown positive results from the application of increasing supply of nitrogen and phosphate fertilizers regarding chlorophyll contents, photosynthetic assimilation and leaf area (Yong et al., 2010; Lima et al., 2011; Prates et al., 2012). Thus, collectively, these previous studies support the present data by showing the stimulating effects of mineral nutrition on key biomass formation processes as light absorption and carbon assimilation.

Table 1 presents data relative to the distribution of fresh and dry mass between organs of shoots and roots. In relation to the effects of nutritive solution RC on fresh mass of various organs, the greatest contrasts were observed between RC = 0.25 and RC = 1.0, including significant reductions to the order of 70% for petioles (PFM) and roots (RFM); 52% for leaves (LFM) and 45% for stems (SFM). In all cases, the values corresponding to RC = 0.5 indicated intermediate reductions which reached statistical significance ($P < 0.05$) in the case of roots and petioles (Table 1). When data were expressed on a dry mass (DM) basis, there was an important modification in the reduction patterns induced by dilution of the nutrient solution: significant effects were only restricted to the leaf and petiole fractions (Table 1). However, the relative contribution of each fraction to total mass was affected by nutritional level (Figure 2*A*), particularly in stems, in which the ratio SDM TDM^{-1} varied from 0.436 to 0.36, when RC passed from 0.25 to 1.0. Correlatively, in leaves, the ratio LDM TDM^{-1} was inverted, varying from 0.39 to 0.432 in the same conditions.

Figure 3A presents the relationship FM DM^{-1} for leaves and stems. In leaves, this relationship remained stable in relation to RC values (4.30 - 4.68 g g^{-1}), reducing significantly only in RC = 0.25. In stems, the amplitude of this relationship was much greater, increasing 48% (from 5.48 to 8.11 g g^{-1}, $P < 0.05$), when RC changed from 0.25 to 1.0. Figure 3B presents data relative to specific leaf mass (SLM) showing decreasing values, statistically differentiated, from RC = 0.25 to the full solution (71.6; 48.3 and 39.4 g m^{-2} for RC = 0.25; 0.5 and 1.0, respectively). Maes et al. (2009), studied responses of *J. curcas* plants of 114 days in age to water deficit, obtaining a value of 183 cm^2 g^{-1} for specific leaf area (SLA), equivalent to SLM = 54.6 g m^{-2}, a value of the same magnitude as those presented here. Differences in SLM between and within species are due to variations in leaf density and/or thickness (Niinemets, 1999). It is interesting to observe that various studies have verified that leaf thickness increases are associated with decreasing levels of soil fertility (Hassiotou et al., 2010), a situation simulated in the present work by dilution of the original solution. It has been suggested that in these conditions, increases in leaf thickness are a compensatory mechanism for smaller leaf areas, in order to maintain leaf hydration levels (Mantovani, 1999). Contrastingly, greater values of SLM are associated with greater dilution (Figure 3B).

This suggests that the adaptive characteristics of *J. curcas* plants to their nutritional environments for growth have early expression at the seedling stage. SLM values

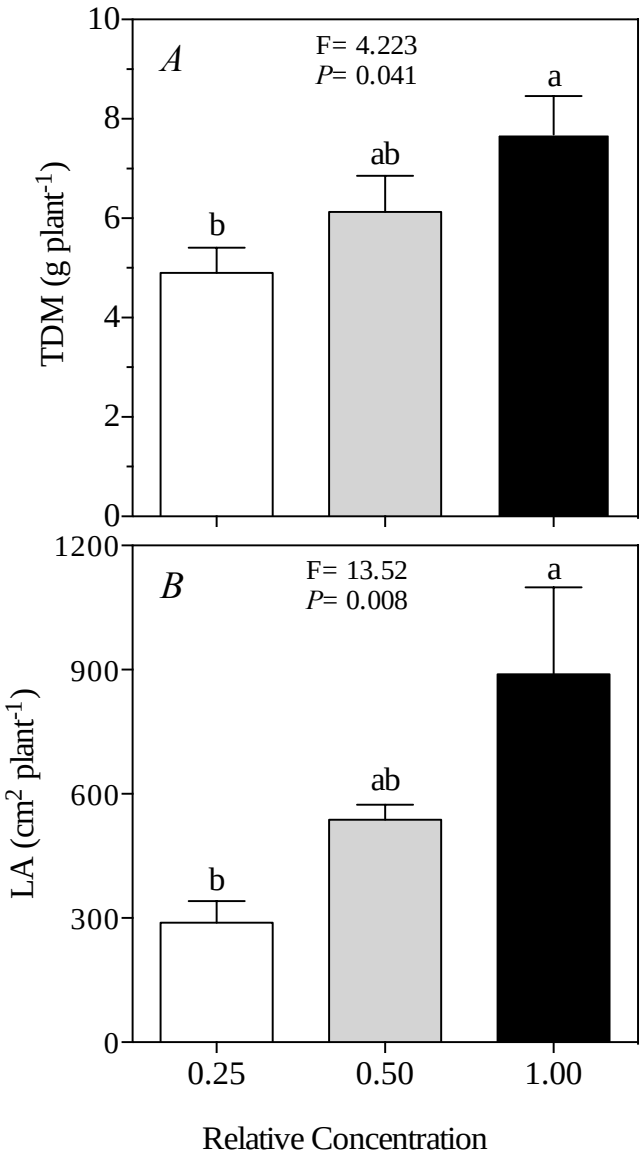

Figure 2. *A*. Total dry mass (TDM); *B*. Leaf area (LA), of *J. curcas* seedlings, grown for 28 days in three levels of relative concentration (RC) of nutritive solution. Values of RC correspond to full solution (1.0) or diluted to half (0.5) or quarter (0.25) of the original nutrient concentrations. Columns with the same lower-case letter on top do not differ significantly (Tukey, P < 0.05). Vertical bars indicate the standard error of means.

resulted inversely related with the corresponding DM FM^{-1} relationships (r = - 0.707; P = 0.032), but this did not affect leaf succulence levels, which oscillated, non significantly, between 140 - 190 g H_2O m^{-2}, with a tendency to increase in RC = 0.25 (Figure 4). These values can be considered low if compared with those reported by Mantovani (1999), and particularly with certain epiphytic bromeliad species with very succulent leaves, in the range of 650 - 850 g H_2O $m\text{-}^{2}$ (Reyes-García et al., 2012).

In relation to stem water content levels (Figure 5), the result was very different, since these responded in a linear, positive form to crescent RC values (SWC = 80.43(± 0.74) + 7.48 (± 1.12) RC; r^2 = 0.773; *n* = 15). By fresh mass unit, stems of plants grown in RC = 1 contained about 6% more water than those cultivated in RC = 0.25 (SWC = 87.6 vs. 81.7%, respectively), while those in RC = 0.5 had an intermediate value. These differences in SWC between treatments are of considerable magnitude, which could be demonstrated by an estimate of its succulence, in approximate terms. Assuming that fresh mass value of an organ can be used as a proxy for their volume (Garnier et al., 1999) and considering its cylindrical morphology, stem succulence for RC = 0.25 corresponded to 1895 ± 60 g H_2O m^{-2}, while in RC = 1 this estimate was 2306 ± 144 g H_2O m^{-2}. These values are between ten and fifteen times greater than those corresponding in leaves (Figure 4). Thus, although the succulence of stems could be a structural characteristic in *J. curcas* (Maes et al., 2009), their water storage capacity by volume or surface area unit is determined by nutritional status available to plants (Figure 5).

Anatomical and morphological traits of shrub species with succulent stems have received research attention in recent years. These succulent stems are differentiated in an outer green photosynthetically active chorenchyma and an internal water storing hydrenchyma (Lüttge, 2008). Cell walls of water storage tissues have a lower modulus of elasticity than those of the chlorenchyma, so that they can absorb and release more water with small changes in turgor potential (Lüttge, 2008). In the cells of the peripheral stem chlorenchyma, that have thin walls and a large central vacuole (Cushman and Bohnert, 1997), water inflow should be favored by increases in the availability of inorganic solutes that regulate osmotic potential, as K^+, Cl^- and NO_3^- (Jones, 1980; Rodrigues et al., 2013). This could contribute to explaining the positive association observed between external nutrient concentration and water capacitance in stem tissues (Figure 5). In fact, in other plant species, a close correlation was previously observed between osmotic pressure increases (or decreases in osmotic potential) and succulence parameters (Lüttge, 2008).It has been suggested that *J. curcas* has the ability to combine C_3/CAM photosynthesis in succulent stems, while leaves are capable of altering their water use efficiency, changing their C_3 metabolism for CAM (Jongschaap et al., 2009). In *Euphorbia tirucalli*, a drought tolerant species with potential as a biofuel source, photosynthetic pathways include C_3 metabolism in non-succulent leaves and CAM in succulent stems (Hastilestari et al., 2013). Nevertheless, in the case of *J. curcas*, this strategy still needs experimental verification. Succulence is a plant characteristic with clear ecophysiological implications (Mantovani, 1999; Mwine and Van Damme, 2011), which in *J. curcas* plants, until now, has been mainly explored in

Table 1. Dry and fresh mass of leaves (L), petioles (P), stem (S) and roots (R), of *J. curcas* seedlings, grown for 28 days in three levels of relative concentration (RC) of nutritive solution.

RC	Fresh mass (g plant^{-1})			
	L	P	S	R
0.25	7.06^{b}*	1.88^{b}	11.72^{b}	3.63^{b}
0.50	10.96^{ab}	3.40^{b}	16.40^{ab}	7.20^{b}
1.00	14.64^{a}	6.01^{a}	21.35^{a}	11.11^{a}
C.V. (%)	21.9	31.4	21.6	27.9
	Dry mass(gplant^{-1})			
	L	P	S	R
0.25	1.93^{b}	0.28^{b}	2.16^{a}	0.58^{a}
0.50	2.58^{ab}	0.35^{b}	2.48^{a}	0.72^{a}
1.00	3.16^{a}	0.56^{a}	2.66^{a}	0.93^{a}
C.V. (%)	24.7	32.8	21.4	27.0

*Means following the same letter do not differ between them by Tukey test at 5%. C.V. = coefficient of variation.

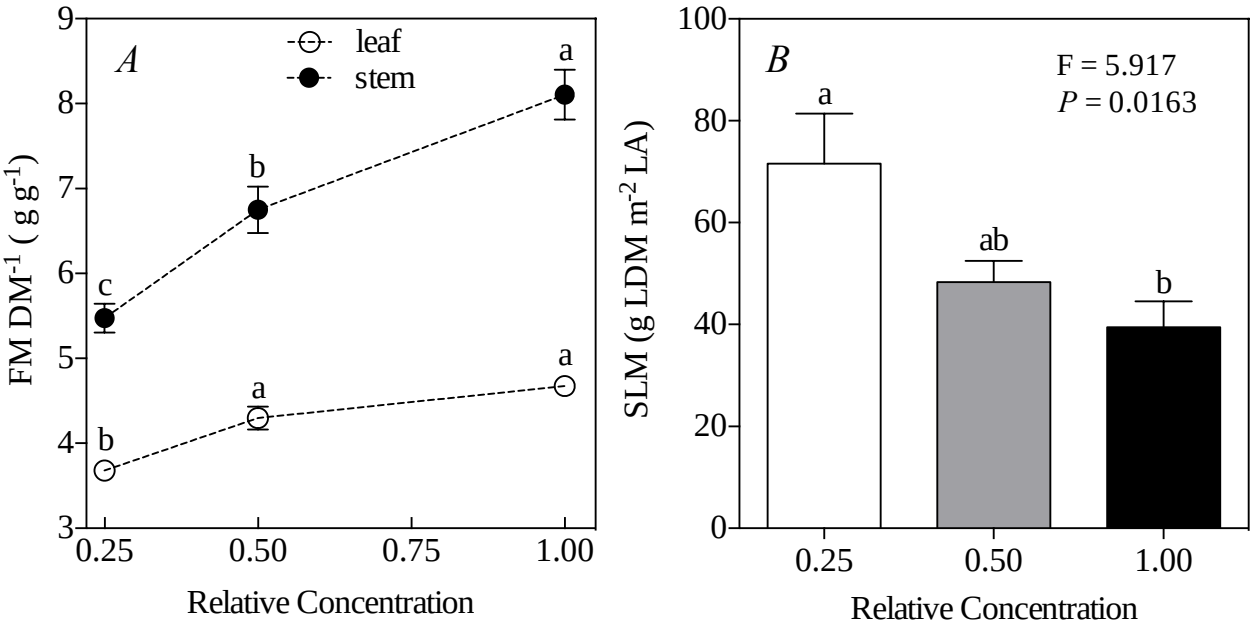

Figure 3. *A*. Fresh mass/dry mass (FM DM^{-1}) ratios; *B*. specific leaf mass (SLM) values of *J. curcas* seedlings, grown for 28 days, in three levels of Relative Concentration (RC) of nutritive solution. Values of RC are the same as Figure 2. Symbols (*A*) or columns (*B*) with the same lower-case letter at the top do not differ significantly (Tukey test P < 0.05). Vertical bars indicate means standard error.

in relation to its adaptation to environments characterized by water deficit or soil salinity (Maes et al., 2009; Arcoverde et al., 2011; Díaz-López et al., 2012; Rodrigues et al., 2013; Sapeta et al., 2013).

Conclusions

Taken together, the present results indicate that there is a potential for the optimization of growth and productivity of *J. curcas* by varying nutritional levels through the application of fertilizers. In particular, the nutrient status of the growth solution of young *J. curcas* plants exerts a direct influence on the production of assimilatory leaf area, as well as on accumulation and allocation of total biomass between different plant organs. At the same time, although nutritional levels do not alter leaf succulence levels, they directly affect the water storage capacity in stems.

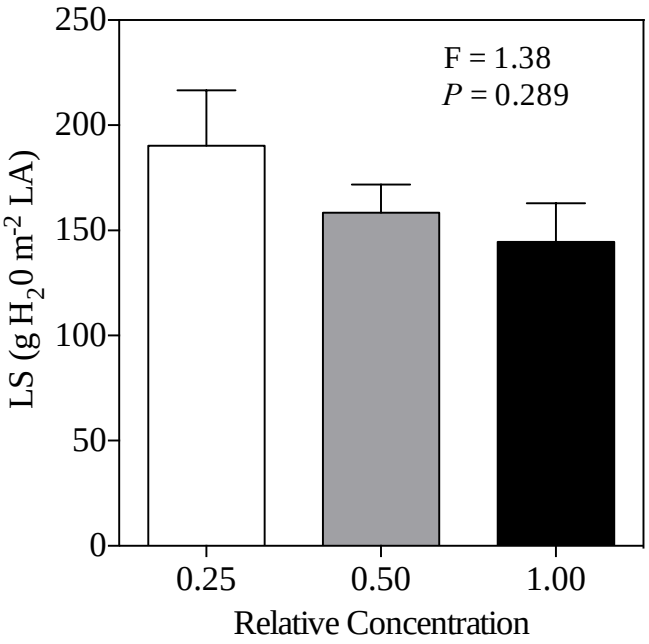

Figure 4. Succulence of fully expanded leaf (LS) of *J. curcas* seedlings, grown for 28 days in three levels of Relative Concentration (RC) of nutritive solution. Vertical bars indicate means standard error.

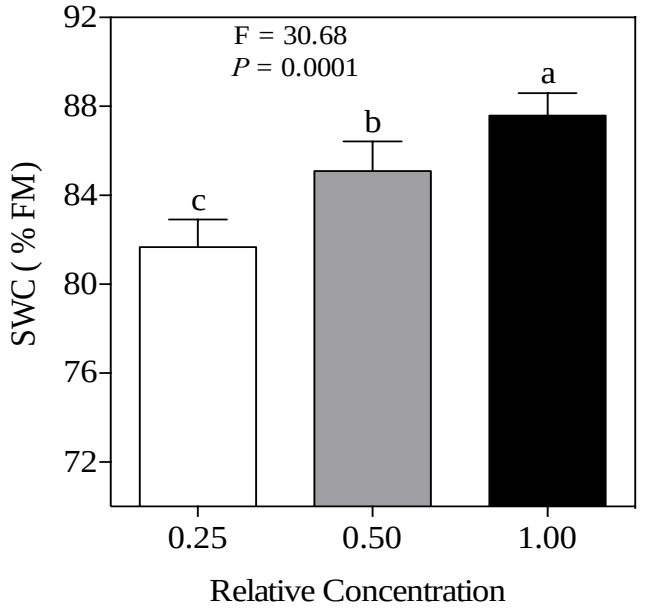

Figure 5. Stem water content (SWC, % FM) of *J. curcas* seedlings, grown for 28 days in three levels of relative concentration (RC) of nutritive solution. Different lower-case letters, at the top of columns, indicate significant differences (Tukey, P < 0.05). Vertical bars indicate means standard error.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Accuracy and genetic progress of agronomic traits in irrigated rice program in Brazil

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The estimate of genetic progress indicates the effectiveness of selection and the need to use new selection methods and strategies. Hence, the objective of the present study was to accuracy estimate the genetic contribution of agronomic traits in the irrigated rice breeding program in the state of Minas Gerais, Brazil between 1998 and 2012. For this goal, the following traits were evaluated: Grain yield (Kg.ha^{-1}), 100-grain weight (g), plant height (cm), days to flowering (days), tillering (score) and lodging (score). These traits were evaluated for 108 inbred lines in advanced yield trials (ATs), conducted in randomised blocks with three to four replicates in four regions of Minas Gerais between 1998 and 2012. Not all sites were included in all crop years, according to the selection accuracy. The restricted maximum likelihood/best linear unbiased predictor Restricted Maximum Likelihood/Best Linear Unbiased Predictor (REML/BLUP) technique was used to obtain reliable genetic value estimates. The genotypes were evaluated for sites within each year and between years by analysis of deviance. Further, genetic and environmental progress were estimated. There was a significant effect for genotype and for the interactions genotype x site and genotype x year. The following results were obtained: 195.91 kg.ha^{-1} for yield, 0.10 g for 100-grain weight, 1.50 cm for plant height, 3.17 days to flowering, 0.01 points for tillering, and -0.18 points for lodging. Although these results are satisfactory, new strategies are suggested to increase the genetic progress values of the agronomic traits of interest in the coming years.

Key words: Genetic contribution, value for cultivation and use, selection accuracy, Restricted Maximum Likelihood/Best Linear Unbiased Predictor (REML/BLUP), *Oryza sativa* L.

INTRODUCTION

The search for agronomic traits that provide higher yields and quality at a lower production cost has been the main objective of Brazilian rice breeding programs. In this context, the effective contribution of

breeding to increasing the performance of rice cultivars available to producers over the years should be estimated periodically (Borges et al., 2009). In addition, these results enable the search for new methods that may expand the program's effectiveness, guiding future research actions and re-evaluating the strategies in use (Soares et al., 2005; Menezes Júnior et al., 2008).

Value for Cultivation and Use (VCU) trials can be used to estimate the genetic gains of a breeding program. Such progress is analysed through the genetic superiority of materials participating in the trials in a given year over that of previous years (Vencovsky et al., 1988). The majority of these studies are restricted to yield, that being the main trait evaluated in these trials (Santos et al., 1999; Rangel et al., 2000; Breseghello et al., 2006). However, it is necessary for the selected material to simultaneously have several favourable attributes that confer superiority over other cultivars. In the case of irrigated rice, traits such as plant height, number of tillers, 100-grain weight, cycle and lodging are crucial for a cultivar's success.

Another aspect to be considered in evaluating genetic progress is the choice of the estimation/prediction method for genetic values. Generally, the analyses are performed considering the phenotypic means and the method of least squares, which, due to the high degree of imbalance (sites, replicates, years, insertion and exclusion of genotypes, among others), does not produce reliable estimates of the true genetic value of the evaluated materials (Resende, 2002; Fritsche-Neto et al., 2010). However, genetic evaluations depend on the accuracy of variance component estimates and the adequacy of assumptions of the models for the nature of the information available. Thus, for situations like this, the use of the Restricted Maximum Likelihood/Best Linear Unbiased Predictor (REML/BLUP) is ideal (Resende, 2002; Fritsche-Neto et al., 2010).

In this context, the specific objectives of the present study were:

1. To accurately estimate the genetic progress for agronomic traits in the irrigated rice breeding program in Minas Gerais, Brazil between 1998 and 2012, and
2. To propose new strategies for increasing the effectiveness of the program in coming years.

MATERIALS AND METHODS

Experimental setup and data collection

A total of 108 genotypes were assessed during the period from 1997/98 to 2011/12 in the VCU trials of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) irrigated rice breeding program in Minas Gerais. Trials were conducted at four distinct sites in this state: Leopoldina, Lambari, Prudente de Morais and Janaúba (Table 1). In each crop year, trials were conducted with 25 genotypes, except for crop year 1998/99, in which 26 genotypes were evaluated (Table 2). There was no selection of inbred lines in 2008/09; thus, that year's data were not considered in the analysis. The 100-grain weight trait was evaluated in the period from 2002/03 to 2011/12, totalling 61 genotypes.

The experimental design used was that of randomised blocks, with four replicates until 2001/02. Since then, three replicates have been used. The experimental plots in the years 1998, 1999 and 2008 to 2012 consisted of five rows of plants 5.0 meters in length with 30-cm spacing, totalling an area of 7.50 m^2. The useful area considered was the central 4.0 m of the three inner rows (3.6 m^2). In the years 2001 to 2007, the plots consisted of six rows of plants, and the central 4.0 m of the four inner rows were considered, totalling a useful area of 4.8 m^2.

At the experimental farm of Leopoldina, the seedlings were initially sown in nurseries and transplanted to the row at a spacing of 0.20 m. In the other sites, sowing was performed in rows with a density of 300 seeds. m^{-2}. The irrigation started approximately 10 to 15 days after seedling emergence, in the case of the sowing of seeds, or when the seedlings had rooted in the soil. The irrigation was stopped approximately 10 days before the maturity of the last line included in the trial. The irrigation depth was gradually increased according to the plants' development. Other crop management practices were performed according to that recommended for the rice crop in each region (Soares et al., 2005).

Plant height was measured by selecting 10 random plants per plot and measuring their height from the ground to the tip of the panicle at the time of harvest; flowering was measured in days as the period in days from planting or sowing in the nursery until 50% of the plants in each plot had flowered; tillering was assessed by means of scores of 1-9, measured at the time of flowering, where 1 = Excellent, 3 = Good, 5 = Fair, 7 = Bad, and 9 = Very Bad; lodging was assessed by scores of 1-5, measured at the time of maturity (harvest) where 1 = no lodging, 2 = 1-25% of lodged plants, 3 = 26-50% of lodged plants, 4 = 51-75% of lodging and 5 = 76-100% of lodged plants; grain production was measured in grams per useful plot, further converted to kilograms per hectare and corrected for the moisture content of grains; the 100-grain weight of panicles obtained from a sample of 10 plants from each plot was also determined (Embrapa, 1977).

Genetic-statistical analyses

The data were analysed using the REML/BLUP method to obtain the variance components and genetic parameter estimates for each variable, as described by Resende (2007). Initially, analysis of deviance was conducted for sites within each year, using the likelihood ratio test (LRT) for comparing the model effects as described by Sturion and Resende (2010). For this purpose, the following model was used:

$$\mathbf{y} = \mathbf{Xr} + \mathbf{Zg} + \mathbf{Wi} + \mathbf{e}$$

where **y** is the vector of phenotypic means of inbred lines; **r** is the vector of replicate effects within a site (assumed to be fixed) added to the overall mean; g is the vector of the genotype effects of inbred lines (assumed to be random), where $g \sim N(0, G)$ and

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Table 1. Environments and the number of Value for Cultivation and Use (VCU) trials considered in analyses[1] based on the selection accuracy of the irrigated rice breeding program of Minas Gerais for the following traits: 100-grain weight, plant height, days to flowering, tillering and lodging, during the period from 1998 to 2012.

Selection accuracy															
Year	Yield				NT	100-Grain weight				NT	Plant height				NT
	Sites[1]					Sites[1]					Sites[1]				
	JA	LA	LE	PM		JA	LA	LE	PM		JA	LA	LE	PM	
1998	0.79*	0.54	0.95*	0.94*	3	-	-	-	-	-	0.87*	0.87*	0.96*	0.97*	4
1999	0.78*	0.79*	0.93*	0.92*	4	-	-	-	-	-	0.77*	0.97*	0.97*	0.95*	4
2000	0.88*	0.83*	0.96*	0.92*	4	-	-	-	-	-	0.94*	0.86*	0.97*	0.92*	4
2001	0.82*	-	0.48	0.42	1	-	-	-	-	-	0.90*	-	0.53	0.80*	2
2002	0.85*	-	0.48	-	1	-	-	-	-	-	0.84*	-	0.99*	-	2
2003	0.54	0.81*	0.87*	-	2	0.97*	0.99*	0.99*	0.98*	4	0.80*	0.82*	0.92*	0.90*	4
2004	0.80*	-	0.9*	0.88*	3	0.98*	-	1.00*	-	2	0.93*	-	0.75*	0.99*	3
2005	-	-	0.91*	-	1	-	-	0.99*	-	1	-	0.92*	0.98*	-	2
2006	0.85*	-	0.52	-	1	1.00*	-	0.99*	-	2	0.79*	0.86*	0.91*	-	3
2007	0.84*	0.91*	0.91*	-	3	0.98*	0.99*	0.99*	-	3	0.81*	0.98*	0.93*	0.65	3
2008	0.85*	-	0.84*	-	2	0.99*	0.99*	0.97*	-	3	0.90*	0.98*	0.90*	-	3
2010	-	0.74*	0.32	-	1	1.00*	0.99*	0.99*	-	3	0.80*	0.74*	0.91*	-	3
2011	0.72*	-	0.76*	-	2	1.00*	0.99*	0.99*	-	3	0.83*	0.41	0.93*	-	2
2012	0.87*	0.55	0.82*	-	2	0.99*	-	0.93*	-	2	0.90*	0.87*	0.84*	-	3
Total	11	5	10	4	30	8	5	9	1	23	13	10	13	6	42

	Days to flowering					Tillering					Lodging				
Year	Sites[1]				NT	Sites[1]				NT	Sites[1]				NT
	JA	LA	LE	PM		JA	LA	LE	PM		JA	LA	LE	PM	
1998	1.00*	1.00*	0.98*	1.00*	4	-	0.80*	0.92*	0.80*	3	1.00*	-	1.00*	1.00*	3
1999	1.00*	0.99*	0.99*	0.97*	4	-	0.53	0.95*	0.83*	2	0.76*	-	1.00*	1.00*	3
2000	1.00*	1.00*	0.99*	-	3	0.64	0.91*	0.98*	0.86*	3	1.00*	-	1.00*	1.00*	3
2001	1.00*	-	0.99*	0.98*	3	0.96*	-	0.95*	0.17	2	1.00*	-	1.00*	1.00*	3
2002	0.99*	-	0.99*	-	2	0.61	-	1.00*	-	1	1.00*	-	1.00*	-	2
2003	1.00*	1.00*	0.98*	0.97*	4	0.50	0.51	0.98*	0.85*	2	1.00*	-	1.00*	1.00*	3
2004	1.00*	1.00*	1.00*	0.95*	4	0.79*	0.94*	1.00*	0.78*	4	1.00*	1.00*	1.00*	1.00*	4
2005	-	1.00*	1.00*	-	2	-	0.88*	1.00*	-	2	-	1.00*	1.00*	-	2
2006	1.00*	1.00*	0.90*	-	3	0.63	0.95*	0.56	-	1	1.00*	1.00*	1.00*	-	3
2007	1.00*	1.00*	0.97*	0.91*	4	0.58	-	0.51	0.90*	1	1.00*	-	-	0.20	1
2008	0.97*	0.99*	0.97*	-	3	0.92*	-	0.69	-	1	1.00*	-	0.90*	-	2
2010	1.00*	0.95*	1.00*	-	3	0.74*	-	0.76*	-	2	1.00*	1.00*	0.95*	-	3
2011	1.00*	0.92*	0.99*	-	3	0.83*	0.69	0.87*	-	2	1.00*	-	0.96*	-	2
2012	0.85*	0.97*	1.00*	-	3	0.58	-	0.66	-	0	-	-	0.96*	-	1
Total	13	12	14	6	45	5	5	10	6	26	12	4	13	6	35

* Values greater than 0.70 indicate that the environment was considered in the analyses. [1]Sites (JA: Janaúba; LA: Lambari; LE: Leopoldina; PM: Prudente de Morais); NT, number of trials in the year.

$G = I\sigma_g^2$; i is the vector of the line x site interaction (assumed to be random), where $i \sim N(0, I_{gl})$ and $I_{gl} = I\sigma_{gl}^2$; and e is the vector of errors, being $e \sim N(0, R)$, where $R = I\sigma_e^2$. X, Z and W are incidence matrices that relate the effects of r, g and i to vector y, respectively.

The mixed model equations for the prediction of **r**, **g** and **I** are as follows:

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} & \mathbf{X'W} \\ \mathbf{Z'X} & \mathbf{Z'Z}+\lambda_1 & \mathbf{Z'W} \\ \mathbf{W'X} & \mathbf{W'Z} & \mathbf{W'W}+\lambda_2 \end{bmatrix} \begin{bmatrix} \mathbf{r} \\ \mathbf{g} \\ \mathbf{i} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{Z'Y} \\ \mathbf{W'y} \end{bmatrix}$$

where $\lambda_1 = \frac{1-h_g^2-h_i^2}{h_g^2}$ and $\lambda_2 = \frac{1-h_g^2-h_i^2}{h_i^2}$, in which h_g^2 is the broad-sense heritability of the inbred lines and h_i^2 is the coefficient

Table 2. Likelihood ratio test (LRT) values for the line effects within year, line x year interactions, estimates of broad-sense heritability (h^2_g) and mean values for yield (Kg.ha^{-1}), 100-grain weight (g), plant height (cm), days to flowering, tillering and lodging of the value for cultivation and use (VCU) trials of the irrigated rice breeding program in Minas Gerais, in the period from 1998 to 2012.

Effects [1]	Yield (kg ha^{-1})	100-Grain Weight (g)	Plant Height (cm)	Days to Flowering	Tillering	Lodging
Line	9.65***	139.05***	74.53***	116.25***	30.16***	43.25***
Line x Year	2.29 N.S	1.35 N.S.	42.60***	93.69***	15.18***	0.01 N.S.
h^2_g	0.13	0.61	0.26	0.44	0.22	0.11
Mean	5691	2.63	91.21	101	2.75	1.14

[1] Values obtained by the Likelihood Ratio Test (LRT), in which the effect is significant at ***p=0.01, **p=0.05, *p=0.10 and ns, non-significant by the $\chi 2$ test with 1 degree of freedom.

of determination of the line x site interaction.

Aiming to determine the ratio of the coefficients of genetic and residual variation (CVg/ CVe), termed the coefficient of relative variation (CVr), the estimates of the genotypic and residual variance components, given by $\hat{\sigma}_g^2$ and $\hat{\sigma}^2$, respectively, were used to calculate the CVr:

$$CV_r = \frac{CV_g}{CV_e} \quad CVg = \frac{\hat{\sigma}_g}{\bar{x}} x100 \quad CVe = \frac{\hat{\sigma}_e}{\bar{x}} x100$$

in which the genetic and residual standard deviations are given by $\hat{\sigma}_g$ and $\hat{\sigma}_e$, respectively, and $\bar{x}$ is the overall mean. This ratio is important to calculate the selection accuracy ($\hat{r}gg$), whose expression, according to Resende and Duarte (2007), is given as follows:

$$\hat{r}gg = \left[1 - \frac{1}{1 + b.CVr^2}\right]^{1/2}$$

where *b* is the number of blocks of the statistical design, and *CVr* is the coefficient of relative variation. According to these authors, values greater than 0.70 indicate a high accuracy class, allowing the inclusion of the assessment site in the analyses.

Then, global analyses of deviance were performed considering all years and sites, according to the following model:

$$\mathbf{y} = \mathbf{Xa} + \mathbf{Zg} + \mathbf{Wu} + \mathbf{e}$$

where **y** is the vector of phenotypic means of inbred lines; **a** is the vector of replicate effects within a year (assumed to be fixed) added to the overall mean; **g** is the vector of the genotype effects of inbred lines (assumed to be random), being $g \sim N(0, G)$, where $G = I\sigma_g^2$; **u** is the vector of the line x year interaction (assumed to be random), where $u \sim N(0, U_{ga})$, and $U_{ga} = I\sigma_{ga}^2$; and **e** is the vector of errors, being $e \sim N(0, R)$, where $R = I\sigma_e^2$. **X**, **Z** and **W** are incidence matrices that relate the effects of **a**, **g** and **u** to vector **y,** respectively.

The mixed model equations for prediction of **a**, **g** and **u** are as follows:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + \lambda_1 & Z'W \\ W'X & W'Z & W'W + \lambda_2 \end{bmatrix} \begin{bmatrix} a \\ g \\ u \end{bmatrix} = \begin{bmatrix} X'y \\ Z'Y \\ W'y \end{bmatrix}$$

where $\lambda_1 = \frac{1 - h_g^2 - h_u^2}{h_g^2}$ and $\lambda_2 = \frac{1 - h_g^2 - h_u^2}{h_u^2}$; in which h_g^2 is the broad-sense hereditability of genotype and h_u^2 is the coefficient of determination of the inbred line x year interaction.

To solve the mixed model equations and estimate genetic values, the genetic and non-genetic variance components were assumed to be unknown. These were estimated by the REML method using the Selegen-REML/BLUP statistical package (Resende, 2007).

In the case of nominal polytomous categorical variables such as tillering and lodging, Resende (2002) explains that a number of categories equal to five, such as the ones used in the present study, still allows for a correct estimation and prediction of genetic values and variance components by means of REML/BLUP without the need for data transformation.

From the estimates of genotypic values of the inbred lines, the genetic progress of assessed traits was estimated by the contrast between means of genotypic values (g) of the inbred lines evaluated in the last year compared to those of the first year studied.

Finally, to estimate the percentages of included (I), excluded (E), maintained (M), and replaced (R) genotypes, the methodology used was that described by Moresco et al. (2004):

$$\%I = \frac{100xI}{M+E+I} \quad \%E = \frac{100xE}{M+E+I} \quad \%M = \frac{100xM}{M+E+I} \quad \%R = \frac{100xI}{M+I}$$

For this purpose, a genetics and statistical software program was used-Genes (Cruz, 2006).

RESULTS AND DISCUSSION

Number of sites, analysis of deviance and genetic parameters

Data from 30 trials were used for the genetic progress analysis of yield, 23 for 100-grain weight, 42 for plant height, 45 for days to flowering, 26 for tillering and 35 trials for lodging data. In this context, only sites that exhibited selection accuracy greater than 0.7 were considered for the trait in question (Table 1). The number of environments considered would likely be higher, particularly for yield and tillering, if there were a greater number of replicates per trial, which would consequently

minimise the environmental effect and increase the selection accuracy (Resende and Duarte, 2007). Resende (2002) explain that the selection accuracy ($\hat{r}gg$) is a better alternative to field trials validation comparing with experimental coefficient of variance, because the first refers to the correlation between the true genotypic value of the genetic treatment and that estimated or predicted from the experimental data.

The genetic variability between inbred lines tested in the irrigated rice breeding program of EPAMIG became evident through the significant differences between genotypes identified by the analysis of deviance for all study variables (Table 2), which then allowed the estimation of their genetic progress. However, a line x year interaction was detected only for plant height, days to flowering and tillering, showing for these cases a change in the performance of the genotypes over the years.

Among the evaluated traits, the highest broad-sense heritability was obtained for the 100-grain weight (0.61), similar to that observed by Akhtar et al. (2011). Conversely, the lowest value was observed for lodging (0.11) (Table 2). Many variables are involved in resistance to lodging, which partly explains the low heritability value obtained such as elongation and thickness of internodes, stem strength, leaf angle, epidermis thickness and cuticle layer (Mahbub et al., 2006). In addition, the organisation of the vascular bundles also influences this trait regarding the compression of sclerenchyma and parenchyma cells, showing lower amounts of lacunae and, consequently, a higher density and lodging resistance. As for the 100-grain weight variable, despite being controlled by many genes as reported by Song et al. (2007), it suffered little environmental influence, as evidenced by the high values of both selection accuracy (Table 1) and the heritability values (Table 2).

Program dynamics

There was a balance in the irrigated rice breeding program of EPAMIG regarding the inclusion and exclusion of materials, with the percentage of both being 18% in the study period, whereas the mean maintenance rate was 63% (Table 3). Atroch and Nunes (2000) and Soares et al. (1999) observed values of 56 and 38%, respectively, in their breeding programs. According to these authors, relevant values for the mean maintenance rate allow for good estimates of environmental variation between the assessment years, as this effect is due to the contrast between common genotypes in different years. However, high maintenance rates, as occurred in the breeding program between the years 2002 and 2012, limit the genetic gains for the traits in question, primarily because of the little exploitation of the genetic basis for the crop available in germplasm banks. This restricts the potential genotypic variability of elite materials to be exploited. The ideal situation was for replacement rates to be equal to,or even higher, than that observed in the period from 1998 to 2012 (26%) (Table 3). Higher results were observed by Atroch and Nunes (2000) in the Amapá program (46%).

Genetic progress

The mean genetic value of inbred lines in the first year was lower than in the last year for most traits, the exception being the tillering variable (Table 4). This indicates that the varieties inserted every couple of years were, in general, genetically superior to those excluded, promoting a genetic gain of 195.91 $kg.ha^{-1}$ for grain yield in the period considered. That is equivalent to an increase of 13.99 $kg.ha^{-1}.year^{-1}$ obtained by the genetic improvement of the cultivars in the program. The genetic progress results for yield are similar to those observed by Soares et al. (1999) for upland rice in Minas Gerais and Souza et al. (2007).

The first study achieved a mean annual genetic gain of 1.26% for early-cycle materials and of 3.37% for mid-cycle and late-cycle ones. As for the second study, the authors obtained 0.3% for early-cycle materials and 2.09% for late-cycle materials. This indicates that the irrigated rice breeding program of Minas Gerais is continuing the genetic progress of rice in this state, as reiterated by analysing cultivars launched by the program during the period in question.

Conversely, the tested genotypes indicate that the program obtained low genetic progress for the 100-grain weight (0.10 grams) in the period from 2002/03 to 2011/12 (Table 4). However, it is worth mentioning that according to Breseghello et al. (2006), the elite germplasm of the Embrapa Rice and Beans (1977), which is the basis of the program under study, recently achieved relative uniformity of grains in the long-thin class (‘Agulhinha’). This is a difficult trait to change because changes in the grain pattern will lead to difficulties in the commercialization of rice due to low market acceptance. This further indicates that other priorities by the rice breeding programs, such as increasing the average yield, the efficient use of resources such as nutrients and water and the tolerance to diseases.

The genetic progress of plant height was positive (1.50 cm), but in the opposite direction of the program objectives for the species (Table 4). In this context, Souza et al. (2007) found, in six decades of upland rice breeding in Brazil, a reduction of 21 cm in plant height. Hence, Tabien et al. (2008) state that breeding programs will likely no longer obtains satisfactory progress for the reduction of size. Thus, it is worth highlighting that the overall mean for plant height observed in this study was 91.21 cm (Table 2), very close to the ideal (Soares et al., 1999), thus reiterating that the program inbred lines have achieved satisfactory size.

Table 3. Percentages of inclusion, exclusion, maintenance and renewal of inbred lines in the irrigated rice breeding program in Minas Gerais in the period from 1998 to 2012.

Year	Inclusion (%)	Exclusion (%)	Maintenance (%)	Replacement (%)
1998/1997	-	-	-	-
1999/1998	31	28	42	42
2000/1999	38	40	21	64
2001/2000	38	38	25	60
2002/2001	0	0	100	0
2003/2002	19	19	61	24
2004/2003	29	29	43	40
2005/2004	22	22	56	28
2006/2005	11	11	79	12
2007/2006	11	11	79	12
2008/2007	17	17	67	20
2010/2009	22	22	56	28
2011/2010	4	4	92	4
2012/2011	0	0	100	0
Mean	18	18	63	26

Table 4. Genetic progress (GP) estimates obtained for yield (Kg.ha^{-1}), 100-grain weight (g), plant height (cm) and days to flowering in the irrigated rice breeding program of Minas Gerais, in the period from 1998 to 2012.

Year	Yield (Kg.ha^{-1})		100-Grain Weight (g)		Plant Height (cm)	
	μ_g[a]	PG	μ_g	PG	μ_g	PG
1997/98	15.25		-		0.04	
2002/03	-	195.91	0.05	0.10	-	1.50
2011/12	211.16		0.16		3.00	

Year	Days to flowering		Lodging		Tillering	
	μ_g	PG	μ_g	PG	μ_g	PG
1997/98	-0.31	3.17	0.00	0.01	-0.01	-0.18
2011/12	4.47		0.06		0.40	

[a]. μ_g, Mean of the genetic values of inbred lines evaluated in the crop year in question.

According to the differences in the genetic values of inbred lines in the study period, there was an average increase of 3.17 days in the time to flowering (Table 4). It is worth noting that this trait was not the main trait used by the program for selection of the best materials during these 14 years (Souza et al., 2007). These authors reported that there was an increase of 10 in days to flowering for early cultivars and a reduction of 14 days for late ones between the decades of 1950 and 2000. In this study, the mean cycle of the inbred lines was 101 days (Table 2), which characterises them as early-cycle in the state of Minas Gerais. However, super-early cultivars (Manfron et al., 2004), as well as size reduction, are something to be sought for in a breeding program. Therefore, it will be necessary to give a greater weight to these traits during the selection process through the use of selection indixes that aim to obtain superior cultivars not only in terms of yield.

The genetic progress values for lodging (0.01) and tillering were low, with the latter being a reason for concern as, although small, it was negative (-0.18) (Table 4). That is the case because, despite the fact that sowing density has remained constant over the assessment years, an increase in the number of tillers while maintaining the uniformity standard of grain maturity would be ideal, as the consumer market is quite demanding about endosperm translucency. This translucency depends on good compaction of the starch grains and proteins, which prevents the formation of chalky grains (Bangwaek et al., 1994). For that reason, the need for the use of selection indices in the breeding program is reinforced.

Conclusion

Although the results are satisfactory, new strategies should be used in the breeding program increase the selection accuracy, such as an increase in the genetic base of the inbred lines, as well as an increase in the replacement rate and reduction in the maintenance rate, selection in specific environments, the use of selection indexes in the intermediate phase of the program to obtain higher values of genetic progress for the main traits of interest, and an increase in the number of replicates.

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Conflict of Interests

The authors have not declared any conflict of interests.

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Nutrient recycling from sanitation and energy systems to the agroecosystem- Ecological research on case studies in Karagwe, Tanzania

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Open cycles of organic carbon and nutrients cause soil degradation. Procedures such as ecological sanitation (EcoSan), bioenergy and Terra Preta practice (TPP) can contribute to closing nutrient cycles and may, in addition, sequester carbon. This paper introduces three projects in Karagwe, Tanzania, and their applied approach of integrated resource management to capture carbon and nutrients from different waste flows. Substrates derived from these case studies, biogas slurry, compost and CaSa-compost (containing biochar and sanitized human excreta), were assessed for their nutrient content by analysis of the total element composition. Evaluation focused on potential impacts of the tested amendments on the nutrient availability in the soil as well as on the local soil nutrient balance. Results revealed that all substrates show appropriate fertilizing potential compared to literature, especially for phosphorus (P). CaSa-compost was outstanding, with a total P concentration of 1.7 g dm^{-3} compared to 0.5 and 0.3 g dm^{-3} in compost and biogas slurry respectively. Furthermore, these soil amendments may reduce acidity of the soil, with a calculated liming effect of 3.4, 2.6 and 7.8 kg CaO for each kg of nitrogen added for biogas slurry, compost and CaSa-compost respectively. To offset negative P balances in Karagwe, about 8100, 6000 and 1600 dm^3 ha^{-1} are required for biogas slurry, compost and CaSa-compost respectively. We conclude that especially CaSa-compost might offer immediate positive effects to crop production and nutrient availability in the soil.

Key words: Ecological sanitation, bioenergy, Terra Preta practice, biochar, biogas slurry, compost, soil amendments, soil improvement, waste as resource.

INTRODUCTION

Open cycles cause agronomic problems

Since more nutrients are taken out of the agroecosystem than are put back, anthropogenic activities create open cycles of mineral nutrients and carbon (C) (Lal, 2006). Such activities comprise among others: Excessive

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deforestation for firewood, exploitation of phosphate rocks for fertilizer production, and energy consumption for production of synthetic fertilizers. Furthermore, most current sanitation systems waste nutrients from human excreta (especially nitrogen (N), phosphorus (P) and potassium (K) as well as micronutrients) since they are either disposed in the ground (pit latrine, ashes of incinerated sewage sludge) or enter the aquatic system (pit latrine, flush toilet), where they cause eutrophication and lead to contamination of the groundwater with fecal microorganisms(Esrey et al., 2001;Graham and Polizzotto, 2012; Meinzinger, 2010). In general, open cycles can cause soil degradation and loss of soil fertility since cultivated soils become increasingly deficient in essential plant nutrients when long term cropping takes place without replacement of nutrients (Hartemink and Bridges, 1995). In addition, soil organic matter (SOM), which is the major building block of a fertile soil, might be depleted by continuous cropping if the plant residues are not put back into the soil after harvesting (Batjes and Sombroek, 1997). Consequently, the soil might show declining water and nutrient retention capacity and an increasing tendency to soil erosion (Horn et al., 2010). Tropical climate conditions aggravate such soil degradation; with year-round elevated temperature, SOM is lost due to fast microbial decomposition of organic matter; heavy rains during the rainy season in turn cause leaching of mineral nutrients (Lal, 2009). It is widely agreed that in order to secure sustainable food supply for everyone, soil degradation must be reversed and soil productivity enhanced.

Problems of using synthetic fertilizers in Sub-Saharan Africa

Agricultural practices using synthetic fertilizers often add too much N to the soil and sometimes neglect input of P, K and micronutrients, which can result in imbalanced plant nutrition (Lal, 2009). Furthermore, nutrients added by synthetic fertilizers often are immediately available and thus can be subject to high losses via leaching and volatilization (Finck, 2007; Savci, 2012). Moreover, the *International Assessment of Agricultural Knowledge, Science and Technology for Development* (IAASTD) showed that in some parts of Sub-Saharan Africa (SSA), especially poor farmers do not have access to synthetic fertilizers (Markwei et al., 2008). Those who have access often lack adequate information on their appropriate use (*ibid.)*. Inappropriate use of synthetic fertilizers, however, may result in soil acidification, pollution of water bodies, and emissions with global warming potential to the atmosphere (Markwei et al., 2008; Savci, 2012). Furthermore, the production of synthetic fertilizers requires energy; for example about one third of the total energy input to crop production of the United States of America is required to produce, to package, to transport and to apply synthetic fertilizers (Gellings and Parmenter, 2004).

Solutions based on using locally available organic fertilizers

Kiers et al. (2008) concluded that in African countries reversing soil infertility might be achieved “through the use of locally available resources”, because the use of synthetic fertilizers is not a feasible option for many subsistence farmers. In “Agriculture at a crossroads“ McIntyre et al. (2009) called for a focus on efficient, small-scale agroecosystems with almost closed nutrient cycles. In addition, the IAASTD demanded that research in a SSA context should reorient “towards integrated nutrient management approaches” (Markwei et al., 2008). Kimetu et al. (2004) demonstrated in Western Kenya that “inorganic N additions can be fully substituted by organic N additions if the appropriate source of organic matter is applied”. Furthermore, the intensified use of organic fertilizer can reduce the cost of fertilization in crop production in SSA (Markwei et al., 2008).

In order to create positive C and nutrient budgets, SOM can be enhanced through addition of organic amendments, as Lal (2009) pointed out. He further suggested that both organic residues, such as compost and animal manures, and biological N-fixation should be included in the nutrient management (*ibid.*). Stoorvogel (1993) particularly emphasized the efficient use of organic household waste as a means to supply nutrients. Beardsley (2011) pointed out that human excreta “is an abundant but often ignored source of P available for recycling worldwide”. Another important soil management practice to strengthen the nutrient cycling process in SSA is acidity management through liming, as described, for example, by Batjes and Sombroek (1997).

Approaches towards closing the loop

In our research, we focus on the following practices for local nutrient and C recycling: (1) Composting in general, as well as co-composting of human excreta and ecological sanitation (EcoSan); (2) Provision of bioenergy combined with agricultural use of residues; (3) Terra Preta practices (TPP) – using biochar as a soil amendment.

Composting and ecological sanitation

Composting is a globally common method in agriculture whereby organic residues are mixed with mineral components and subsequently aerobically decomposed by macro- and microorganisms (for East-Africa see work of e.g. Amoding et al. (2005), Karungi et al. (2010) and

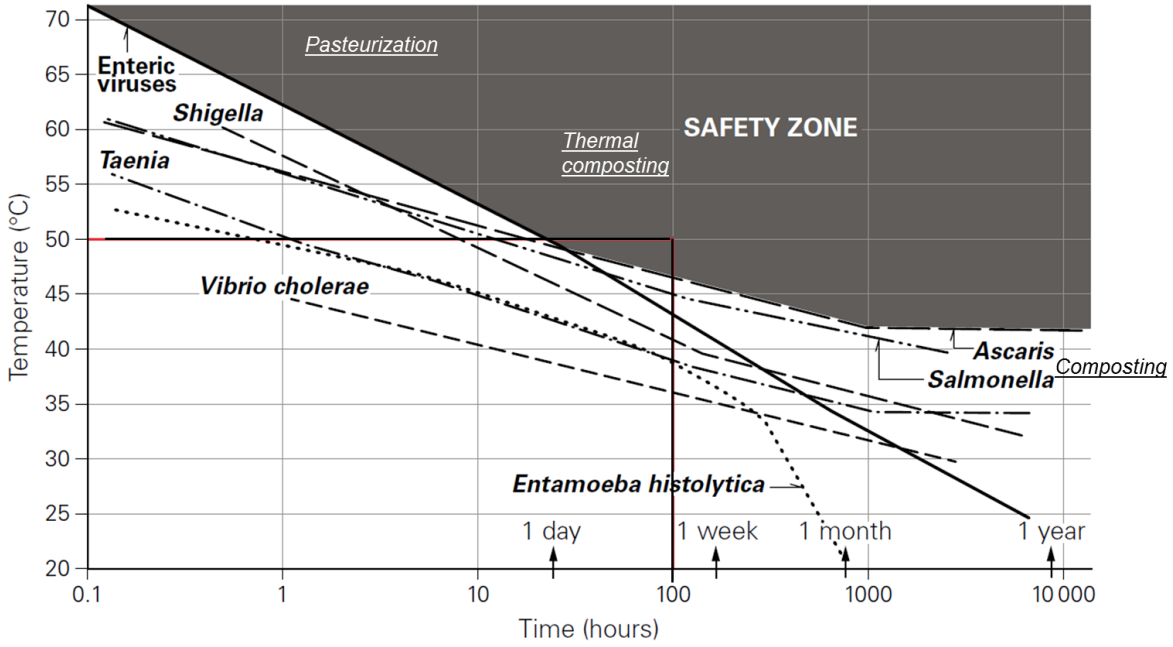

Figure 1. Relationship between temperature and time required to inactivate certain pathogens (according to Feachem et al., 1983, graphic adopted from Vögeli et al., 2014; corresponding combinations of time and temperature for the described possible treatments are indicated)

Tumuhairwe et al. (2009). EcoSan facilitates co-composting of human excreta as an alternative to conventional sanitation systems. EcoSan aims at (i) "closing the loop" by recycling nutrients from humanexcreta in order to improve soil fertility; (ii) avoiding potential human health risks by sanitizing urine and feces; (iii) preventing the pollution of freshwater and marine environments by avoiding waste water discharge into natural water bodies (Winblad et al., 2004). Further benefits of EcoSan, according to Esrey et al. (2001), are that it is: (i) A decentralized system based on household and community management and, thus, omits investment in large-scale infrastructure; (ii) Particularly appropriate in areas with water shortages or irregular water supply since no or very little water is required; (iii) Feasible in both rural and urban areas as well as for rich and poor people alike. Usually, urine and feces are stored and processed on-site. A number of different types of composting toilets are in use in EcoSan, e.g. the urine diverting dry toilet (UDDT), which collects human excreta separately (see Morgan, 2007, for further description and discussion of "Toilets That Make Compost - Low-cost, sanitary toilets that produce valuable compost for crops in an African context"). According to the World Health Organization (WHO, 2006) urine is safe for use as a fertilizer, untreated or after short storage. However, feces mostly contain pathogens (such as viruses, bacteria and worm eggs) and require treatment (*ibid*.). Techniques for sanitation include: dehydration or drying, e.g. through UDDT with a separation of the solid parts and the liquid fraction of the excreta and improved ventilation system (Winblad et al., 2004); disinfection by using additives, e.g. urea (Vinnerås, 2002) or lactic acid bacteria (Factura et al., 2010); disinfection through exposure to elevated temperatures over time, e.g. mesophilic or thermophilic composting (Niwagaba et al., 2009; Ogwang et al., 2012) or pasteurization (RKI, 2013; Schönning and Stenström, 2004). In general, thermal sanitation relies on a temperature/time relationship to inactivate certain pathogens, as described by Feachem et al. (1983) (Figure 1).

Currently, there are no national regulations for the treatment of human excreta, in neither Tanzania nor Germany, but different guidelines for thermophilic composting exist. The WHO (2006) recommended a treatment at 55 to 60°C over several days up to one month depending on the conditions (e.g. constant control of the temperature). In Germany, the following thermal treatments are required for organic waste in general: 55°C for two weeks, 60°C for six days or 65°C for three days (German BO, 2013).

Bioenergy and the agricultural use of its residues

Bioenergy technologies focus on energy recovery from biomass. Also, by-products and residues from bioenergy provision can be recycled back into the agroecosystem. The main principle is the conversion of biomass to heat for either the consecutive production of electricity or

direct provision for productive processes (e.g. for a bakery, green-house heating) and consumption in households or institutions (e.g. for cooking and heating) (Kaltschmitt et al., 2009). In this study, our focus is onprovision of cooking energy at household level and the applied technologies include: three stone fire, charcoal burner, microgasifier and a system using a biogas digester and biogas burner. The use of firewood, three stone fires and charcoal burners is currently most common in many countries of SSA. Ash is the main residue from these bioenergy applications and contains mineral nutrients such as P and K as well as calcium (Ca) and magnesium (Mg), but hardly any C, N or sulphur (S) since these elements volatilize during the oxidation process. Ash is therefore often used as a soil amendment or addition to compost. Another small-scale technology is the biogas digester, which is used for cooking both in households and institutions, such as schools or hospitals (Vögeli et al., 2014). Organic wastes are anaerobically digested via microbiological activity in a closed fermenter, resulting in a methane-rich combustible gas as the main product and biogas slurry as a liquid residue (*ibid*.). Small-scale and low-tech biogas digesters usually operate in a mesophilic range of about 30 to 40°C and a retention time of around 40 days (Kossmann *et al.*, undated). Biogas is accumulated inside the digester or in a separate storage tank and is usually combusted in a biogas burner. Biogas slurry can be used as a fertilizer since it contains most of the mineral nutrients from the digested organic waste in an already plant-available form (Vögeli et al., 2014). Caution and additional treatment of the biogas slurry is required, however, in case human excreta is also digested since pathogens are not inactivated under the mesophilic conditions mentioned above (Figure 1). In Nepal, for example, Lohri et al. (2010) showed that the biogas slurry from mixed fermentation of human excreta and kitchen waste contained pathogens such as helminth eggs. Moreover, inappropriate use of the liquid biogas slurry can cause eutrophication if it is applied in excess or discharged directly to a receiving body of water (Kossmann et al., undated). Finally, households can meet their energy demand by using microgasifiers, which are improved cooking stoves that use dry biomass and spatially separate the transformation of biomass into combustible wood-gas from the subsequent oxidation of the gas (Mukunda et al., 2010; Roth, 2013). One particularly prominent stove design is called the TLUD ("Top-Lit Up Draft"), which is licensed as an open source technology (Anderson and Reed, 2007). Apart from heat, the stove provides charcoal of about 10 to 30% of the fuel fresh weight as a by-product (Roth, 2013). As for ash, charcoal preserves mineral nutrients. It also contains C in a concentration of about 60 to 75% of its dry matter (DM) (McLaughlin et al., 2009). The charcoal can be used for further provision of energy by directly pouring the hot charcoal onto a conventional charcoal burner, to continue cooking immediately, or by making charcoal briquettes in a separate process with an accumulated amount of charcoal. Charcoal can also be used as a soil amendment, which is then termed biochar (Taylor, 2010). Altogether, residues from bioenergy processes have a potential for use as soil amendments; however, their quality depends on the composition of the feedstock used and the application practice. There is a need for field experiments to evaluate the impact of biogas slurry on the local carbon balance as well as on soil characteristics and productivity (Bogdanski and di Caracalla, 2011). The positive effects of pyrolitic charcoal as a soil amendment are historically evident in findings of Terra Preta soils, which we will introduce in the following section. However, there is still a lack of scientifically rigorous field experiments using biochar derived from microgasifiers on tropical soils.

Terra Preta practices (TPP) - using biochar as a soil amendment

One particularly interesting and promising holistic approach for improving or remediating degraded soils is the principle of "Terra Preta" (Portuguese for "Black Soil" = "Udongo Meusi" in Swahili), as practiced by people in the Amazon basin in Brazil, South America, centuries ago (Sombroek, 1966; Glaser et al., 2002). Lehmann et al. (2003b) classified Terra Preta as Anthrosol, a human-made, fertile, black soil. Glaser and Birk (2012) found that it mainly contains charcoal, animal and human excreta as well as other organic and inorganic wastes. Compared to surrounding soils, including Ferralsol, Acrisol or Arenosol, the Terra Preta soils show significantly higher availability of P, Ca, manganese (Mn), and zinc (Zn) (Lehmann et al., 2003a). For example, Falcão et al. (2009) found up to 40 times larger concentrations of plant-available P in Terra Preta than in surrounding natural soils. Other characteristics include high water and nutrient retention capacity as well as a pH of around 5.7, adequate for plant growth (Lehmann et al., 2003a; Horn et al., 2010). Biochar plays a major role for the specific properties of Terra Preta because it builds up a stable stock of SOM. Biochar shows an aromatic C structure with many micro pores, large surface, high adsorption capacity and a C-concentration of about 70 to 80% of DM (Lehmann and Joseph, 2009). In some soils, biochar can significantly improve the availability of both nutrients and water by effecting chemical and hydraulic characteristics of the soil. It can also positively affect the activities of soil microbial communities (Lehmann and Joseph, 2009; Glaser and Birk, 2012). According to Taylor (2010), biochar works as a catalyst in the soil, because it "facilitates reaction beneficial to soil dynamics without being consumed in the process". This means that much of the biochar persists in the soil and is not decomposed

in the way many other organic materials are (*ibid*.). Therefore, biochar amendments may enhance plant growth in some cases, although nutrient inputs from biochar are low (Lehmann and Joseph, 2009).

Consequently, its application was tested in combination with mineral fertilizers (Kimetu et al., 2004; Jeffery et al., 2011), in combination with compost that releases nutrients over time (Liu et al., 2012; Schulz et al., 2013), and as compost-additive to be enriched and loaded with nutrients during the composting process (Kammann et al., 2015).

Recently, Frausin et al. (2014) revealed the presence of so-called African Dark Earth at more than 134 locations in several West-African countries including Liberia, Sierra Leone, Guinea and Ghana. This Terra Preta-like African Anthrosol is preferably located in the vicinity of towns and mainly is the product of women doing appropriate management of wastes from housing and farming (*ibid*.). Altogether, TPP - using biochar as compost-additive and soil amendment - is seen as a "suitable technique helping to refine farm-scale nutrient cycles" (Schulz et al., 2013).

Research objectives

Based on the context described in the introduction, we hypothesize that new approaches which combine EcoSan, bioenergy and TPP can contribute to soil improvement and resource protection by recycling of nutrients and C, if sanitation is taken into account and integrated appropriately. Especially the use of biogas slurry from fermentation of organic waste as a fertilizer and the combined composting of residues from microgasification and sanitized human excreta are promising methods. However, there is need for practice-oriented experiments and assessment of the local ecological impacts under the specific conditions of tropical regions. Hence, the objectives of this paper were (i) to introduce three case studies from Karagwe, Tanzania, and their applied approach of integrated resource management; (ii) to assess the substrates derived from these projects with respect to their nutrient concentrations; and (iii) to evaluate potential impacts of the tested amendments on the nutrient availability in the soil as well as on the local soil nutrient balance.

MATERIALS AND METHODS

Farming activities in Karagwe, Tanzania

Karagwe district is located in Kagera region in northwest Tanzania, a hilly area situated at an altitude of about 1200 m up to 1800 m.a.s.l., semi-arid with equatorial-tropical climate (Baijuka and de Steenhuijsen Piters, 1998). The average daily temperature is about 21°C, with a range from 10°C at night to > 40°C during the daytime (Blösch, 2008). Rainfall is bimodal with rainy seasons from March to May (long rainy season) and October to November (short rainy season), with crop cultivation taking place during both seasons (Tanzania, 2012). Precipitation ranges between 1000 and 2100 mm $a^{-1,}$ with annual and regional differences (Blösch, 2008).

According to the national sample census of agriculture 2007/2008 for Kagera region, most families in Karagwe districtsubsist on farming activities (Tanzania, 2012): about 45% of the population work full-time on their farms and more than 86% of the households sell agricultural products grown on their farms. On average, around 0.75 ha usable land is available per household out of which around 83% is planted. The most important permanent crops are banana and coffee, while beans, sorghum and maize dominate annual cropping. Most of the planted land is used multiply in mixed cropping systems and only some 16% of the land is used for temporary mono-cultural cultivation. A majority of approximately 78% of the farmers in Kagera region who apply fertilizers on their land, use organic fertilizers which are according to Baijukya and de Steenhuijsen Piters (1998) mainly grasses (mulch) and farmyard manure. However, the supplied amount only suffices for roughly 5% of the planted land (distributed to 0.7 and 4.3 %. of the planted land in the long and short rainy season respectively). Synthetic fertilizers are used on less than 1% of the planted land in Kagera region. In 2010 we conducted a preliminary study in Karagwe district including a survey on 10 households and soil sampling at three different farms. We found that small-scale farmers in Karagwe live on an average with six people in one household. In addition, we found that some major problems of local agriculture are a very low soil pH of 3.8 to 4.2, low nutrient availability (especially P) and soil erosion due to a hilly landscape. Concerning sanitation services, a majority of more than 90% of the rural population of Karagwe district use pit latrines, around 6% do not have any toilet so use bushes and only 1% uses flush toilets in combination with septic tanks (Tanzania, 2012). Hence, for 91% of rural households in Karagwe district, excreta are disposed in a pit or tank after dropping without any treatment or use. Concerning energy supply, the most common source of energy for cooking is biomass, with about 96% of the rural households using firewood and 3% using charcoal (Tanzania, 2012). It is common in Karagwe to add ashes from three stone fires to the compost.

Grassroots projects in Karagwe realizing integrated resource management

Since 2008, two local non-governmental organizations, namely MAVUNO Project Improvement for Community Relief and Services (MAVUNO; meaning "harvest" in Swahili) and CHEMA Programme for Community Habitat Environmental Management (CHEMA), have initiated projects in cooperation with the German association Ingenieure ohne Grenzen e.V. (Engineers Without Borders, EWB) and Technische Universität (TU) Berlin. These projects follow a community-participatory approach to appropriate development of technologies and aim at resource protection, autonomous energy supply and safe sanitation services. Together, these projects present an integrated approach to resource management as well as recycling of nutrients and C (Figure 2). Their process combines three systems: The energy system, whereby cooking energy is provided as heat by either burning biogas from a small-scale biogas digester or by microgasifiers; the sanitation system based on EcoSan; finally, the recycling of by-products from both systems, namely biogas slurry, biochar and sanitized human excreta, back into the agroecosystem. In the latter, composting and the principles of TPP are applied to capture nutrients and C from different waste flows.

One of the expected results is soil improvement, to ensure long-term food security and income generation for the rural population. The respective technologies were developed and tested in Karagwe within three pilot projects:

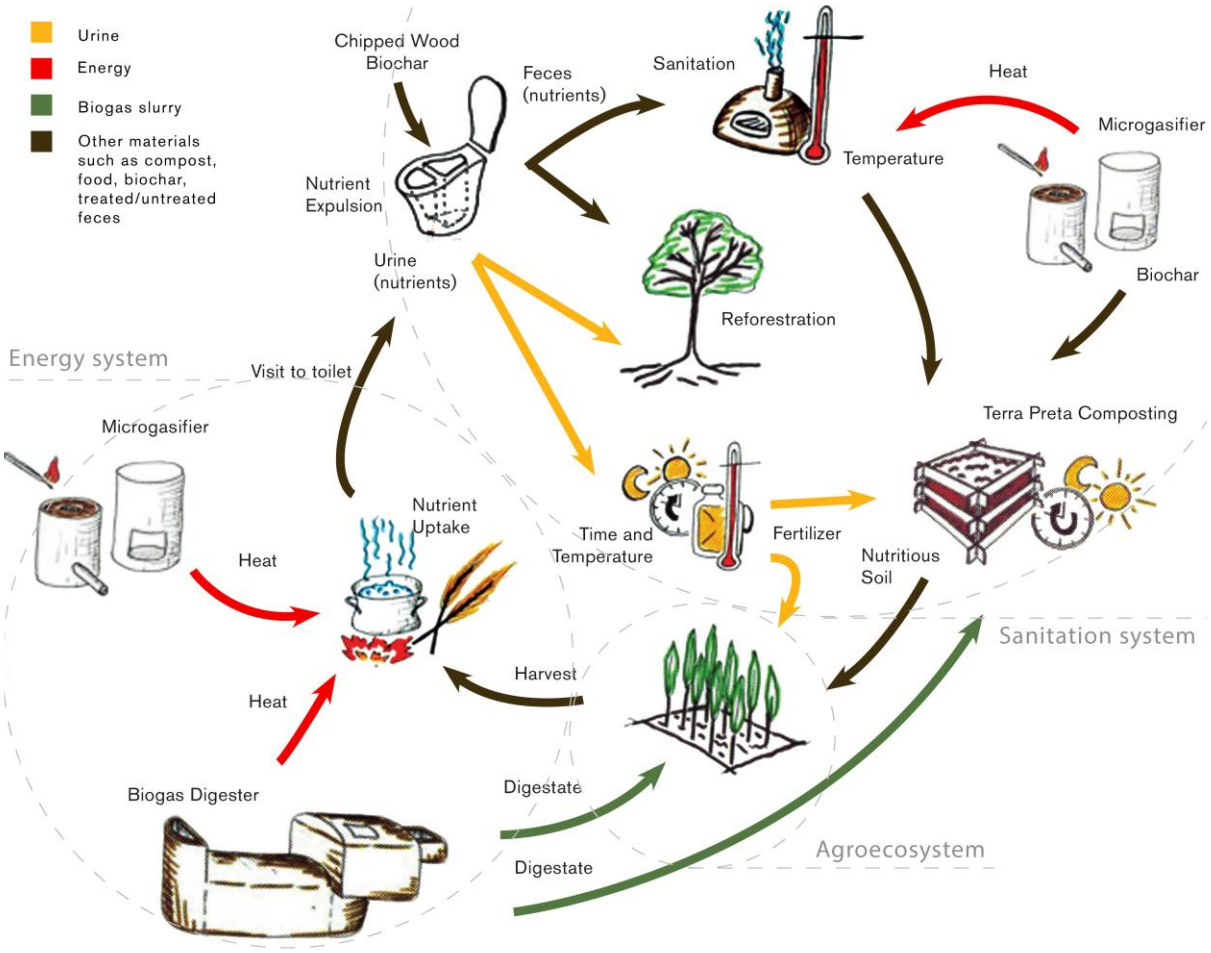

Figure 2. Illustrated concept of the integrated approach of bioenergy, EcoSan, TPP for sustainable food production where waste is considered a resource, as realized by three projects in Karagwe, Tanzania (own picture; with graphical assistance of Daniel Mutz and Lusi Ajonjoli).

(1) The project “Carbonization and Sanitation” (CaSa) aims at closing the cycle of nutrients on a local scale by recycling human excreta without health hazards. This project is a cooperation of MAVUNO, EWB and TU (CaSa, 2011). The approach is called CaSa because the heat of the carbonization process is used for thermal sanitation (Figure 2). The process starts in a UDDT, where a mixture of dry materials like biochar, sawdust, loam soil and ash is added after defecation to improve and accelerate drying of the feces. All solid parts including toilet paper are collected in aluminum pots and remain inside the UDDT for two to four weeks in order to dry. Afterwards, the pot is brought to a loam oven for thermal sanitation via the process of pasteurization, where microgasifiers are used to provide the required heat. Finally, the co-produced biochar, sanitized human feces and stored urine are composted together with other organic and mineral residues. The pilot project for testing the technologies started in 2012 and finished in 2014; since then the implementation has begun with the construction of eight toilets, a sanitation area and a composting area in a boarding school in Karagwe.

(2) The project “Biogas Support for Tanzania” (BiogaST) focuses on the sustainable provision of cooking energy through small-scale biogas digesters, which use organic residues from farming. It is a cooperation of MAVUNO, EWB and the University of Hohenheim in Stuttgart, Germany. The technology follows the design of a plug flow reactor and uses mainly cut pieces of banana tree stump, mixed with cow dung and kitchen waste. Water, together with the anaerobic microorganisms, is recycled and nutrient-rich biogas slurry is produced (Becker and Krause, 2011). Since 2010, two pilot digesters have been in operation to study (i) the effect of using different organic wastes in different mixtures and (ii) the design of a heating system to raise the temperature inside the fermenter and consequently increase biogas production. In 2015, implementation will start with the construction of a larger digester to provide a school canteen with cooking energy.

(3) The project “Efficient Cooking in Tanzania” (EfCoiTa) conducts research on advanced designs of microgasifiers including TLUD and improved sawdust stove (Ndibalema and Berten, 2015). In this project, CHEMA and EWB work in close cooperation with the Center for Research in Energy and Energy Conservation (CREEC) based at Makerere University and Awamu Biomass Energy Ltd, both located in Kampala, Uganda. In 2014, a series of so-called water boiling tests were performed to assess the resource efficiency and currently, in 2015, so-called controlled cooking tests are in progress together with kitchen performance tests to evaluate the practical use of the stoves in local households (Ndibalema and Berten, 2015).

Technically, these projects are connected through the use of microgasifiers for thermal cooking energy in the EfCoiTa-project as well as for the sanitation process in the CaSa-project. Furthermore, they collectively consider waste as a resource and exercise the use

of by-products as soil amendments according to the principles of TPP. Hence, the assessment of these substrates regarding their fertilizing effect and the evaluation of potential impacts on the local soil's nutrient budget was among the first tasks of the accompanied ecological research.

Substrates tested as soil amendment.

The following substrates derived from the CaSa- and BiogaST-projects were tested:

1. Urine collected in UDDT and stored for two months in closed jerry cans for sanitation.
2. Biogas slurry from the first pilot digester using banana tree stump mixed with cow dung for fermentation (mixture 1:1 by volume).
3. Grass is included in the assessment because, according to local practice, plots where biogas slurry is applied are covered with grasses.
4. Compost prepared by local farmers containing a mixture of fresh and dried grasses (91 vol%), ash (3 vol%) and kitchen waste (6 vol%). In addition, water was added to improve the moisture content of the mixture and topsoil was added to introduce microorganisms. Composting was done in one batch for about three months in a shallow pit in the ground, covered with soil and grasses to mitigate evaporation.
5. CaSa-compost containing sanitized human feces (15 vol%), biochar (17 vol%; residues from microgasification of eucalyptus-sawdust with pyrolitic temperature conditions of over 500°C, residence time ≥ 120 min), kitchen waste and harvest residues (15 vol%; beans straw, banana peels), mineral material (31 vol%; ash from three stone fire with eucalyptus wood, brick particles, local soil to add minerals and soil microorganisms) and woody material (22 vol%; sawdust, grasses). In addition, 1.2 dm^3 of stored urine was added per 10 dm^3 of solid material. Urine was mixed with sawdust or charcoal two days before addition to the compost pit so that N contained in urine could be adsorbed to the charcoal. Composting was done continuously with weekly addition of one pot of about 20 dm^3 of sanitized feces and the other materials in the respective amounts. The compost pit was located in a shallow hole under the shade of a tree and covered with grasses.

Analytical assessment of the soil amendments

A series of analyses were carried out to assess the fertilizing potential of the tested amendments. Total concentrations of nutrients, P_{tot}, K_{tot}, Ca_{tot}, Mg_{tot}, Zn_{tot}, Mn_{tot}, aluminium (Al_{tot}), and iron (Fe_{tot}), were determined after nitric acid (HNO_3) digestion under pressure using inductively coupled plasma optical emission spectrometry (ICP-OES; with iCAP 6000, Thermo Scientific, Waltham, USA) and method according to König (2005). Total concentrations of C (C_{tot}) and N (N_{tot}) were analyzed after dry combustion of oven-dry material using a thermal conductivity detector (with CNS-Analyzer, Vario ELIII, Elementar, Hanau, Germany) and method according to ISO DIN 10694 (1995) for C_{tot} and ISO DIN 13878 (1998) for N_{tot}. Mineral nitrogen (N_{min}) was extracted with potassium chloride (KCl) and analyzed using test strips (AgroQuant 114602 Soil Laboratory, Merck, Darmstadt, Germany). The method involved the suspension of 50 g material of the amenders in 0.1 dm^3 of 0.1 mol KCl. Within the same solution, pH was measured by using a glass electrode (pH 330i, WTW, Weilheim, Germany). In addition, gravimetric determination of water content of the fresh matter (wc_{FM}) was made for each material by weighing the materials before and after drying in a laboratory oven, at 105°C and 24 h for compost and at 65°C and 72 h for biogas slurry. Bulk density (ρ) of the composts was determined by filling 20 dm^3 buckets with equally poured fresh matter (FM) and measuring the weight respectively. Total concentrations of nutrients and C were measured at the laboratory of TU Berlin at the department of soil science. Other analyses were done on-site in MAVUNO's laboratory.

Data analyses

We calculated mean values (x) and standard deviations (σ) using MS Excel. For the experimental measurements, the numbers of replications (n) varied and were n=1, 2 and 5 for grasses, biogas slurry and compost as well as CaSa-compost respectively. We compared the assessed data considering the interval of $x \pm \sigma$. Furthermore, we applied propagation of errors to determine the uncertainty of the calculated values.

RESULTS AND DISCUSSION

Nutrient concentrations in substrates derived from case studies

The pH of all tested substrates was similar and slightly alkaline (Table 1). According to literature, the pH of fresh urine depends on the nutrition and varies between 4.8 and 7.5. During storage the pH rises to 8.8 or 9.2 (Schönning and Stenström, 2004). The wc_{FM} ranged from 25.0 ± 13.1% to 33.6 ± 5.3 and 32.5 ± 1.9 up to 95.6 ± 0.5 % of the FM for grasses, compost, CaSa-compost and biogas slurry respectively. With 770.5 ± 8.9 g dm^{-3}, CaSa-compost had a higher bulk density of FM as compared to the local compost with 546.5 ± 1.5 g dm^{-3}. This might be related to the differences in content of C_{tot} in FM because CaSa-compost showed with 60.1 ± 6.9 g dm^3 nearly two times higher concentration than compost while concentration in biogas slurry was about half of that for CaSa-compost. With 5.3 ± 0.2 g kg^{-1} and 6.0 ± 0.5 g kg^{-1}, compost and CaSa-compost showed comparatively low N_{tot}, with a concentration of N_{tot} in DM typically around 12 g kg^{-1} for composts (Horn et al., 2010); compared to 19.9 ± 0.1 g kg^{-1} in biogas slurry. The dominant forms of available N_{min} were ammonium (NH_4^+) in biogas slurry and nitrate (NO_3^-) in compost and CaSa-compost, while the concentration was highest in biogas slurry and similar in both composts. Furthermore, CaSa-compost showed adequate fertilizing potential with concentrations of P_{tot} in DM of 3.2 ± 0.2 g kg^{-1}, compared to literature for composts made of organic residues with an average value of about 1 g kg^{-1} (Finck, 2007). With P_{tot} in FM of 1.7 ± 0.1 g dm^{-3}, the concentration was 3.6 times and 5 times higher compared to compost and biogas slurry respectively. In addition, concentrations of K_{tot}, Mg_{tot}, Ca_{tot}, Zn_{tot} were higher in CaSa-compost compared to the other amendments.

Furthermore, the ratios of C and N, P, S need to be considered to avoid immobilization of N, P or S during organic decomposition after the application of the soil amendments. Thresholds are C/N > 25, C/P > 150

Table 1. Analytical assessment of the tested soil amendments.

	pH	wc	C_{tot}	N_{tot}	N_{min}	S_{tot}	P_{tot}	K_{tot}	Mg_{tot}	Ca_{tot}	Al_{tot}	Fe_{tot}	Zn_{tot}	Mn_{tot}
	$_{KCl}$	% (FM)	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}
Gras		25.0 ± 13.1	426.3	1.9	ua.	1.7	1.0	13.8	2.8	8.6	4.9	4.0	24.1	172.4
Biogas slurry	7.7	95.6 ± 0.5	347.8 ± 6.4	19.9 ± 0.1	16.0 ± 0.8	3.1 ± 0.02	7.6 ± 0.2	92.9 ± 8.4	12.2 ± 0.1	17.4 ± 0.9	4.0 ± 0.7	4.3 ± 0.07	115.3 ± 1.7	282.7 ± 8.8
Compost	7.4	33.6 ± 5.3	90.6 ± 7.7	5.3 ± 0.2	0.12 ± 0.04	1.2 ± 0.05	1.2 ± 0.1	8.5 ± 1.2	3.2 ± 0.2	10.0 ± 1.2	77.5 ± 1.6	65.2 ± 10.3	59.5 ± 4.3	641.4 ± 105.6
CaSa	7.5	32.5 ± 1.9	115.6 ± 11.4	6.0 ± 0.5	0.36 ± 0.07	1.3 ± 0.1	3.2 ± 0.2	14.6 ± 1.4	5.1 ± 0.5	29.6 ± 2.8	54.5 ± 1.4	83.5 ± 17.5	67.0 ± 4.7	480.2 ± 47.7
	ρ_{FM}	ρ_{DM}	C_{tot}	N_{tot}	N_{min}	S_{tot}	P_{tot}	K_{tot}	Mg_{tot}	Ca_{tot}	Al_{tot}	Fe_{tot}	Zn_{tot}	Mn_{tot}
	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	g dm^{-3}	mg dm^{-3}	mg dm^{-3}
Gras	77.4 ± 0.7	58.0 ± 30.4	24.7 ± 13.0	0.1 ± 0.1	ua.	0.1 ± 0.1	0.1 ± 0.03	0.8 ± 0.4	0.2 ± 0.1	0.5 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	1.4 ± 0.7	10.0 ± 5.2
Biogas slurry	1000 ± 50*	44.0 ± 2.2	15.3 ± 0.8	0.9 ± 0.04	0.7 ± 0.05	0.1 ± 0.01	0.3 ± 0.02	4.1 ± 0.4	0.5 ± 0.03	0.8 ± 0.1	0.2 ± 0.03	0.2 ± 0.01	5.1 ± 0.3	12.4 ± 0.7
Compost	546.5 ± 1.5	362.9 ± 57.2	32.9 ± 5.9	1.9 ± 0.3	0.04 ± 0.02	0.4 ± 0.1	0.5 ± 0.1	3.1 ± 0.7	1.1 ± 0.2	3.6 ± 0.7	28.1 ± 4.5	23.7 ± 5.3	21.6 ± 3.7	232.8 ± 53.1
CaSa	770.5 ± 8.9	520.1 ± 31.0	60.1 ± 6.9	3.1 ± 0.3	0.2 ± 0.04	0.7 ± 0.1	1.7 ± 0.1	7.6 ± 0.9	2.7 ± 0.3	15.4 ± 1.7	28.3 ± 1.8	43.4 ± 9.5	34.9 ± 3.2	249.7 ± 28.9
Urine **	1030	30	8.0	9.2	n.a.	1.5	0.5	2.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Element concentrations in DM of the tested soil amendments [g kg^{-1} and mg kg^{-1}] and bulk density of FM (ρ_{FM}) [g dm^{-3}] were analyzed and are displayed with mean value and standard deviation with n=1, 2 and 5 for grasses, biogas slurry and compost as well as CaSa-compost respectively. Element concentrations in FM based on volume [g dm^{-3} and mg dm^{-3}] and bulk density of DM (ρ_{DM}) [g dm^{-3}] are calculated by using wc and displayed with mean values and standard error calculated applying propagation of error. *Density of slurry was unanalyzed (ua.); assumption is based on literature for liquid biogas slurry (Vögeli et al., 2014). ** Values are based on literature for stored urine (Berger, 2008; some concentrations were not available, n.a.)

and C/S > 150 (Finck, 2007). With C/N of about 18, 17 and 14for biogas slurry, compost and CaSa-compost respectively, the immobilization of N is not likely. The same was shown for the immobilization of P with C/P ratios of 46, 73 and 36 and immobilization of S with C/S ratios of 114, 74, 63 for biogas slurry, compost and CaSa-compost respectively. Compared to the assessed amendments, N_{tot}-concentration in urine is comparatively high and concentration of P_{tot} and K_{tot} are in the range of compost with 0.5 and 2.2 g dm^{-3} respectively (Berger, 2008). However, according to Finck (2007), plants will initially utilize only a certain proportion of the added nutrients of the assessed fertilizing amendments. The remaining amount will stay in the soil and be taken in the next cropping seasons, if not leached out (e.g. for S, N), volatilized (e.g. for N) or taken away through erosion (e.g. for P). Hence, the total concentrations we presented here should be considered as “apparent” utilizations (Finck, 2007) or specific nutrient recycling potential.

Assessment of the tested amendments with respect to nutrient availability in the soil

The availability of nutrients in the soil is, among other factors, a function of soil pH. The optimum range of pH for agricultural soils depends on the clay content as well as on the concentration of SOM and is, on an average, between 5.5 and 6.5 (Horn et al., 2010; Finck, 2007). An increase of soil pH in the topsoil, depending on the treatment and the respective nutrient addition, has often been considered to have an immediate impact on harvest yield (Jeffery et al., 2011; Liu et al., 2013). Falcão et al. (2009) argued that the high productivity of plants growing on Terra Preta is inter alia due to the improved pH and consequent reduction of Al-toxicity.

As mentioned earlier, in preliminary studies we found very low values of about 3.8 to 4.2 for soil pH in Karagwe. Commonly, lime ($CaCO_3$) is used to neutralize soil acidity (Horn et al., 2010). However, organic material also has the potential to buffer acids in soils (Wong et al., 1998). Furthermore, Biederman and Harpole (2013) concluded that the addition of biochar can improve the availability of nutrients in the soil through soil liming effects.

Table 2. Effects on soil acidification or alkalization of the tested soil amendments in comparison to organic (Jobe et al., 2007) and synthetic fertilizers (Sluijsmansen, 1970; KTBL, 2009; Fink, 1979) expressed in kg of CaO in 100 kg of DM and in kg of CaO in each kg of N_{tot}.

Treatment	E	
	kg_{CaO} $100\ kg_{DM}^{-1}$	kg_{CaO} kg_{N}^{-1}
Tested soil amendments		
Biogas slurry	+ 6.8	+ 3.4
Compost	+ 1.4	+ 2.6
CaSa-compost	+ 4.7	+ 7.8
Organic fertilizers		
Poultry manure I	+ 14	+ 10.0
Fish waste I	+ 3.5	+ 0.8
Fish waste II	+ 3.5	+ 0.8
Poultry manure II	+ 13.6	+ 9.7
Sugar molasses	+ 3.5	+ 1.4
Cattle manure	+ 2.7	+ 2.1
Synthetic fertilizers		
Ammonium sulfate	- 63	- 3
Calcium ammonium nitrate (22% N)	- 4	0
Urea	- 46	- 1
Calcium nitrate	+ 13	+ 1

Wong et al. (1998) proposed an acid titration method to quantify the acid neutralizing capacity of compost (ANC). Jobe et al. (2007) used this method and estimated ANC ranging between 95 and 500 cmol H^+ kg^{-1} for six different composts. If complete mineralization of the compost and oxidation of organic N and S are considered, which is reasonable under tropical soil conditions, the ANC may, however, simply be calculated as the difference between metal- (M+) and non-metal-equivalents (A-) in the compost. This is possible, because the mineralization of M+ is a H^+-sink and the mineralization of A- is a H^+-source (Van Breemen et al., 1983). Under these conditions the formula which was developed by Sluijsmans (1970) for the prediction of the liming effect E, expressed as kg CaO equivalent of 100 kg of DM of any fertilizer may be applied:

$$E = (1.0 \times CaO + 1.4 \times MgO + 0.6 \times K_2O) - (0.4 \times P_2O_5 + 0.7 \times SO_3 + 2 \times N) \quad (1)$$

The amounts of nutrients (CaO, MgO etc.) are to be inserted into the equation in kg of nutrient per 100 kg of fertilizer. Overall, the compost application will cause acidification if E < 0 and alkalization if E > 0.

The results of our calculation using Equation 1 are presented in Table 2 and compared with literature for selected organic and synthetic fertilizers (Sluijsmansen, 1970; KTBL, 2009; Fink, 1979; Jobe et al., 2007). In addition, we calculated the liming effect related to N in the various fertilizers.

Additions of 100 kg of DM of, respectively, biogas slurry, compost or CaSa-compost are equivalent to 6.8, 1.4 and 4.7 kg of CaO. Thus, all products will cause alkalization and reduce acidity of the soil. Our results are well in line with the range of pH buffering capacity of different composts given by Jobe et al. (2007). The liming effect related to N_{tot} in the tested amendments is similar for biogas slurry and compost, with 3.4 and 2.6 kg of CaO per kg of N_{tot} respectively, while the value is more than doubled for CaSa-compost. In comparison with our results, most synthetic N-fertilizers that are commonly used would cause soil acidification. For example, if 100 kg of urea are applied as N_2-fertilizer, about 46 kg CaO are needed to buffer the acidification effect in the soil. Among the synthetic N-fertilizers only calcium nitrate ($Ca(NO_3)_2$) has a positive value for E with 100 kg of calcium nitrate being the equivalent of 13 kg of CaO and 1 kg N addition being the equivalent of 1 kg CaO.

Since Batjes and Sombroek (1997) pointed out that “stable increase in SOM in deeply weathered tropical soils occur especially with addition of phosphate and lime”, we deduce that all of the assessed soil amendments can contribute to sustainable soil improvement through P-recycling and liming with this holding true especially for CaSa-compost. Increased P-levels in the soil may also contribute to mitigation measures since crops may root deeper and, thus, are less vulnerable to droughts and render P-cycling through organic residues more effective (Batjes and Sombroek,

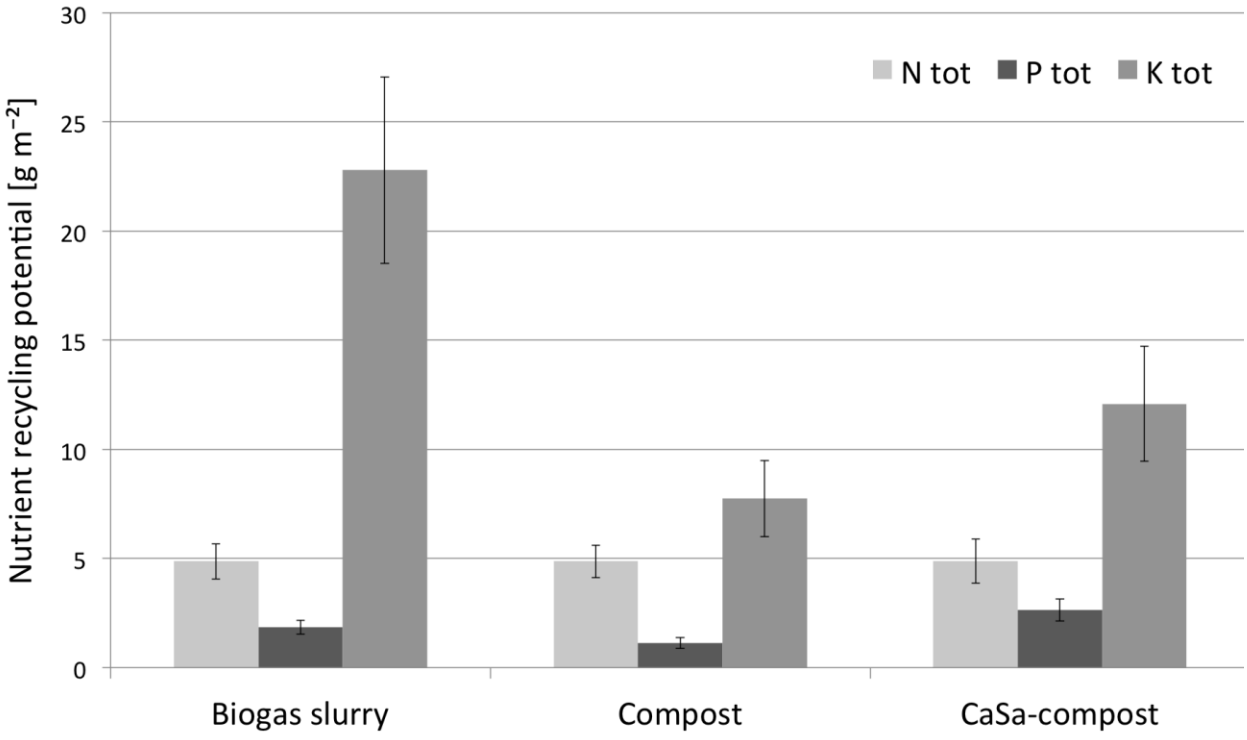

Figure 3. Total nutrient recycling potential expressed in nutrient addition [g m^{-2}] for N_{tot}, P_{tot}, and K_{tot} corresponding with application doses of 5.5, 2.5 and 1.6 dm^3 m^{-2} for biogas slurry, compost and CaSa-compost respectively.

1997).

Estimation of the total nutrient recycling potential in agricultural practice

According to Mafongoya et al. (2007), the amount of manure applied by farmers in SSA is on an average within a range of 1 to 1.5 kg m^{-2} per year which is equivalent to about 1.8 to 2.7 dm^3 m^{-2} (calculated with ρ_{FM} as presented in Table 1). Hence, we estimated the total nutrient recycling potentials for N_{tot}, P_{tot} and K_{tot} in g m^{-2} in the tested soil amendments (Figure 3).

An application of the tested local compost in FM with 2.5 dm^3 m^{-2} per year resulted in a potential nutrient addition to the soil of 4.9 ± 0.8, 1.1 ± 0.3 and 7.7 ± 1.8 g m^{-2} a^{-1} for N_{tot}, P_{tot} and K_{tot} respectively. According to the premise, that the same dose of N should be obtained with the other tested soil amendments, we subsequently calculated the necessary application of CaSa-compost and biogas slurry in FM to be 1.6 ± 0.3 and 5.5 ± 0.9 dm^3 m^{-2} a^{-1} respectively. Thus, to reach the same level of N-application, the required amount of CaSa-compost is, on average, only about 65% of the required amount of conventional compost. In other words, an available amount of 1000 dm^3 of compost material in FM would suffice for application on 400 m^2 by using compost and on about 630 m^2 by using CaSa-compost. Given these specific application doses, the resulting addition of P_{tot} by CaSa-compost would be about 1.4 and 2.3 times higher compared to biogas slurry and compost respectively. Ranging from 1.1 up to 2.6 g m^{-2} a^{-1} the estimated recycling potentials for P_{tot} are very low, especially on soils with low P-concentrations (KTBL, 2009; Finck, 2007). The calculated recycling potential for K_{tot} is about 7.7 g m^{-2} for compost and 1.6 and 2.9 times higher for CaSa-compost and biogas slurry respectively. With the estimated K-additions, the local compost as well as CaSa-compost meet the requirements for appropriate K-fertilization on soils, with an adequate K-supply of about 13 to 19 g m^{-2} on an average (KTBL, 2009; Finck, 2007). Only biogas slurry exceeds this fertilizing recommendation. According to Finck (2007), an increasing addition of K lowers the uptake of Ca and Mg during plant growth (“antagonism of nutrient uptake”). Given the K-addition with biogas slurry, it is recommendable to mix (or compost) biogas slurry prior to its application with other materials containing more N and P compared to K to reach a better balanced nutrient ratio of N:P:K. This ratio was 4:1:7, 2:1:5 and 3:1:12 for compost, CaSa-compost and biogas slurry respectively. Furthermore, the corresponding input of C_{tot} would be about the same for all tested soil amendments with 86 ± 14, 82 ± 15 and 96 ± 21 g m^{-2} for biogas slurry, compost and CaSa-compost respectively. However, the kind of C differed in the materials, as CaSa-compost

contains biochar, that is, a source of stable C.

Estimation of the local potential to close the loop

Stoorvogel et al. (1993) calculated soil nutrient balances for African countries for the year 2000. They considered mineral fertilizer, animal manure, dry deposition, biological N-fixation and sedimentation as inputs to the agricultural land, while the removal of harvest products and crop residues, leaching, gaseous emissions and erosion were accounted for as losses. Their results showed an average negative balance per year and per square meter of 3.2 g N, 0.5 g P, and 2.1 g K on arable land in Tanzania. Looking at a neighboring country, bordering Kagera region, Jönsson et al. (2004) assessed that the human excreta of one Ugandan person contains in total 2.5 kg N and 0.4 kg P per year. Combining this data, we estimated the recycling potential of EcoSan for one family with 6 people to be about 15 kg N and 2.4 kg P per year, which would be sufficient to cover the negative balance of approximately 4800 m^2. Furthermore, Baijukya and de Steenhuijsen Piters (1998) calculated "Nutrient balances in the banana-based land use systems of northwest Tanzania", including Karagwe district. In addition to the balances of Stoorvogel et al. (1993), they also considered mulching and subsoil exploitation by perennial trees as input flows. Their balances were done for farms with different nutrient management levels. For farms without cattle and without brewing activities (lowest management level), they calculated an average loss per year of around 2.8 g N, 0.3 g P, and 3.0 g K on one m^2. They concluded that "substantial amounts of nutrients are lost through human feces and end up in deep pit latrines" and demanded changes in the sanitation system to "facilitate the recycling of nutrients in feces" (*ibid*.). On this basis, we assessed the potentials of the tested soil amendments to contribute to the local nutrient budget to close the loop. As P-scarcity was identified as a major problem in our pre-studies and since N-fertilization can more easily be realized with the use of urine as a fertilizer, we calculated the required amounts for compensation of the negative P-balance.

Our results show that the estimated required amount of FM is approximately 6 and 3 times higher for biogas slurry and compost respectively as compared to CaSa-compost with about 0.1 kg m^{-2} a^{-1} (Figure 4). Respective amounts based on volume are considered feasible, ranging from around 0.2 to 0.8 dm^3 m^{-2} a^{-1}. Given the fact, that one farmer household in Karagwe cultivates on average 6,225 m^2 (Tanzania, 2012), the required total amounts of FM per household to close the loop for P would be 5.0, 2.0 and 0.8 t a^{-1} for biogas slurry, local compost and CaSa-compost respectively. However, by adding the respective substrates to the soil, negative balances for N and K still remain. Considering calculated amounts and N_{tot}-concentration of the substrates, we calculated that the N-deficit would be covered by 26, 42 and 18% for biogas slurry, compost and CaSa-compost respectively. Additional nutrient requirements could be covered, for example, by applying urine as fertilizer with about 0.2 dm^3 m^{-2} a^{-1} according to own calculations. Hence, the total amount of urine required to cover the remaining N-deficit on one small-scale farm in Karagwe with 6,225 m^2 cultivated land would be about 1.7, 1.3 and 1.8 m^3 a^{-1}. According to Winblad et al. (2004) the excreta of person includes 1 dm^3 urine per day so that one family with 6 people has about 2.2 m^3 urine available per year and could finally close the local nutrient balance on their farmland.

CONCLUSION AND RECOMMENDATION

The introduced projects and case studies of this research present an integrated approach of resource management where different substrates rich in mineral nutrients, such as ash, biogas slurry, stored urine and sanitized feces are recycled in combination with C-rich materials such as biochar. The results of our first investigations support our hypothesis that new approaches that combine EcoSan, bioenergy and TPP can contribute to the recycling of nutrients and C-sequestration as well as to soil improvement. The analytical assessment of the substrates derived from these projects showed that all of the tested substrates are feasible soil amendments due to their sufficient nutrient concentrations and adequate nutrient ratios compared to literature. Based on the more practice-oriented volume [dm^3], CaSa-compost showed the highest concentration of all nutrients as well as C, followed by compost and biogas slurry. Furthermore, all tested soil amendments have good liming potential compared to other soil amendments. As $CaCO_3$ is usually quite expensive, we conclude that all tested substrates are a feasible low-cost option for liming. Especially the locally produced CaSa-compost is promising due to the comparatively high P-concentration and E-value for liming. Under the circumstances given in Karagwe, sufficient application rates of CaSa-compost can contribute to mitigating existing P-scarcity and acidification in the soil and, consequently, to increasing biomass production. Furthermore, our final evaluation revealed that amounts of FM of less than one dm^3 m^{-2} a^{-1} of the assessed materials in combination with urine are required to close existing open nutrient cycles (for P and N) in Karagwe. However, higher amounts of the soil amendments are required if they should be applied as a major source of nutrients, in order to provide a full substitution of the existing input of mineral fertilizer and animal manure. We conclude that EcoSan combined with TPP as well as the use of biogas slurry are promising practices to close the loop in the agroecosystems in SSA (as well as elsewhere). However, there is a need for practice-oriented experiments to assess short and long-term effects of these amendments on biomass production

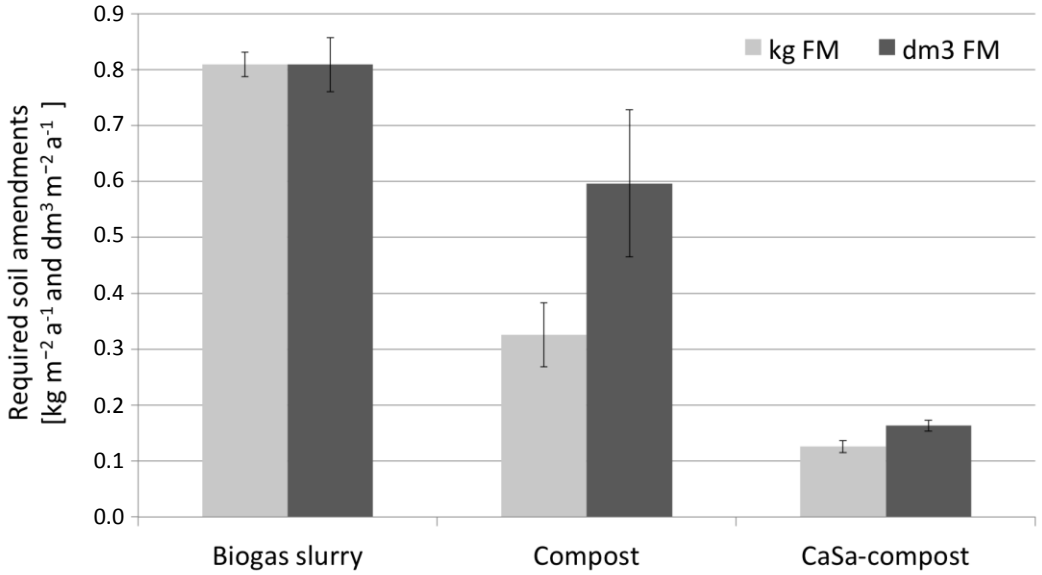

Figure 4. Calculated required amounts of FM of the tested substrates [kg ha^{-1} a^{-1} and dm^3 ha^{-1} a^{-1}] to compensate the negative P-balance of 0.3 g m^{-2} a^{-1} in banana-based land use systems of northwest Tanzania (Baijukya and de Steenhuijsen Piters, 1998).

and soil properties. Altogether, the strategies to investigate further potentials of the substrates derived from the projects include (1) practice-oriented field experiments to compare and to assess the short-term effectiveness of urine, biogas slurry, compost and CaSa-compost as a fertilizer with respect to crop productivity and crop nutrition as well as potential soil improvements. Furthermore, the applied resource management approach, as it is practiced in the introduced projects, should be (2) integrated in the local nutrient and C balance by using methods such as Material Flow Analysis and (3) should finally be evaluated including other perspectives than only the ecological one (e.g. socio-economic) by using Multi-Criteria Analysis. In addition, long-term field experiments are required to investigate the sustainable effects on SOM and other fertility-related soil parameters, such as the water holding capacity.

Conflict of Interests

The authors have not declared any conflict of interests. All partner organizations of the projects agreed to the ecological research on the projects and that the products will be assessed and that the results will be published.

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Abbreviations

Biochar, Charcoal used as soil amendment; **BiogaST,** Project “Biogas Support for Tanzania”; **CaSa,** Project “Carbonization and Sanitation”; **CaSa-compost,** Product of CaSa-project containing composted biochar and sanitized excreta; **CHEMA,** Community Habitat Environmental Management; **CREEC,** Center for Research in Energy and Energy Conservation; **EcoSan,** ecological sanitation; **EfCoiTa,** Project “Efficient Cooking in Tanzania”; **EWB,** Engineers Without Borders; **IAASTD,** International Assessment of Agricultural Knowledge, Science and Technology for Development; **ICP-OES,** Inductively coupled plasma optical emission spectrometry; **IGZ,** Leibniz Institute of Vegetable and Ornamental Crops; **MAVUNO,** MAVUNO Project Improvement for Community Relief and Services;

("mavuno" meaning "harvest" in Swahili); **m.a.s.l.,** meter above sea level; **SOM,** soil organic matter; **SSA,** Sub-Saharan Africa; **TLUD,** top-lit up draft; **TPP,**Terra Preta practice; **TU,** Technische Universität; **UDDT,** urine diverting dry toilet; **WHO,** World Health Organization.

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Postharvest shelf-life and fruit quality of strawberry grown in different cropping systems

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The strawberry is highly perishable, has high metabolic activity and considerable levels of favorable substrates for proliferation of pathogenic organisms, such as humidity, organic acids and sugars. These organisms lead to further, therefore reducing post-harvest shelf-life. With this in mind, the research objective was to evaluate the post-harvest shelf life in cooled storage of two cultivars of strawberries (Oso Grande and Festival) in the hydroponic cultivation system in coconut fiber substrate, and then compare them with the quality obtained in conventional farming. The strawberries were stored under refrigeration (2 ± 2°C and 85% ± 10%) using modified atmosphere, with 9 µ PVC film for 14 days. The experiment was conducted in a completely randomized design, distributed in a sub-subdivided plot with the cropping systems in the plot, cultivars in the subplot and in the sub-subplot with storage times of 0, 3, 6, 10 and 14 days with three replicates. The use of cooling associated with modified atmosphere increased the shelf-life of the strawberries of both systems, indicating the potential of this combination to maintain the post-harvest quality of the fruit. Although the shelf life of cultivars of both systems increased, the fruits produced in the conventional system showed better physical-chemical characteristics.

Key word: *Fragaria x ananassa*, cold storage, conservation, modified atmosphere.

INTRODUCTION

In Brazil, the cultivation of the strawberry plant (*Fragaria x ananassa* Duch.) is being carried out in tropical areas, more specifically on small farms in the mountainous region of the state of Ceará. This is feasible due to the fact that the environmental conditions present similarities to those found in the main producing regions. The first crops in the region have shown good adaptation to the climate, reducing the time for the start of the harvest and have a lower incidence of pests and diseases. Since 2009, strawberries have been cultivated in the mountainous region of the state of Ceará, Brazil, predominantly the cv. Oso Grande in the conventional system, which presents some disadvantages. These disadvantages include leaf wetness, increased susceptibility

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to pests and diseases, and the need to rotate its planting areas. The areas need to be rotated since the successive use of the same area culminates in frequent problems like the spread of diseases in the plants causing reduction in crop productivity (Resende et al., 1999), which leads to an increased use of agricultural defense systems, hindering the acceptance and safety of the product for the consumers.

Despite being a non-climacteric fruit (Pineli et al., 2011), the strawberry is highly perishable, with high metabolic activity, greater susceptibility to mechanical injury and considerable levels of substrates favorable to proliferation of pathogenic organisms, such as moisture, organic acids and sugars. These organisms lead to a higher probability of deterioration, and therefore considerably reducing the post-harvest shelf-life. In order to extend the shelf-life through the reduction of the metabolic rate of the fruit and fruit rot control, cooled storage, is the main tool that has proven to be effective. However, for extended storage, it is still not enough to maintain good fruit quality, therefore an addition of other post-harvest preservation techniques, such as modified atmosphere are needed.

The modified atmosphere has had success in many vegetables. This technique consists of an increase in the partial pressure of the CO_2 and a decrease in the O_2 (Silveira et al., 2014), caused by the gas exchanges of the container with the ambient air. The packaging with low partial pressure of oxygen can reduce the breathing rate and maintain the shelf-life for a longer time or with better quality than the package with normal air. However, the extremely low oxygen content may result, in some cases, in fermentation resulting in an accumulation of unpleasant odors and tastes, reducing the aroma biosynthesis and tissue damage (Li et al., 2014). In this type of atmosphere, the partial pressure of O_2 and CO_2 are not controlled, and vary with time, temperature, type of film and the respiratory rate of the product (Chitarra and Chitarra, 2005).

Since the recent strawberry crop in the mountainous region of Ceará, is still adopting conducting techniques from other producing regions, there is a need for studies in the region with other cropping systems and crop cultivars, and the post-harvest shelf-life of the fruit. With this in mind, the objective of the research was to evaluate the post-harvest shelf life of two cultivars of strawberries (Oso Grande and Festival) in the hydroponic cultivation system in coconut fiber substrate, and compare them with the quality obtained in conventional farming, with cooled storage under modified atmosphere.

MATERIALS AND METHODS

Experimental conditions

The experiment was conducted in 2012 in the county of Ibiapina-CE, located in the Serra da Ibiapaba (mountainous region), 360 km from Fortaleza-CE, Brazil. The average temperature and relative humidity in the conventional system were 21.58°C and 61.76% and in the greenhouse 23.38°C and 71.24%, respectively.

The strawberries were stored under refrigeration (2 ± 2°C and 85% ± 10%) using modified atmosphere, with 9 µ PVC film for 14 days. The experiment was conducted in a completely randomized design, distributed in sub-subdivided plots with cropping systems (hydroponic and conventional) in the plot, Oso Grande and Festival cultivars in the subplot and the sub-subplot with storage times of 0, 3, 6, 10 and 14 days, with three replicates, represented by a tray with approximately 200 g of strawberries.

In the treatments in hydroponic crops, two wooden benches were used that were installed under a high tunnel with a tubular structure in galvanized steel, covered with white polyethylene film of 150 µm that was 3 m wide and 2 m high. The Golden Mix Misto type 80 coconut fiber substrate was used. The nutrient solution used presented itself with electrical conductivity ranging from 1.3 to 1.5 dS m^{-1} and a pH ranging from 5.4 to 6.5. The plots in conventional farming followed the technology already used by the farmer, which were installed outside of the tunnel in a garden that was 0.2 m high and 1 m wide, that was covered with black-white plastic (mulching), and before planting, the garden was fertilized with 180 g m^{-2} of simple superphosphate.

Quality evaluation

The fruits were harvested when they appeared to be ripe, meaning they had fully red skin, then transported to the laboratory, where they went through a selection process, where those with cuts or slots and insect attacks were discarded. Initially they were analyzed for the average mass variables (g) of the fruit, obtained by weighing each fruit individually with a semi-analytical scale; including the length (mm) and diameter (mm) of the fruit, determined with the use of a caliper rule. The other physical and physical-chemical variables were determined throughout the course of storage (0, 3, 6, 10 and 14 days). In each storage interval, the fruit samples were removed from the cooled chamber and physically analyzed and then processed using a domestic Walita® centrifuger, with the pulp stored at -20°C for a subsequent completion of the physical-chemical analysis.

The mass loss (%) was obtained through the difference between the initial mass and the mass obtained in each analysis interval and the firmness (N) was determined in the middle region of the whole fruit, with a manual Magness-Taylor model FT 011 penetrometer using a 8 mm tip. For the appearance we used a subjective grading scale, which considered the extra category - grade 4 (absence of serious defects and up to 5% of small defects), I - grade 3 (10% defects, considering up to 3 and 10% severe and mild defects, respectively) II - grade 2 (100% of defects, whereas up to 10 and 100% of severe and mild defects, respectively) (PBMH; PIMo, 2009). The severe defects that were analyzed were: Rot, mechanical damage, deep injury and severe deformation. Mild defects: Slight deformation, presence of foreign material and healed superficial damage. Grade 3, category I, was the minimum grade considered acceptable for consumption.

The titratable acidity (% citric acid) obtained through titration of the pulp with 0.1 M NaOH solution, according to the AOAC methodology (2005). Soluble solids (°Brix), the pulp, was filtered with filter paper and the content was measured with an Atago® model PR-101 Pallete digital refractometer, according to the AOAC (2005). The SS / TA ratio was determined by the ratio between the soluble solids and the titratable acidity values. The vitamin C (mg ascorbic acid 100 g^{-1}) was measured immediately after the processing of the fruit through titration with DFI solution of (2.6-

dichloro-phenol indophenol 0.02%) until it had a permanent pink color (Strohecker and Henning, 1967). Total anthocyanins and Yellow flavonoids (mg 100 g^{-1}) were extracted and determined through the method developed by Francis (1982).

Statistical analysis

Data was subjected to variance analysis (ANOVA) performed with the help of the SISVAR software version 5.3, and for the comparison of means, we used the Tukey test with 0.05 significance. Regression analysis was performed for data in which significant effects occurred.

RESULTS AND DISCUSSION

There were differences ($p<0.05$) between the crops and the crop systems that were isolated for the physical variables of the strawberries that were analyzed before storage. An average mass of 19.95 and 10.95 g, with a diameter of 31.64 and 24.01 mm and length of 40.86 and 36.97 mm for the hydroponic and conventional systems, respectively, was observed. Comparing the cultivars amongst themselves, the average mass of 16.21 and 14.47 g, diameter of 29.00 and 26.65 mm for the cvs. Oso Grande and Festival, respectively, was observed. The length of 39.21 mm for cv. Oso Grande and 38.63 mm for cv. Festival did not differ ($p>0.05$).

Similar results were observed by Resende et al. (2010), while evaluating the influence of different cropping systems on the average mass of strawberries in Guarapuava, in the state of Paraná. They found the highest and lowest values for the cultivar in greenhouses and field, respectively.

The largest fruits were obtained when produced hydroponically. This is probably due to the fact that plants are more healthy and vigorous when grown hydroponically, because of the nutritional and climatic conditions provided by this environment. This allows a greater expression of physiological activities and, consequently, a greater accumulation of carbohydrates which result in the increase of biomass (Taiz and Zeiger, 2013), resulting in larger strawberries. Meanwhile, the plants in the conventional system proved to be less vigorous, perhaps due to edafoclimatic stress conditions, resulting in smaller strawberries.

The storage time did not influence ($p>0.05$) the firmness of the pulp of the strawberries. As for systems and the cultivars, an isolated difference was observed ($p<0.01$), with higher values for the conventional system and the cv. Festival, averaging at 8.80 and 9.75 N respectively. As for the hydroponic system, it was 7.52 N and for the cv. Oso Grande it was 6.57 N.

The maximum firmness in the conventional cultivation system probably occurred due to higher weight loss in these fruits, because when water is lost, a stiffening of the cell walls of the fruit occurs. It may also be the result of the difference in nutrient availability between the systems, the culture management in the systems and the lowest temperature in the conventional, when compared to hydroponics.

A fact that was also confirmed by Pinto et al. (2012) while they studied the induction of weight loss in post-harvest quality of 'Eragil' peaches was an increase in firmness with an increase in mass loss.

Hoppula and Karhu (2006) observed that the low firmness in the strawberry is strongly related to high temperatures. They found a stronger correlation with the average temperature three weeks before the harvest. This indicates that the temperature conditions may affect the properties of the cell wall responsible for the firmness of the strawberry in the initial stages of the development of the fruit.

Time interaction was observed ($p<0.01$) between the systems and between the cultivars (Figure 1A and B) in relation appearance of the strawberries. The systems reduced 20 and 18.25% over time in the hydroponic and conventional systems, respectively, not differing from each other. The cvs. Oso Grande and Festival decreased over time, represented by 26.75 and 11.75%, respectively, showing a difference between them, only in the last storage time, with the highest value for the cv. Festival (average grade 3.53). However, at the end of the storage time, the fruits of both cultivars still had attractive features to consumers, such as firmness, external color and absence of injuries and rot.

The mass loss showed interaction ($p<0.05$) between the time, system and cultivars (Figure 2A). The largest losses were observed in the conventional system with the cv. Festival, which differed from other treatments at 10 and 14 days, presenting on the day 14 a mass loss of 2.1%. For other treatments, similar behavior was observed, not differing from each other at any point. The cv. Festival in the hydroponic system and the cv. Oso Grande in the conventional system showed a growing behavior throughout the storage period, reaching 1.18 and 1.33% at 14 days of mass loss, respectively. As for the cv. Oso Grande in the hydroponic system, it showed an increase up to day 10 (1.19%) followed by a drop until the end of the storage time. It was also observed that the cv. Oso Grande, unlike the cv. Festival was not different in both systems. According to Calegaro et al. (2002), the maximum loss commercially tolerated for strawberries is 6%, being that in this study, lower values were found.

The most massive loss recorded in strawberries in the conventional system may have been influenced by the smaller size of the fruit, which probably has a higher transpiration rate, compared to fruits produced in the hydroponic system. It is assumed that the transpiration rate of vegetables is proportional to its relative surface area / mass. In other words, with the increase in the size of the fruit, there is a reduction in the ratio surface area / mass and consequently a reduction of the transpiration

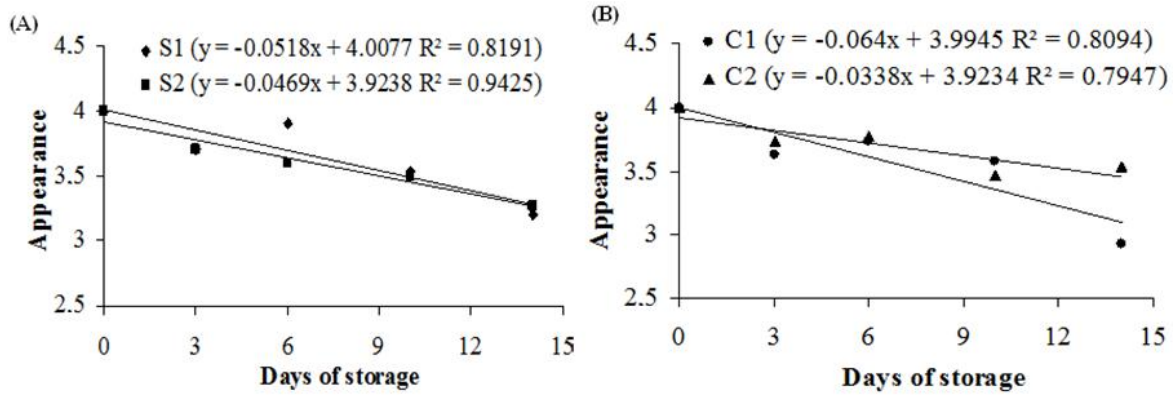

Figure 1. External appearance for system (A) and cultivars (B) of fruits of two strawberry cultivars and in two cropping systems and stored under modified atmosphere at 2 ± 2°C and 85 ± 10% U.R. S1 - hydroponic system; S2 - conventional system; C1 - Oso Grande; C2 - Festival.

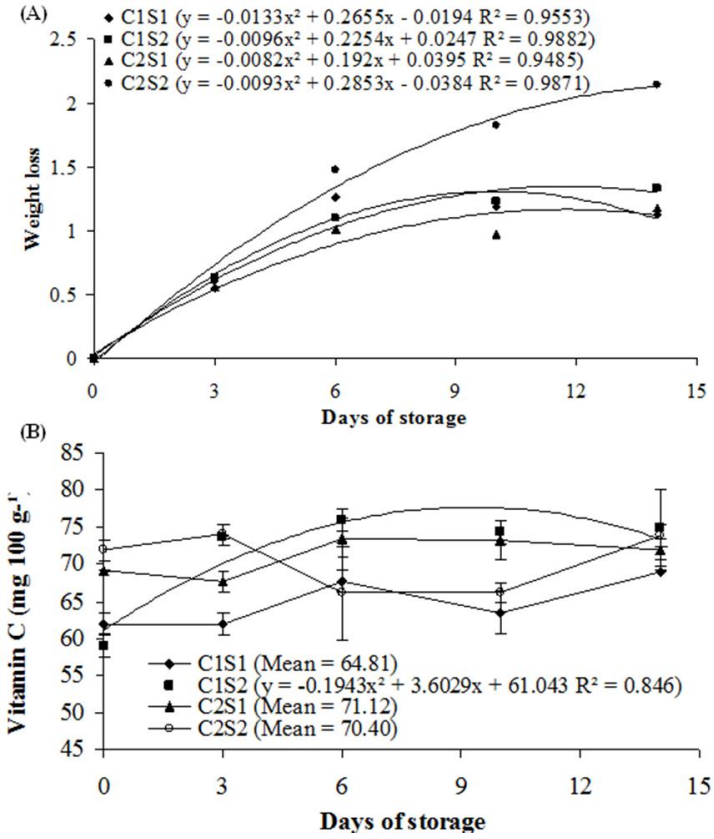

Figure 2. Weight loss (%, A) and Vitamin C (mg ascorbic acid 100 g^1, B) of fruits of two strawberry cultivars and in two cropping systems and stored under modified atmosphere at 2 ± 2 °C and 85 ± 10% U.R. S1 - hydroponic system; S2 - conventional system; C1 - Oso Grande; C2 – Festival.

rate, as shown in eggplants (Pérez, 1998).

In vitamin C, interaction was observed ($p<0.01$) between the storage time, systems and cultivars (Figure 2B). The hydroponic system with the cvs. Oso Grande and Festival and the conventional system with the cv. Festival showed variations over time, but at the end of

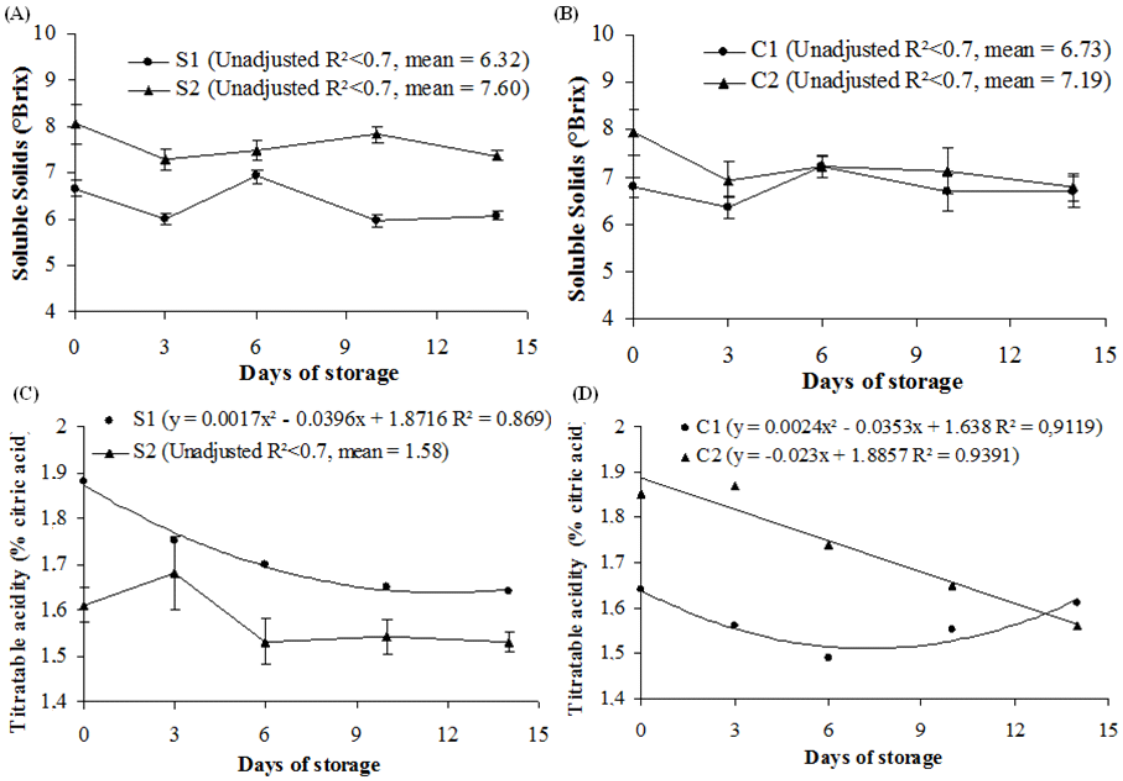

Figure 3. Soluble solids (°Brix, A and B) and Titratable acidity (% citric acid, C and D) of fruits of two strawberry cultivars in two cropping systems and stored under modified atmosphere at 2 ± 2 °C and 85 ± 10% U.R. S1 - hydroponic system; S2 - conventional system; C1 - Oso Grande; C2 – Festival.

the storage time, it maintained values similar to the original ones. For these treatments, there was not an equation that would fit. However, the cv. Oso Grande in the conventional system showed a higher level of variation, starting the storage time with 58.9 mg 100 g^{-1} and ending with 74.8 mg 100 g^{-1}. These variations in the quantification of the vitamin C may have been caused by the determination method that was used, using the titrimetric. With this tool, systematic errors may occur. And example of this is the difficulty in the visualization of the final titration point which presents a pink color, therefore when the strawberry presents an intense red color, it makes this visualization difficult.

The values of this study were higher than those observed by Atress et al. (2010), who while evaluating edible films in the cv. Festival strawberries in Egypt, found variations of approximately 10 to 35 mg 100 g^{-1} during the storage time.

There was interaction ($p<0.01$) between the storage time and the systems, as well as between the time and the cultivars in relation to the soluble solids content (Figure 3A and B). In both cases, there were fluctuations of soluble solids over time. They did not present an equation that would fit. These soluble solids results are in agreement with those obtained by Borges et al. (2013), who found similar values and also saw variations during the storage time.

The cultivars only differed until the third day, with a greater amount of soluble solids for the cv. Festival, maintaining similar values after this period. As for the systems, there were differences from the beginning to the end of the storage time, with the highest averages presented by the conventional system (Table 1). The highest concentration of SS in the conventional system may be due to direct sunlight, and also, the lowest water absorption by the plant, when compared to the hydroponic system, which has water more frequently. In the conventional system, the amount of water applied does not reflect the absorbed, since various factors affected the absorption of water. Among these is the evapotranspiration. This is influenced by several climatic parameters, the most important being the temperature, relative humidity, wind speed and solar radiation (Valipour, 2014a, b).

According to Kader (1999), the minimum amount recommended for the strawberry flavor to be acceptable is 7°Brix. In this study, lower values were observed when produced in the hydroponic system. These lower values

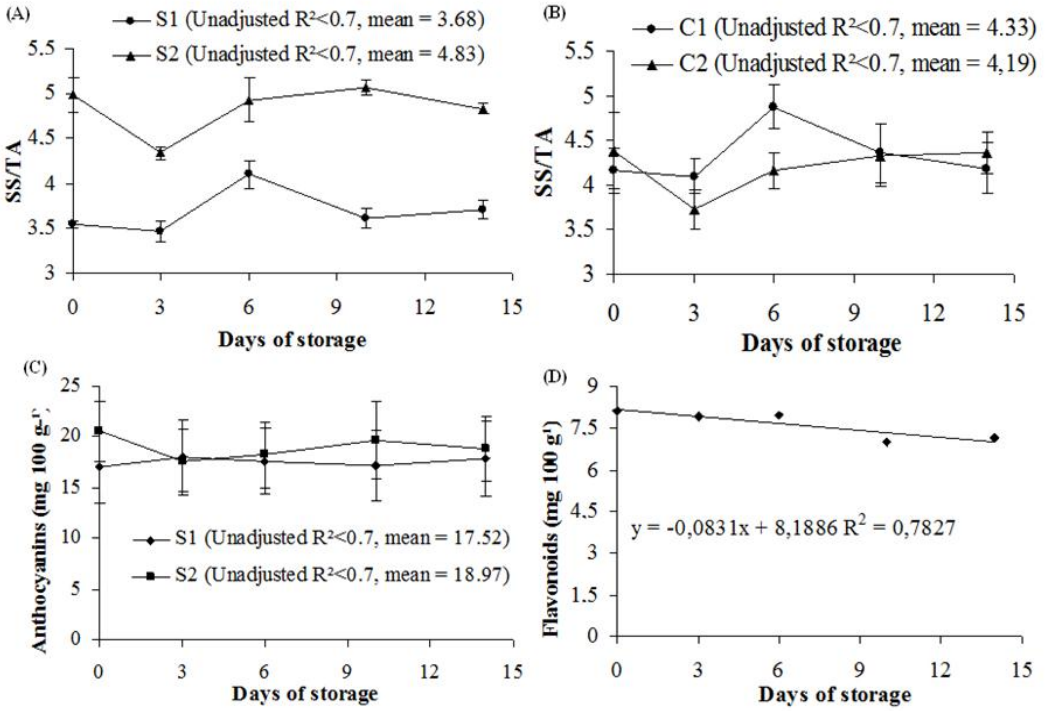

Figure 4. SS/TA ratio (A and B), Total anthocyanins (mg 100 g^{-1}, C) and Flavonoids (mg 100 g^{-1}, D) of fruits of two strawberry cultivars in two cropping systems and stored under modified atmosphere at 2 ± 2°C and 85 ± 10% U.R. S1 - hydroponic system; S2 - conventional system; C1 - Oso Grande; C2 – Festival.

are probably due to weather conditions that are unfavorable to the crop, that are present in the system that was used. The fruit that was produced in the conventional system showed values that fall within the acceptable minimum.

The titratable acidity showed interaction ($p<0.05$) between the storage time and the systems, and between the time and the cultivars (Figure 3C and D). During the study, one could observe that the acidity decreased over time, probably due to the use of organic acids as a substrate in respiratory metabolism during the storage time and /or as carbon skeletons for the synthesis of new compounds (Sólon et al., 2005). The systems differ from each other in all storage time intervals, except for the third day, that has the highest values observed in the hydroponic system, reducing throughout the storage time, presenting on day 14, 1.64% of citric acid (Table 1). For the conventional system, there was not an equation that adjusted over time. While the cultivars differed among themselves until the tenth day, with the cv. Festival presenting the highest rates, which decreased linearly during the storage time; however, the cv. Oso Grande showed a lower value of 1.49% of citric acid for the 6 days (Table 1).

The results of this study were higher than those found by Cunha Júnior et al. (2012), in the municipality of Valinhos-SP, for the cv. Oso Grande, that varied from 0.79 to 0.85% of citric acid during the storage time. The elevated acidity of the fruits can be attributed to the high temperatures of the Ceará region, when compared with traditional cultivation. Since the strawberry plant is a crop that under high temperature, during the day and / or night, may cause the strawberry to become excessively acidic.

As far as the SS / TA, an interaction ($p<0.01$) was observed between the storage time and the systems, and between the time and the cultivars (Figure 4A and B), in which there was no equation that would fit in both cases. Farming systems showed differences in all the storage intervals, with the highest values represented by the conventional system. The cultivars showed variation over time, but only showed a difference on the third and sixth day, and at the end of the storage time the final values were very close to the original ones, represented by a difference of 0.48 and 0.46% for the cvs. Oso Grande and Festival, respectively (Table 1).

The results of this work were inferior to those observed by Borsatti et al. (2009), who found SS /TA values of 6.29 and 7.27 for the cvs. Oso Grande and Festival, respectively, being lower than recommended (8.75) by

Table 1. Soluble solids (°Brix), Titratable acidity (% citric acid) and SS/TA ratio of fruits of two strawberry cultivars in two cropping systems and stored under modified atmosphere at 2 ± 2 °C and 85 ± 10% U.R.

Soluble solids**					
Cultivars	**Days of storage**				
	0	**3**	**6**	**10**	**14**
Oso Grande	6.77[b]	6.35[b]	7.20[a]	6.67[a]	6.68[a]
Festival	7.93[a]	6.93[a]	7.20[a]	7.13[a]	6.77[a]
Systems					
Hydroponic	6.65[b]	6.00[b]	6.92[b]	5.97 [b]	6.08[b]
Conventional	8.05[a]	7.28[a]	7.48[a]	7.83 [a]	7.37[a]
CV(%)-a = 6.42		CV(%)-b=5.58		CV(%)-c=5.15	
Cultivars			**Titratable acidity.**		
Oso Grande	1.64b	1.56b	1.49b	1.55b	1.61[a]
Festival	1.84[a]	1.87[a]	1.74[a]	1.65[a]	1.56[a]
Systems					
Hydroponic	1.88[a]	1.74[a]	1.70[a]	1.66[a]	1.64[a]
Conventional	1.61[b]	1.68[a]	1.53[b]	1.55[b]	1.53[b]
CV(%)-a = 4.66		CV(%)-b=3.17		CV(%)-c=4.65	
Cultivars			**SS/TAratio****		
Oso Grande	4.15[a]	4.08[a]	4.87[a]	4.34[a]	4.17[a]
Festival	4.38[a]	3.71[b]	4.16[b]	4.33[a]	4.36[a]
Systems					
Hydroponic	3.54[b]	3.46[b]	4.10 [b]	3.61[b]	3.71[b]
Conventional	4.99[a]	4.34[a]	4.93 [a]	5.07[a]	4.82[a]
CV(%)-a = 4.81		CV(%)-b=5.51		CV(%)-c=6.24	

Means followed by the same letter in columns do not differ by the Tukey test at 1% (**) and 5% (*) probability.

Kader (1999). Although the soluble solid values were lower than recommended in some treatments, what affected the reduction of the SS / TA relation the most was the acidity of the fruit. However, even with low SS / TA relation, the local consumer market and from the state of Piauí, Brazil, routinely consume these products.

As for the total anthocyanins, there was interaction ($p<0.01$) between the storage time and the cultivation systems (Figure 4C), with variation over time. Differences were noted among the systems at harvest and on day 10, with the highest values in the conventional system, however not very expressive (Table 2). In both systems, there was no equation that would fit, with the averages represented in the chart. The cultivars and the systems differ in an isolated way, with 10.7 and 25.8 mg 100 g^{-1} of anthocyanins for the cvs. Oso Grande and Festival and 17.53 and 18.97 mg 100 g^{-1} of this pigment for the hydroponic and conventional systems, respectively.

The flavonoids showed linear reduction throughout the storage time, represented by 11.96% ($p<0.01$) (Figure 4D). Interaction was observed ($p<0.01$) between the systems and the cultivars, which was the best performance of the hydroponic and conventional systems with the cvs. Festival and Oso Grande, respectively (Table 2).

The contents of anthocyanins and flavonoids were greater in the conventional system, probably due to activation of the biosynthesis of these compounds, as a result of high solar radiation in this system when compared with the hydroponic system, which was conducted in a greenhouse. Corroborating Jaakola et al. (2004), that while studying the activation of flavonoid biosynthesis in *Vaccinium myrtillus* L., observed activation of expression of the flavonoid pathway genes, as a result of high solar radiation. The influence of solar radiation in the phenolic compounds has also been observed by Cortell and Kennedy (2006) who studied *Vitis vinifera*, and noted in a cloudy system, a decrease of some anthocyanins, such as delphinidin, cyanidin, petunidin and malvidin. In studies with strawberries by Josuttis et al. (2010), a decrease in the content of cyanidin 3-glucoside, quercetin 3-glucuronide and Kaempferol 3-glucoside in the fruit cultivated with the blocking of ultraviolet radiation in comparison with the strawberries

Table 2. Total anthocyanins (mg 100 g^{-1}) and Flavonoids (mg 100 g^{-1}) of fruits of two strawberry cultivars in two cropping systems and stored under modified atmosphere at 2 ± 2°C and 85 ± 10% U.R.

Total anthocyanins					
Systems	**Days of storage**				
	0	**3**	**6**	**10**	**14**
Hydroponic	16.98[b]	18.01[a]	17.63 [a]	17.20 [b]	17.79 [a]
Conventional	20.58[a]	17.61[a]	18.22 [a]	19.67 [a]	18.77 [a]
CV (%)-a = 8.42		CV(%)-b=8.70		CV(%)-c=5.62	
	Flavonoids				
	OsoGrande		Festival		
Hydroponic	6.79[bB]		7.30[aA]		
Conventional	9.44[aA]		7.09[aB]		
CV (%)-a = 8.10	CV (%)-b = 4.99		CV (%)-c = 9.85		

Means followed by the same lowercase letters in columns and uppercase letters in rows do not differ by the Tukey test at 1% probability.

cultivated in the open field.

Although the strawberry's exposure to sunlight adds benefits to the quality, such as accumulation of phenolic compounds, the intensity of the sunlight should not be too strong. This is because if sunlight too strong, it results in increased temperature of the fruit, which may cause a decrease in phenolic compounds, such as anthocyanins, as observed in 'Aki Queen' and red-wine by Yamane and Shibayama (2006) and Mori et al. (2007), respectively.

Conclusions

The fruit shelf life was extended by the use of cooling along the modified atmosphere, indicating the good potential of this combination to increase the post-harvest life of the strawberry, with fruit produced in conventional and hydroponic systems with the best physical-chemical quality and greater size, respectively.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Cucurbita pepo nitrogen fertigation in greenhouse environments

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Greenhouse crops afford higher quality and competitiveness. The objective of this work was to evaluate the effect of nitrogen doses (80, 110, 140 and 170 kg ha^{-1}) applied through fertigation in the yield of two cultivars of *Cucurbita pepo* (Anita F1 and Novita Plus), in a 4 × 2 factorial design. The experiment was carried out in an entirely randomized design with eight replications. The study evaluated the sum of the fresh biomass of fruits per plant, number of fruits per plant and mean fruit biomass. Analysis of the collected data made it possible to assume that increasing doses of N led to a linear yield increase for cultivar Anita F1 and a quadratic increase for cultivar Novita Plus.

Key words: *Cucurbita pepo*, nitrogen, drip fertigation, vegetables.

INTRODUCTION

Vegetable production is an intensely competitive activity. The market demands constancy and production volume, which vegetable farmers cannot meet due to lack of investment in technology for production systems. In this context, greenhouse crops emerge as an alternative for the seasonality of production and increased productivity, as crops are not exposed to environmental variability and disease control is more efficient. Moreover, greenhouse crops afford higher quality, such as with leafy vegetables, and earlier production (Purquerio et al., 2007).

Nitrogen is linked to photosynthesis, respiration, root development and activity, cellular growth and differentiation. It is among the nutrients that promote the most significant morphological changes in the plant, capable of altering the number and mass of fruits. On the other hand, excessive doses of nitrogen promote vegetative growth in detriment of reproductive growth (Marschner, 1995). Nitrogen fertilization of squash promotes plant growth from the seedling stage (Higuti et al., 2010), which may lead in the future to larger leaf area and greater supply of photoassimilates for fruits. To obtain high yields, it is essential to avoid excess or deficient nitrogen fertilization.

Fertigation is the technique of adding solubilized nutrients to irrigation water. The success of fertigation depends on a combination of factors, including the uniformity of water application, which reflect directly on the uniformity of nutrient distribution in the area. For this

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Table 1. Physical and chemical properties of the soil of the experimental sites before planting.

Parameter	Results
Physical properties (%)	
Coarse sand	5.0
Fine sand	8.3
Silt	12.0
Clay	74.7
Textural class	Clay
Chemical properties	
pH in Water	6.80
pH in $CaCl_2$	6.10
Organic matter (g dm^{-3})	26.08
Total Carbon (g dm^{-3})	15.13
Available P (mg dm^{-3})	4.27
Exchangeable K (cmol $dm^{-3)}$	0.27
Exchangeable Ca (cmol $dm^{-3)}$	5.07
Exchangeable Mg (cmol $dm^{-3)}$	1.45
Cation exchange capacity (cmol dm^{-3})	9.53

reason, fertigation combined with a drip irrigation system shows higher efficiency compared to other irrigation methods. In addition, fertigation is the better technique for nitrogen application in *Cucurbita pepo* compared to solid-form application followed by irrigation, as it increases the efficiency of nitrogen and water use (Mohammad, 2004). Improper technique leads to several problems, such as soil salinization and fertilizer waste, which can decrease productivity, increase the incidence of disease (Zatarim et al., 2005) and reduce the viability of the production system. This reinforces the need for more information on nitrogen fertilization in greenhouse environments, in order to support proper handling and obtain maximum yield per unit of nitrogen applied.

The adequate dose of nitrogen varies according to the technology of the production system, edaphoclimatic conditions and crop characteristics. Currently there is scarce information on *C. pepo* crops grown in greenhouse environments to evaluate growth and yield by using the fertigation technique. Several works can be found in the literature on nitrogen fertigation of other cucurbitaceous species, such as watermelon, melon and cucumber. However, extrapolating the results of other crops may not be adequate. The objective of this work was to evaluate the production of two cultivars of *C. pepo* (Anita F1 and Novita Plus) by applying different nitrogen doses (80, 110, 140 and 170 kg ha^{-1}) through fertigation in a greenhouse.

MATERIALS AND METHODS

The experiment was carried out at the Irrigation Technical Center (CTI) of the Agronomy Department at Maringá State University (UEM), located in Maringá, PR, at an elevation of 542 m and coordinates 23° 25' S and 51° 57' W. The greenhouse structure featured an arch-type cover, was 30 m long, 6.9 m wide and 3.5 m tall. The experiment was installed in a distroferric red Nitosol area with moderate A horizon, clayish texture, subperennial tropical forest phase (EMBRAPA, 2006).

The microirrigation system consisted of a 0.5 m^3 reservoir in which the fertilizers were solubilized, and a valve that allowed water to enter the SC-30SM motor-pump set, installed below the bottom of the reservoir. A total of 14 kPa of operating pressure was used, providing mean flow of 0.9 L h^{-1} per drip feeder. A slide valve and manometer adaptation were installed at the pump output, enabling control of pressure in the system. The main line consisted of PVC tubes, 0.032 m in diameter. The main line had a return to the reservoir, characterizing a closed system and making it possible to clean the main line after each fertigation process. Seven irrigation lines were installed with high-density polyethylene pipes, 0.016 m in diameter, and 19 IRRITEC drips with 0.24 m of micro tube attached at the end of each drip, with the objective of placing the drip point 0.03 m from soil level.

To analyze the system and irrigation uniformity, the amount of water emitted by all drips individually was collected, for a period of 0.46 h. Data collection was performed with the aid of plastic containers, labeled and with a defined tare. The water mass collected in each drip was quantified using a GEHAKA BG8000 digital scale, accurate to 0.1 g. Given specific water mass equal to 1 kg L^{-1}, the flow of each drip was calculated in L h^{-1}. The distribution uniformity coefficient (CUD) was calculated according to Bralts (1986) and was equal to 91.3%.

The treatments were the result of the combination of four nitrogen doses (80, 110, 140 and 170 kg ha^{-1}) and two cultivars of *C. pepo* (Anita F1 and Novita Plus), totaling eight treatments arranged in an entirely randomized design in a 4 × 2 factorial treatment arrangement. Eight replications were used per treatment, totaling 64 plots.

To prepare the experimental area, the 0 to 0.15 m layer of soil was tilled with a rotary hoe over the full area. Holes were prepared

Table 2. Mean effect of nitrogen doses on yield attibutes of the varieties.

Variety	FFB	NF	AFB
Anita F1	538.80	2.67	200.62
Novita Plus	707.17	3.53	202.84
CV (%)	3.72	11.75	1.56
F test (P <0.01)	0.0029	0.0035	ns

FFB, Fruit fresh biomass; NF, number of fruits; AFB, average fruit biomass.

manually, at a depth of 0.20 m. The soil was analyzed at the Maringá Rural Laboratory. According to the results of the soil analysis (Table 1), Trani and Raij (1996) recommend increasing base saturation to 80% and providing 400 kg ha^{-1} of P. Dolomite with 84% relative efficiency and monoammonium phosphate (MAP) were used to prepare the holes. In addition, 2.1 g of urea were added to each hole, equivalent to 19.5% of the dose in the lowest treatment, so that the soil solution showed an adequate concentration of nutrients for early crop development (CARRIJO et al., 2004).

Sowing took place on March 17, 2012 in 72-cell Styrofoam trays previously filled with substrate. Transplantation took place 19 days after sowing (DAS), at a spacing of 0.80 m between rows and 0.75 m between plants. Acephate was sprayed at 14 and 19 DAS, Metamidophos at 32 DAS, Thiophanate-methyl and Chlorothalonil at 35 DAS, and Mancozeb at 35 and 47 DAS. Female flowers were manually pollinized every morning.

Potassium fertilization was equally distributed in six fertigations during the cycle, considering a crop extraction of 247.52 kg ha^{-1} of K and expected yield of 13600 kg ha^{-1} (Furlani et al., 1978; Carrijo et al., 2004). Nitrogen doses were equally distributed in five weekly fertigations starting 27 days after sowing (DAS) and on different days than potassium fertilizations. According to Vidigal et al (2007) , nitrogen and potassium have to be apllied in several doses throughout the cycle.

Urea dilutions for the treatments took place in the reservoir filled to the 150 L inner mark of water. 50 L of solution was used to stabilize the system, in which 26 L was used to fill the inner volume of the pipes and 24 L were used as a safety margin. During stabilization, the entire pool applied was collected in containers located under the drips. After stabilization, the motor-pump set was turned off, the containers below the drips corresponding to treatment were removed, and the motor-pump set was turned on again, applying 50 L. The remaining volume of solution in the reservoir was discarded. The applications of nitrogen doses in solid form were carried out near the drip point.

Soil moisture control was carried out with the aid of three tensiometers with Bourdon vacuum gauge, installed 0.20 m deep throughout the experimental area. The objective was to maintain soil water tension between 10 and 30 kPa, applying the same pool in all plots throughout the experiment.

Harvest began 52 DAS, lasting until 84 DAS. Fruits longer than 0.15 m were collected every morning. Immediately after harvest, the fruits were taken to the laboratory for analysis. The measurements of fruit fresh biomass were obtained using a GEHAKA BG8000 digital scale, accurate to 0.1 g.

Data on the sum of fruit fresh biomass (SFFB) per plant, number of fruits per plant (NF) and mean fruit biomass (MFB) were converted through a base 10 logarithm, so that residues would stand near normal distribution and feature homoscedasticity. They were later subjected to analysis of variance. The quantitative variables were subjected to regression analysis and tested by the test t (P <0.01).

RESULTS AND DISCUSSION

Table 2 shows the mean values for SFFB, NF and MFB of the cultivars in the average doses. Although cultivar Novita Plus showed higher yield per plant, mean fruit size did not show significant differences. Higher yields depending on N dose can be explained by the greater supply of photoassimilates to the fruits due to increased leaf area and liquid photosynthesis. N is absorbed by the mass flow and transported to the leaves, where approximately 70% of this nutrient is found in the chloroplasts, taking part in the synthesis and structure of chlorophyll molecules (Marenco and Lopes, 2005). Strassburguer et al. (2011) affirm that *C. pepo* shows higher harvesting rate if grown during spring-summer, when the availability of solar radiation is greater than during summer-fall, which suggests the importance of leaf area in capturing solar energy, producing photo-assimilates and supplies for fruits, resulting in higher yields.

Figure 1 shows the graphs of production as a function of tested doses. For cultivar Anita F1, the data fit the linear regression, which suggests that the dose corresponding to maximum yield is greater than 170 kg N ha^{-1}. For cultivar Novita Plus, variables SFFB and NF, data fit more adequately a polynomial regression, with maximum yield per plant of 942.4 g and 4.6 fruits corresponding to doses 142.1 and 133.3 kg N ha^{-1} (8.52 and 8.00 g of N per plant, respectively). For MFB, the increase was linear in response to application of the doses. The increase in the variables can be explained by the favoring towards formation of the vegetative canopy and extraction of N to form fruits (Carrijo et al., 2004). This result agrees with Zotarelli et al. (2008), who observed that the productivity of *C. pepo* cultivar Wildcat at the dose of 145 kg ha^{-1} of N was statistically superior to the 82 kg ha^{-1} dose. However, it did not show statistical difference to the 217 kg ha^{-1} dose.

The magnitude of the productivity increase in response to doses of N depends on the division of photoassimilates among plant parts. Although the vegetative part is usually responsive to nitrogen most of the time, the reproductive part may not show biomass increase at the same rate (Huett and Belinda, 1991). In melon plants, fruits are a

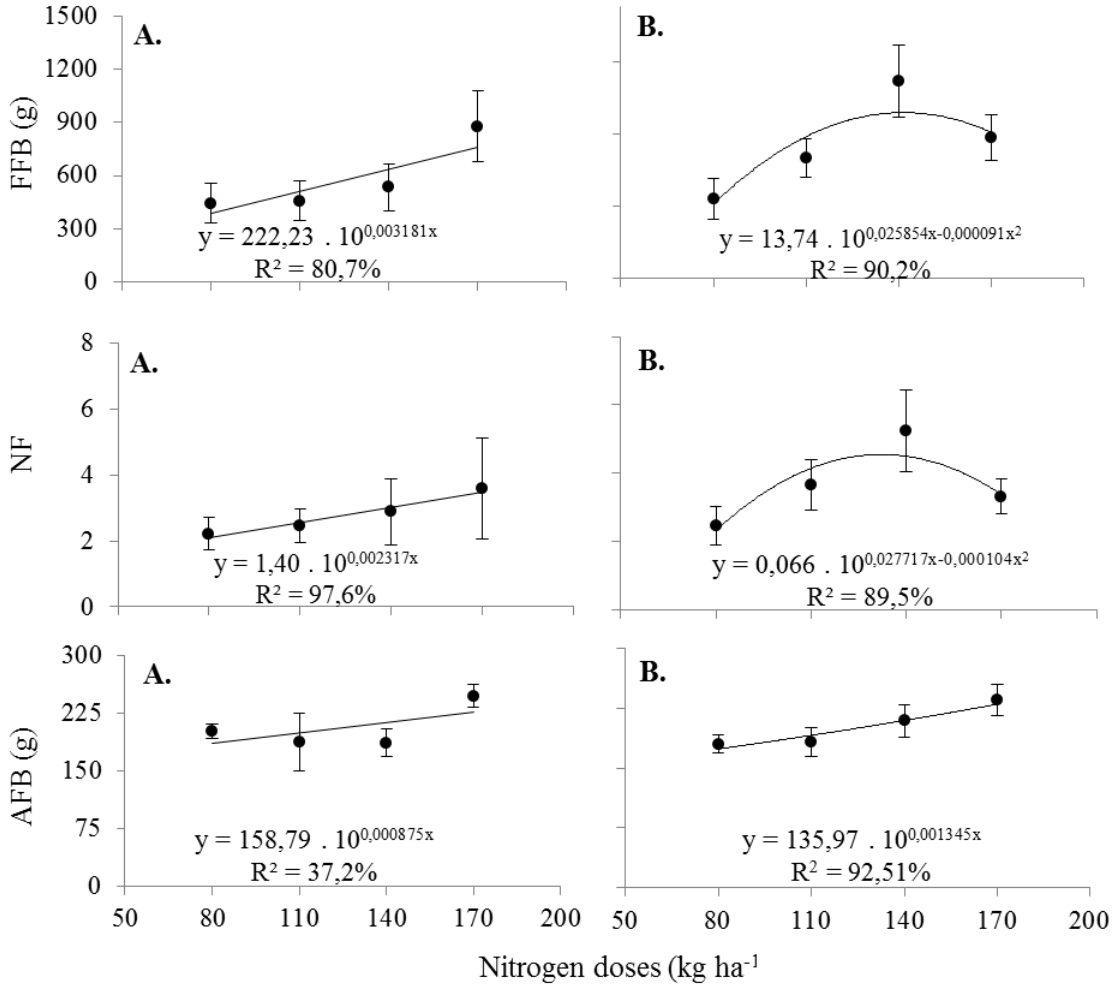

Figure 1. Anita F1 (A) and Novita Plus (B) yield in response to application of nitrogen through fertigation. Vertical bars refer to the standard deviation in the treatment. FFB, Fresh fruit biomass; NF, number of fruits; AFB, average fruit biomass.

powerful sink of photoassimilates to the plant (Duarte et al., 2008), which may represent a reduction in the rate of increase for vegetative biomass during the reproductive period. However, Strassburguer et al. (2011) did not detect this characteristic for *C. pepo*, which suggests a positive interaction between N doses, rate of leaf area increase and yield. At suboptimal levels of nitrogen availability, the fruit does not seem to be a strong sink (Huett and Belinda, 1991). The same authors affirm that at low level of N availability, a decrease is observed in the reproductive period and delayed fruit set, which results in smaller and less plentiful fruits.

Conclusions

Cultivar Novita Plus shows higher average yield compared to cultivar Anita F1. Cultivar Anita F1 shows a linear increase in productivity, number of fruits and da mean biomass per fruit when N doses were raised up to 170 kg ha^{-1}. In cultivar Novita Plus, the increase in productivity and number of fruits is quadratic, and doses of 142.1 and 133.3 kg N ha^{-1} applied via fertigation correspond to the maximum respective values. Higher N doses provide a linear increase in mean fruit biomass in the tested dose interval.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Quantifying biomass and carbon stocks in oil palm (*Elaeis guineensis* Jacq.) in Northeastern Brazil

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Oil palm (*Elaeis guineensis* Jacq.) is a major raw material for biofuel and food industries in the world. In Brazil, cultivation of this species has evolved much in recent years, but basic information on its growth in the country is still scarce. This study analyzes the biomass and carbon storage in plants selected in three stands located in southern state of Bahia, northeastern Brazil, considering the full rotation cycle. Plants aging 3 to 36 years were cut and measured at biometric variables: Diameter at 50 cm from the ground level, crown diameter, stipe length and total height. Relationships among the biometric variables and the stipe, foliage, root, and total biomasses were analyzed. The Chapman & Richards model was fitted to the total biomass and carbon as a function of age. All the linear correlations between variables were significant at 95% probability. Total height and stipe length were more strongly correlated with age than with the diameter at 50 cm and crown diameter. The total biomass was highly correlated with the stipe variables and age. The percentage participation of stipe and total biomasses increases with age unlike the biomass foliage. The proportion of roots does not change with age. The total dry biomass and carbon stocks at the age of 25 years were estimated at ca. 90 and 35 t.ha^{-1}, respectively. It was concluded that oil palm, because of its rapid growth and due to the fact that it is a permanent culture, is able to stock a high amount of biomass and carbon per unit area. If implemented in appropriate places, oil palm cannot be considered a carbon debt crop and represent an important alternative to regional socioeconomics.

Key words: Growth, oil palm, biomass expansion factor, biomass partitioning, biometric relationships.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is originating from the African continent, whose natural range includes all the west coast of Africa, from Senegal (parallel 16°N) to Angola (Hartley, 1977; Wahid, 2005). It can also be found in the interior of the continent in direction to Congo, and in East Africa, including the Madagascar (Moretzsohn et al., 2002). The adaptability of thisplant has contributed to the spread of its cultivation to other parts of the world, incorporating to the local flora, both by the formation of oil palm spontaneously regenerated stands or through conventional commercial plantations.

In Brazil, the oil palm was probably introduced with slaves in the 16th century, on the occasion of the people trade from Africa (Savin, 1965). At the time, the Africans,

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primarily from Angola, Benin and Mozambique, have transported seeds inside the vessels, which gave rise possibly to the first oil palm spontaneous stands on the coast of Bahia state (Chavez, 1984).

The fruits of oil palm produce two types of oil: palm-oil or dende oil, extracted from the mesocarp (outer part of the fruit); and the nut-oil (palm kernel oil), extracted from the seed. It is possible to obtain up to 22% of oil of the bunch weight from the pulp and up to 3.5% of oil from the nuts (Cardoso, 2010). With extensive use throughout the world, the palm-oil constitutes nowadays a major raw material for food, medicinal, chemical and industrial uses.

Palm crop may reach a yield per unit area of 4 to 5 tons of oil per hectare per year (Moura, 2008). Indonesia and Malaysiaarethelargestproducers ofpalm-oil, accounting for more than 85% of world production. Brazil occupies the 15th position among the producers, but it has gradually increased its production and has the largest suitable area to this crop in the world (Butler and Laurance, 2009). However, the country still imports more than half of the palm-oil necessary to its factories (Becker, 2010). Para state is the largest Brazilian producer, with about 100 thousand tons per year and 50 thousand hectares planted, accounting for 93% of Brazilian yield (Brazil, 2006; Harada et al., 2008; Furlan et al., 2006; Lange, 2012). Other Brazilian states producers are Amazonas, Amapa and Bahia, which also have relevant crop areas (Lange, 2012).

In the biological and environmental context, the most important aspects for the crop yield are those related to the plant, soil and climate (Brazilio et al., 2012). Nutritional requirements of this plant vary widely, depending on the expected yield, type of genetic material used, spacing, plant age, type of soil and environmental factors (Santos, 2010). The density of planting practiced is of 143 plants per hectare, arranged in an equilateral triangle of 9 m, that is, a spacing of 7.8 m between rows and 9 m between plants in the row (Berthaud et al., 2000). This spacing is also adopted in Southeast Asia.

Today there is a concern with a possible increase of deforestation rates in the Amazon region due to the expansion of the biofuel plantations, based on the critical examples in the Asian continent (Butler, 2011). The cultivation of this species in appropriate areas, on the other hand, can represent social, environmental and economic benefits. Formerly deforested areas for purposes agriculture and cattle ranching should be priority to oil palm plantations, avoiding the practice of shifting cultivation and extensive livestock farming. This can provide more jobs and income for the local population, aggregation of technological and economic benefits, as well as increase in carbon stocks in crop biomass, mitigating partially the emissions generated by deforestation

Most of the studies on biomass and carbon in oil palm plantations have been conducted in Southeast Asia. Works in Africa have been also reported elsewhere (Aholoukpe et al., 2013; Thenkabail et al., 2010). However, in Brazil this issue has not been yet addressed. Little information exists in the literature on biomass partitioning by compartments and carbon stocks from the oil palm stands (Syahrinudin, 2005), even in the most developed countries. This study aims to analyze the biometric relationships of oil palm coming from three industrial plantations located in southern Bahia state, northeastern Brazil. The study evaluates the entire range of cultivation ages, analyzes the biomass partitioning by compartments and provides a growth model for biomass and carbon stock by unit area for this crop. It can be helpful to oil palm management in Brazil and contribute to the formulation of plans to mitigate climate change from land use and land use change.

MATERIALS AND METHODS

Study area

The study was conducted in the municipality of Taperoa, southern of Bahia state, northeastern Brazil. The reference UTM coordinates are the following: X - 486.212 m and Y - 8,504.380 m.The regional climate is tropical, hot and humid, with average temperature of 24°C, classified as Af according to Köppen and Geiger (1928). Rainfall is abundant and happens more frequently between April to August and in the first three months of spring (September to November). Precipitation can reach, on average, up to 100 mm per month. The study site is situated in a zone with the greatest precipitation in the state, with around 1,500 to 2,000 mm per year (Santos, 2010).

Study material and methodological procedures

Commercial plantations with wide range of planting age were selected in the region, varying from 3 to 36 years. Forty two individuals were selected to be harvested in the three selected stands. A minimum distance of 100 m was kept between the palms sampled, being all located far from the edges of the planting area. All sampled palms showed normal phenotype and vitality, without damage or other defects caused by physical or pathological agents.

In the field, the following biometric variables were measured, which can also be visualized in Figure 1:

1. Diameter of the palm at 1.30 m above ground level (*dbh*), measured with a tape in *cm*;
2. Diameter of the palm at 50 cm above ground level (d_{50}), measured with a tape in *cm*;
3. Crown diameter (*dc*) in *cm*, using a tape in two cross-measures taken at 90° angles;
4. Total height (*ht*), measured in *m* with a tape, after felling the palm;
5. Stipe length (*he*), measured in *m* with a tape, after felling the palm.

The 41 palmswhich were cut had their biomasses separated in field by stipe, foliage, roots, bunches and fruits, but bunches and fruits data were not analyzed in this paper. The fresh biomass compartments were weighed separately in the field using a digital dynamometer with 10g precision. Samples of approximately 200 g were collected from each biomass compartment in the field and transported to laboratory, where they were weighed and oven dried at 70°C to constant weight. The fresh weights of palms were

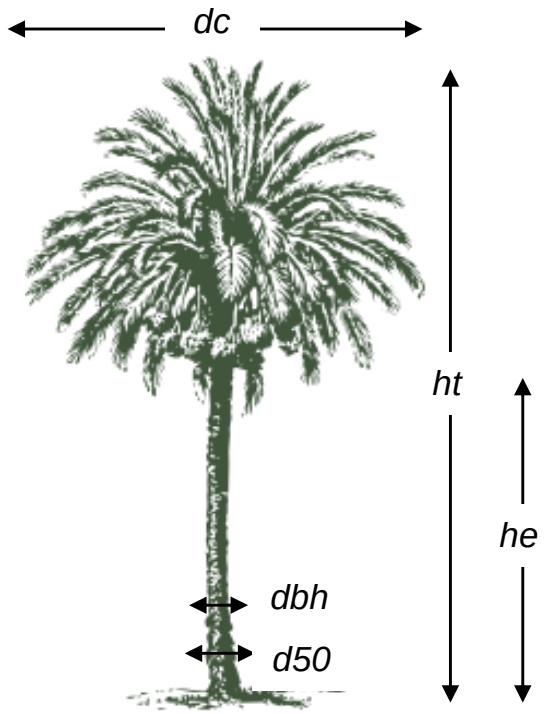

Figure 1. Biometric variables measured in *E. guineensis* in northeastern Brazil.

converted into dry biomass by direct relation between both variables from the samples.

The samples were then manually crushed and processed in a Wiley type mill until reach powder particle size. These were subsequently analyzed in equipment (model LECO C-144) that determines the carbon content of the sample by dry combustion process in an infrared chamber.

The allometric relationships between the biometric variables (Figure 1) with age of the palms and dry biomass of each component were analyzed by the Pearson correlations. The proportions of biomass of foliage and roots in relation to the total biomass of the palm in different ages were also analyzed. Biomass expansion factor of and root-to-shoot ratio were calculated, taking into account the different ages, by the following equations:

$$BEF = \frac{ba}{bt} \quad (1)$$

where: BEF = biomass expansion factor.

$ba = be + bf$ = aboveground biomass (2)

be = stipe biomass; bf = foliage biomass and bt = total biomass.

A growth model for the biomass and carbon stock per unit area (hectare) was adjusted, considering the individual stocks in each age and the planting spacing of 143 palms.ha^{-1}, practiced in the management of the crop. The biological model of Chapman and Richards was used for this purpose, which was fitted by the nonlinear regression method of Levenberg-Marquardt:

$$W = A \times (1 + b \times \exp(-k \times Age))^{(1-1/m)} \quad (3)$$

where: W = total dry biomass (t ha^{-1}); Age = age (years), and A,b,k,m = coefficients of the model.

$$C = \sum_{i=1}^{n} C_i \quad (4)$$

$$C_i = W_i \times TC_i \quad (5)$$

where: TC_i = carbon content of each biomass compartment; W_i = dry biomass of each compartment (t.ha^{-1}), and A,b,k,m = coefficients of the model.

RESULTS AND DISCUSSION

Correlation between the biometric variables

All the linear correlations between the variables were significant at 95% probability. Total height and stipe length were more strongly correlated with age than with the stipe diameter at 50 cm and diameter of the crown. Foliage biomass was more strongly correlated with the palm crown diameter. Stipe biomass was highly correlated with the total height, stipe length and age. The root biomass was more strongly correlated with total height and stipe length, age and stipe biomass. Total biomass was highly correlated with the stipe measures once this compartment composes the largest fraction of the total biomass of the palm. Age also exerts significant influence on total biomass (Table 1), which favors to adjust a growth model for biomass as function of this variable.

Asari et al. (2013) have analyzed the correlations of diameter at breast height (*dbh*), total height, stipe length, age and aboveground biomass in plantations of oil palm in the state of Selangor on the west coast of the peninsular Malaysia. They realized that palm height is more strongly associated with age, particularly the stipe length. They found negative correlations of age with the *dbh*. The biomass was strongly correlated with age and very strongly with stipe length. In this paper *dbh* was not analyzed because this variable could not be measured at younger palms that are lower than 1.3 m. Therefore, the analyses were carried out for d_{50} instead.

The results of Asari et al. (2013), in their majority, corroborate those of the present study, but the reduction of diameter with age was not detected in this work. Jacquemard (1979) also noticed reduction of *dbh* with the advance of age in the studied species. In turn, Hartley (1977) reported a steady increase in the bole diameter during all the first years. However, they noted that the stipe practically ceases its growth in diameter subsequently with the advance of age. This may be related to the absorption of nutrients (Turner and Gilbanks, 1974).

Table 1. Correlations between biometric variables of *E. guineensis* in northeastern Brazil.

	age	***d50***	***dc***	***ht***	***he***	b_f	b_e	b_r	b_t
age	1.00								
d_{50}	0.62	1.00							
d_c	0.78	0.71	1.00						
h_t	0.94	0.72	0.83	1.00					
h_e	0.95	0.65	0.74	0.97	1.00				
b_f	0.63	0.78	0.81	0.76	0.70	1.00			
b_e	0.90	0.73	0.75	0.93	0.93	0.74	1.00		
b_r	0.83	0.74	0.74	0.86	0.85	0.70	0.87	1.00	
b_t	0.88	0.79	0.81	0.94	0.93	0.85	0.98	0.90	1.00

Where: d_{50} = diameter of the palm at 50 cm above ground level; d_c = crown diameter; h_t= total height; h_e = stipe length; b_e = stipe biomass; b_f = foliage biomass; b_r = root biomass; and b_t = total biomass.

Table 2. Carbon content statistics by biomass compartments of *E. guineensis* in northern Brazil.

Compartment	$\bar{X}$	s^2	***CV%***	***n***
Foliage	42.86	0.9942	2.32	41
Stipe	39.73	0.9335	2.35	41
Roots	38.20	2.9985	7.85	41
General (weighted)	40.85	0.9330	2.28	123

Where: $\bar{X}$ = mean, s^2 = variance, *CV%* = coefficient of variation and n = number of cases.

Carbon contents

The carbon contents determined in this study varied among compartments, being highest in foliage. The average carbon content weighted by biomass of each compartment was calculated at 40.85% (Table 2), consistent with the value of 41.3% reported for plantations in Indonesia (Syahrinudin, 2005). That author detected variations in carbon content among biomass compartments, ranging from 32.3% for fine roots to 44.2% for leaves. Such variations were also noticed in thisstudy,althoughthedifferencesareless remarkable in this work in comparison to the formerly mentioned study.

Castilla (2004), analyzing various methods for quantification of carbon in plantations of *E. guineensis* in Colombia at different ages, mentions that the biomass conversion to carbon is made with carbon contents ranging from 45 to 50%. The percentage of 50%, usually employed in many similar studies (Hamburg, 2000), can be considered unreasonably high for *E. guineensis*. That value has already been subject of analysis and criticism in the literature, which motivated the IPCC (2006) to change its default to 47%. For the species studied here even the default value may represent overestimation in the conversion of biomass to carbon stock.

Partitioning of biomass and carbon

The percentage participation of stipe biomass in relation to total biomass increases asymptotically with age, unlike the foliage biomass, which results in a very clear inverse linear correlation between them. Conversely, the roots proportion does not change with age, showing no trend along time. Biomass expansion factor decreases sharply and asymptotically with age, whereas root-to-shoot-ratio does not show clear trend with age (Figure 2).

There is limited research on the partitioning of carbon in biomass for oil palm (Syahrinudin, 2005). Analyzing plantations in Indonesia, with ages ranging from 3 to 30 years, the author concluded that stipe participation in terms of aboveground biomass may range from 56.7 to 75.3%. His findings corroborate the results of this study regarding the evolution of the participation of root biomass with age.

Asari et al. (2013) have reported prevalence of stipe biomass in comparison to leaf biomass in 60 oil palm plantations with ages ranging from 6 to 23 years in Malaysia. Khalid et al. (1999a) found lowest rates of participation of the stipe biomass in mature plantations in Malaysia, evidencing that the root fraction corresponds to approximately 16% of total dry mass (Khalid et al., 1999b). Castilla (2004) examined several methods forquantification of carbon in plantations of *E. guineensis* of various ages in Colombia and also found an increase in the participation of the carbon stock of the stipe at more advanced ages.

Biomass and carbon stock growth model

The Chapman and Richards model fitted well to the data

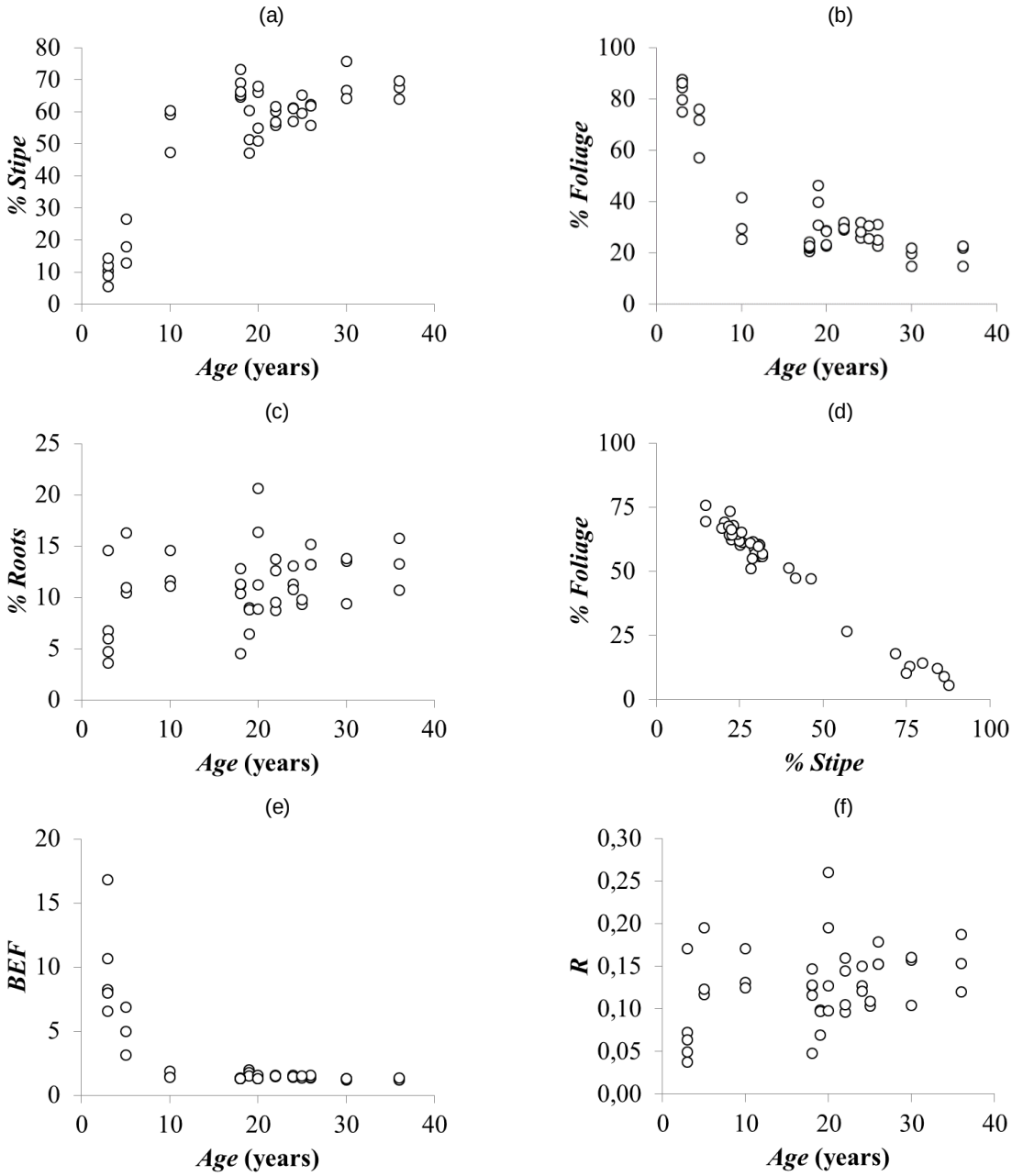

Figure 2. Partitioning of total dry biomass, biomass expansion factor and root-to-shoot ratio as a function of age for *E. guineensis* in northeastern Brazil (a: Stipe participation; b: Foliage participation; c: Root participation; d: Relationship between the stipe and foliage biomass participation; e: Biomass expansion factor; f: Root-to-shoot ratio).

of total biomass of *E. guineensis* as a function of age (Equation 6), as show below:

$$W=142.331(1-(-0.00182\exp(-(0.077365\times Age))^{(1-1/0.000572)} \quad (6)$$

(n = 41; R^2_{adj} = 0.9064)

The total dry biomass growth curve fitted to actual data (Figure 3) indicates a value of ca. 90 t ha^{-1} at age 25 years, which is considered ideal for the rotation of the species due to the height of the palm and of its palm-oil production. At 40 years, age in which oil productivity is already uneconomical (Villela, 2009), biomass stockreaches approximately a value of 120 t.ha^{-1}.

These values are consistent with those found by Asari et al. (2013), in the state of Selangor on the west coast of peninsular Malaysia, though the authors calculated only the aboveground biomass (excluding the roots). Distinct

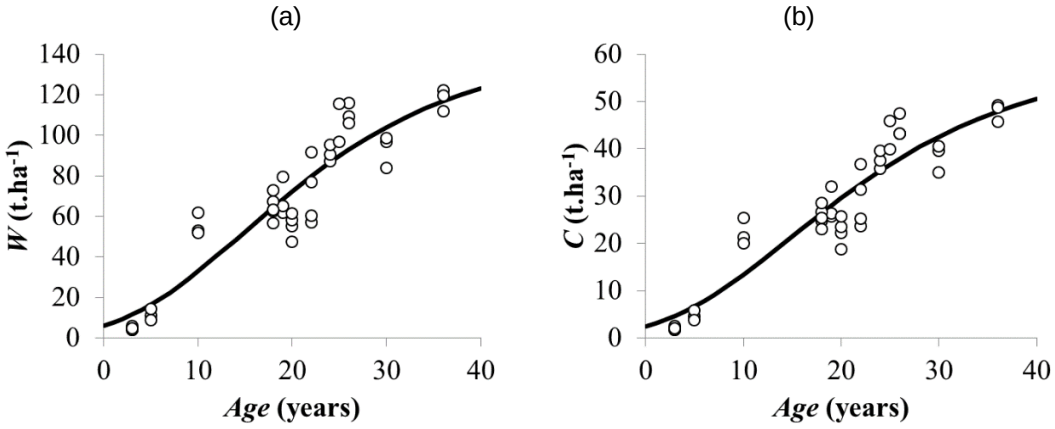

Figure 3. Growth curves of biomass and carbon stocks for *E. guineensis* in northeastern Brazil (a: Total biomass; b: Total carbon). Circles and lines indicate actual and fitted values by the Chapman and Richards model, respectively.

resultsareattributabletodifferencesinclimatic conditions and to variations in the density of plantations in Malaysia, rangingbetween136to148palms per hectare, according to the authors.

Published information on aboveground biomass in oil palm plantations range from 50 to 100 t.ha^{-1} at the end of rotation age, which varies from 20 to 25 years (Klaarenbeeksingel, 2009). Root biomass in Indonesia varied from 40.1 to 52.4 t.ha^{-1} for 20 and 30-year stands, respectively (Syahrinudin, 2005). Therefore, the stocks of biomass found in this study are within the range of values published in Southeast Asia despite the fact that there are differences in climatic conditions and genetic material. This information is also in agreement with the analysis made by Castilla (2004) in Colombia.

Quantifications of carbon in Borneo (Malaysia) made by remote sensing, on the other hand, showed a decrease of aboveground biomass after 20 years due to abscission of foliage (Morel et al., 2011). This was not observed in the present study.

Aboveground carbon stocks for oil palm plantations reported in the literature vary considerably, from 31 to 62 t.ha^{-1} for young cultivations of 10 years and from 96 to 101 t.ha^{-1} in stands of age from 14 and 19 years (Sitompul and Hairiah, 2000). Aboveground carbon stocks reach 9.2 t.ha^{-1} in plantations of 3 years in Sumatra, Indonesia, and 35.4; 41.7 and 55.3 t.ha^{-1} for age classes 10; 20 and 30 years, respectively (Syahrinudin, 2005). The author obtained carbon stocks of 5.4, 10.4, 16.6 and 21.8 t.ha^{-1} for the belowground biomass (roots and stipe base) in 3, 10, 20 and 30 years stands, respectively.

Comparison of carbon stocks in palm-oil cultivation and in other land uses

There are criticisms about the oil palm cultivation in Southeast Asia due to the expansion of this crop for the production of oil, which is preceded by deforestation of tropical forest (Butler, 2011; Fitzherbert et al., 2008). In Malaysia the changes in land use between 1990 and 2007, for example, totaled 1.252 million hectares and 76.3% of this change were resulted from the establishment of oil palm plantations (Malaysia 2007; MPOB, 2008). Wakker et al. (2004) analyzed the social and economic impacts of oil palm crop in Southeast Asia, with emphasis on Indonesia. Their final recom-mendation was a moratorium on any new permitsfor oil palm plantation expansion until the legal framework was modified.

Fargione et al. (2008) evaluated the emissions arising from the establishment of bio-energy crops in Southeast Asia, in the United States and in Brazil. According to the authors the great concern in Brazil is with the expansion of sugar cane biofuel crops, particularly in the Cerrado biome (Savanna). However, criticism of the expansion of oil palm plantations in Brazil, particularly in the Amazon, are constant and of varied background (Becker, 2010).

Despite this criticism, the expansion of oil palm crop in the Amazon has agreat potential to revegetate previously deforested and degraded lands with low economic efficiency.Itcanalso becoupled with increase of biomass and carbon stocks. Obviously, that such expansion shall be made carefully. The appropriate compromise with the social, environmental and cultural territorial development is a condition that will determine the success or failure of this activity (Silva, 2013).

In Brazil oil palm crops have been mostly established in formerly deforested areas occupied by low-production pastures. The regional spatial planning carried out by the Brazilian government encourages the expansion of Oil palmplantationswiththeperspectiveofthe recomposition of disturbed areas and income generation for the local population (Brazil, 2010a).

From the perspective of carbon storage, a permanent

Table 3. Land use and carbon stocks in the region of *E. guineensis* crops in northeastern Brazil.

Land use	tC.ha^{-1}	Main purpose	Biodiversity
Forest*	122.92	Protection	High
Palm (this study)	40.00	Production	Low
Pasture**	8.00	Production	Low
Agriculture**	5.00	Production	Low

Sources: *Brazil, 2010b; **IPCC, 2003.

crop as oil palm shows greater potential compared to temporary agricultural crops and livestock (Table 3). As the cultivation remains producing during 25 years on average, and the palms are large-sized, its carbon storage per hectare is comparably higher, as stated by Castilla (2004).

The expansion of oil palm plantations may represent a carbon debit if the practice is to replace the tropical forest by this crop (Fargione et al., 2008). In the study site natural vegetation type is the Tropical Atlantic Rain Forest (Dense Mixed Forest) whose carbon stocks may reach over 120 tC.ha^{-1} (Brazil 2010b). Loss of this carbon stock may imply in a carbon dioxide emission of about ca. 450 t.ha^{-1}. If one hectare of forest is replaced by oil palm the associated debit related to the emissions of greenhouse gases would be of ca. 304 tCO_2.ha^{-1}, taking into account the carbon stock at age 25 years (Table 3). These figures do not accounted for the other emissions due to management of oil palm crop, such as fertilizers and fossil fuel use and the use of fire during land clearing. However, the replacement of tropical forest by seasonal agriculture or pasture may result in an even higher carbon dioxide emission, of 421.37 and 432.37 tCO_2eq.ha^{-1}, respectively, derived by this change in land use.

The criticism of the expansion of the oil palm crop in Brazil based upon the carbon debit discussion does not make much sense. The key issue to be addressed is the driver of deforestation regardless if it is oil palm, cash crops or livestock ranching. The growth of human population and the higher living standards of the contemporary society is a major indirect force pushing up the deforestation rates of tropical forest. Migration, inexpensive land prices, low-efficiency agriculture and impunity against lawlessness are the direct and decisive factors. The challenge is to increase productivity, that is, produce more in less space to avoid of the advance of deforestation. Oil palm is efficient in terms of oil production per unit area because its yield per unit area is ten times greater than that of soybean, for instance. Occupying only 5% of the cultivated land for oil in the country it produces 38% of the national production. Such conditions make the cultivation of oil palm a profitable and relatively inexpensive business (Becker, 2010).

The central issue of the environmental discussions associated with the culture of oil palm in Brazil has not been addressed adequately. The focal point should be to prevent the expansion of this crop in areas of tropical forests, whose main aim is the protection of the environment and the conservation of biodiversity. It makes no sense to compare the biodiversity of a natural forest with a crop. From the perspective of carbon storage oil palm shows advantages in relation to seasonal crops, as shown here. If this crop represents any carbon debit, most other agricultural crops are comparatively much worse. If the planting of oil palm is done in areas previously occupied by pastures and/or agriculture it may conversely represent a carbon credit.

Animportant aspect along this discussion concerns the fact that oil palm has traditionally been considered a monoculture. However, efforts have been made in recent years on integrated pest management programs and intercropping with other plants (Castilla, 2004), which also promotes social inclusion and a more favorable carbonbalance.Inaddition,highconservationvalue (HCV) areaswithinregionsidentifiedforoilpalmcultivation could be left for conservation to rebut critics on monoculture andenhancecarbonconservationinanoilpalm cultivation belt.Further conservative measure could be keeping preserved forest areas located in riparian zonesandinmountainslopes,withinregionsforoilpalm cultivation.

The cultivation of oil palm in Brazil may be a major alternative to regional development by its potential role in the recovery of deforested lands. It can also provide income and employment generation to local people, diversification of production, as well as produce renewable energy and diminish dependence on imported biofuel (Becker, 2010). As demonstrated here oil palm plantations can also contribute positively to mitigate climate change through carbon sequestration.

The great challenge is to confine the cultivation of this species in already deforested areas, avoid the risks of monoculture by means of agroforestry systems and share their socioeconomic benefits to assure the sustainability of its production chain.

Conclusions

1. The biometric variables of oil palm are significantly

correlated. Biomass is more strongly correlated to stipe measures;
2. In mature oil palm stands the stipe represents the largest fraction of biomass and carbon stocks;
3. Due to the strong relationship to total biomass and carbon, age can be used as a predictor variable for growth modeling;
4. Stipe biomass proportion increases with age, whereas foliagebiomassdecreases,androotsremain
practically unchanged;
5. A mature oil palm stores more carbon than agricultural crops and pastures, but less than a tropical forest;
6. Expansion of oil palm plantations in the country must observe the country's regulation, which is directed to formerly deforested lands, particularly degraded crop and pastures, preventing increase of deforestation of the tropical forest.

Conflict of Interests

The authors have not declared any conflict of interests.

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Short-term effect of different green manure on soil chemical and biological properties

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The use of different legume species, as green manure, may affect differently soil biological and chemical properties. The aim of this study was to evaluate the effect of four legumes species used as green manure on soil biological and chemical properties in short-term. We evaluated the following legume species: *Crotalaria, Cajanus, Mucuna* and *Canavalia*. The study was arranged in a completely randomised design with four replicates. The plants were incorporated into the soil (0-20 cm) by harrow and the chemical and biological properties were evaluated 30 and 60 days from the incorporation. Soil chemical and biological properties showed different trends according to legume species used. Soil P and K contents were highest in plot with *Crotalaria*, while soil Ca content was highest in plot with *Mucuna*. Soil microbial biomass was higher in plot with *Mucuna* as compared with others green manure species. Fluorescein diacetate hydrolysis was higher in plots with *Mucuna* and *Canavalia* than the others plots. Our results supported the hypothesis that different types of legume used as green manure affect differently the biological and chemical properties of soil. In this case, *Mucuna* was more effective to improve soil biological properties, while *Crotalaria* seems to be more efficient in the improvement of chemical properties.

Key words: Organic fertilization, alternative agriculture, legumes.

INTRODUCTION

The use of legumes species as green manure is an important practice for sustainable agriculture as the legumes may fix N and have deep and extensive root system, allowing greater extraction and recycling of plant nutrients. Some studies have shown the great potential of green manure for supplying nutrients to crops (Perin et al., 2004; Adediran et al., 2004) and the improvement of soil properties (Adediran et al., 2004; Ziblim et al., 2013).

However, several legume species may be used as green manure and each one presents different properties which may affect differently the soil properties. Also, the influence of organic residues on soil properties depends upon the amount, type and size of the added organic materials (Zhang et al., 2015). Among the legume species used for green manure in tropical regions, the *Canavalia* is one of the most favorable for agricultural use

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Table 1. Chemical properties of legume species.

Parameter	*Crotalaria*	*Guandu*	*Mucuna*	*Canavalia*
Dry mass (t ha^{-1})	9.0	8.5	13.4	8.1
C/N ratio	22	19	29	24
N (g kg^{-1})	20.1	16.3	21.2	18.7
P (g kg^{-1})	5.4	3.0	1.9	3.5
K (g kg^{-1})	25.7	13.4	13.0	12.4
Ca (g kg^{-1})	4.2	3.0	12.6	3.9
Mg (g kg^{-1})	2.0	1.3	2.8	3.0

by their morphological and physiological characteristics (Heinrichs et al., 2002), while *Crotalaria* is very efficient as producer of plant dry mass (Ziblim et al., 2013). *Mucuna* is other legume specie which has a potential for soil restoration, improving the chemical and physical properties of the soil (Alvarenga et al., 1995). Finally, *Cajanus* presents deep root system with high potential in water absorption and recycling of nutrients from deep layers (Alvarenga et al., 1995).

Although it is widely known that green manure and others crop residues improve the soil chemical and biological properties (Biederbeck et al., 2005; Liu et al., 2006; Shah et al., 2010; Ye et al., 2014; Adediran et al., 2004; Ziblim et al., 2013), there is the need to compare the different legume species in the improvement of soil biological and chemical properties in the short-term as usually these soil properties may respond quickly to soil management according to chemical characteristics of organic residue.

As an important biological property, soil microbial biomass is used as an ecological attribute to evaluate changes in soil properties by soil use and management (Lopes et al., 2010; Santos et al., 2012). In addition, soil microbial biomass releases enzymes which play important functions in soil processes, such as the decomposition of organic matter (Silva et al., 2012). Thus, soil enzyme activity may be used as an indicator of soil quality due to its control on microbial growth (Burns et al., 2013). For chemical properties, Smith and Doran (1996) suggested that soil total organic carbon (TOC), available P, exchangeable K and soil pH are important measurements of soil quality because they provide indicators of soil nutrient supplying capacity.

We hypothesized that each legume species presents different chemical composition and therefore affect differently soil biological and chemical properties in short-term. Thus, the aim of this study was to evaluate the effect of four legumes species used as green manure on soil biological and chemical properties in short-term.

MATERIALS AND METHODS

The experiment was located at the Experimental Area from Agricultural Science Centre, Teresina, Piauí, Brazil. The regional climate is dry tropical (Köppen) and is characterised by two distinct seasons: Rainy summer and dry winter, with annual average temperatures of 30°C and rainfall of 1200 mm. The rainy season extends from January to April, when 90% of the total annual rainfall occurs. The soil is classified as a Fluvisol with the following composition at 0 to 20 cm depth: 10% clay, 28% silt, and 62% sand.

The following legume species were evaluated: *Crotalaria, Cajanus, Mucuna* and *Canavalia*. We included a treatment without green manure as a reference (control). The experimental area was arranged in a completely randomised design with four replicates. Plots measured 10 m^2 each, with 6 m^2 of usable area for soil sampling and rows are spaced 0.4 m apart. *Crotalaria* and *Cajanus* were grown at a density of 10 plant m^{-1}, while *Mucuna* and *Canavalia* were grown at a density of 7 plant m^{-1}. After 60 days from the sowing (flowering), all plants inside the plots were harvested and incorporated into the soil (0-20 cm) by harrow. Chemical characteristics of the legume species are shown in Table 1 (Cavalcanti et al., 2012). After 30 and 60 days from the incorporation, five soil samples were collected (0-20 cm) in each plot (from usable area) using a spade and pooled to form a composite sample per plot. Each soil sample was sieved (< 2 mm) and stored prior to analysis. Soil pH was estimated in water (1:2.5 v:v) and measured using a pH meter (Tedesco et al., 1995). Available P and exchangeable K, Ca, and Mg were evaluated according with Tedesco et al. (1995). Soil organic C was determined by wet combustion using a mixture of 5 mL of 0.167 mol L^{-1} potassium dichromate and 7.5 mL of concentrated sulphuric acid under heating (170°C for 30 min) (Yeomans and Bremner 1988).

Soil microbial biomass C (MBC) was determined according to the methods developed by Vance et al. (1987) with 0.5 M K_2SO_4 extraction of the organic C contents from fumigated and unfumigated soils. The soil respiration was monitored through daily measurement of CO_2 evolution under aerobic incubation at 25°C for 7 days (Alef and Nannipieri, 1995). Fluorescein diacetate (FDA) hydrolysis was measured by spectrophotometry at 490 nm after incubation for 20 min at 30°C, according to the method described by Schnürer and Rosswall (1982).

The data were submitted to the analysis of variance (ANOVA) and the means were compared by the Student's test (5% level). All the statistical analyses were performed with the SPSS (version 15.0) software package. The data from the variables were analysed using multivariate ordination non-metric multidimensional scaling (NMS) with Sorensen distances. Ordination was performed using the PC-ORD v. 6.0 program.

RESULTS

The incorporation of green manure changed the soil

Table 2. Chemical properties of soil after 60 days of incorporation of green manures.

Plots	pH	P*	K**	Ca**	Mg**
		mg kg^{-1}		cmolc kg^{-1}	
Crotalaria	7.3^{a}	62.4^{a}	16.4^{a}	2.2^{b}	0.7^{a}
Guandu	7.2^{a}	44.9^{b}	14.8^{b}	1.7^{b}	0.7^{a}
Mucuna	6.9^{a}	40.4^{b}	15.0^{b}	4.2^{a}	0.6^{a}
Canavalia	7.1^{a}	48.8^{b}	14.3^{b}	2.9^{b}	0.4^{b}
Control***	6.8^{a}	25.1^{c}	9.4^{c}	1.3^{c}	0.2^{c}

* Available P; ** Exchangeable K, Ca, and Mg; *** Control – plot without green manure

Table 3. Soil organic C (SOC, g kg^{-1}), microbial biomass (MBC, mg C kg^{-1}), microbial quotient (qmic, %), basal respiration (BR, mg CO_2 kg^{-1}), and diacetate fluorescein hydrolysis (FDA, mg FDA kg^{-1}) in plots with different green manure.

Plots	SOC		MBC		qmic		BR		FDA	
	30	60	30	60	30	60	30	60	30	60
Crotalaria	6.6^{a}	5.9^{a}	80.3^{b}	98.5^{b}	1.2^{b}	1.6^{a}	5.06^{a}	3.56^{a}	74.1^{b}	81.4^{b}
Guandu	6.3^{a}	5.8^{a}	83.6^{b}	107.6^{b}	1.3^{b}	1.8^{a}	4.97^{a}	3.55^{a}	78.7^{b}	96.0^{b}
Mucuna	6.2^{a}	6.7^{a}	132.5^{a}	138.5^{a}	1.6^{a}	1.5^{a}	5.19^{a}	3.71^{a}	154.1^{a}	142.9^{a}
Canavalia	6.5^{a}	6.8^{a}	78.2^{b}	113.2^{b}	1.2^{b}	1.6^{a}	5.23^{a}	4.69^{a}	175.2^{a}	158.1^{a}
Control*	6.1^{a}	6.3^{a}	46.3^{c}	39.2^{c}	0.6^{c}	0.5^{c}	4.81^{a}	4.06^{a}	52.1^{c}	47.3^{c}

*Control, plot without green manure.

chemical properties as compared with soil without green manure (Table 2), where the incorporation of green manure increased the content of available P and exchangeable K, Ca and Mg. Comparing the plots with green manure, soil chemical properties differed according to the legume species used (Table 2). Soil pH did not differ between evaluated plots. However, available P and exchangeable K contents were highest in plot with *Crotalaria*, while soil exchangeable Ca content was highest in plot with *Mucuna*.

The incorporation of green manure, in short-term, did not promote difference in soil organic C content at 30 and 60 days after green manure application. Soil biological properties changed with the incorporation of green manure (Table 3). The incorporation of green manure promoted an increase in MBC as compared with soil without green manure. However, soil MBC showed different behavior according to the green manure incorporated. Thus, plots whit *Mucuna* showed highest values of MBC at 30 and 60 days as compared with others green manure species. There was an increase in soil MBC from the 30th to 60th days of evaluation for all plots with green manure. At 30th day, the microbial quotient (qmic) was higher in plot with *Mucuna*; while at the 60th day there was not difference between plots.

Soil microbial activity, as measured by basal respiration and FDA hydrolysis, increased with the use of green manure as compared with the plot without green manure (Table 3). However, the soil basal respiration did not differ between plots with green manure. On the other hands, FDA hydrolysis was higher in plots with *Mucuna* and *Canavalia* than the others plots. There were not differences in values of FDA hydrolysis between the 30th and 60th days of evaluation for all evaluated plots.

NMS analysis explained 81% of variation and clustered two main groups according to biological and chemical properties (Figure 1). The first group consisted of plot with *Mucuna* and was linked with soil microbial biomass C and FDA hydrolysis; while the other group consisted of plots with *Guandu*, *Crotalaria* and *Canavalia* and was grouped with soil basal respiration and microbial quotient.

DISCUSSION

The incorporation of green manure, in short-term, increased the content of some chemical elements in the soil, suggesting that this practice may improve the soil fertility shortly. Previous studies had shown the positive effect of green manure on soil fertility in short-term (Astier et al., 2006; Partey et al., 2014). The legume species promoted different responses of soil chemical properties and it occurred due to different composition of each green manure. The higher values reported for soil P and K content in plot with *Crotalaria* are due to decomposition of the green manure, which presents high content of P and K in its composition (Table 1) and contributes for the releasing of these elements to the soil. Similar results

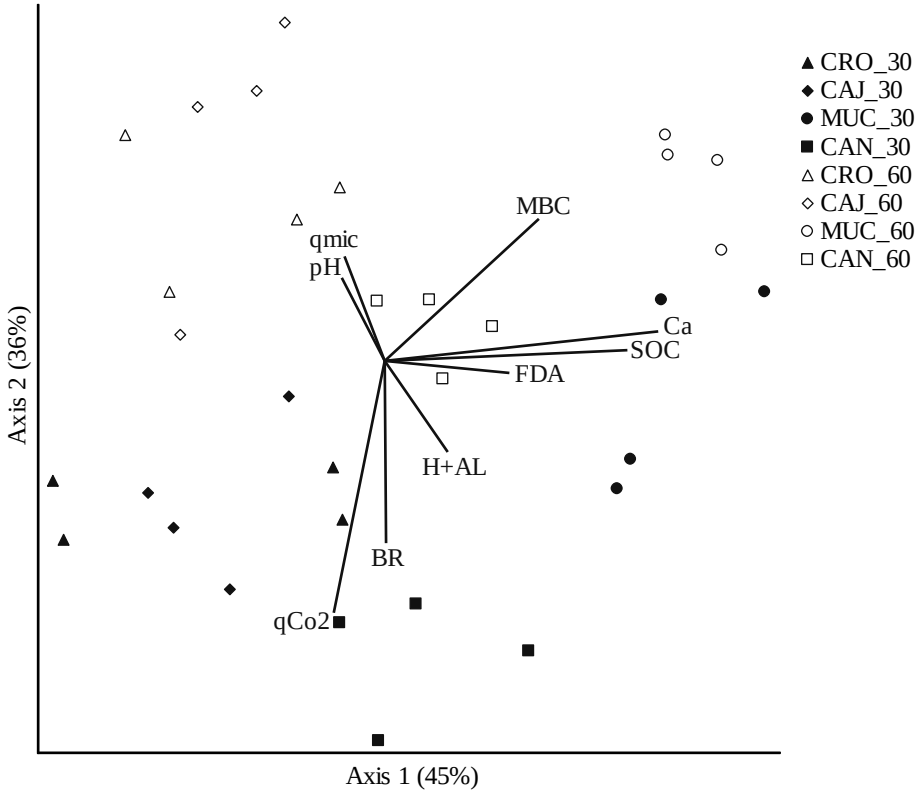

Figure 1. NMS of chemical and biological properties of soil after the incorporation of different green manures.

were found by Ziblim et al. (2013) comparing the potentials of *Mucuna* and *Crotalaria* as green manure on soil fertility, where they observed that *Crotalaria* added a higher amount of available P and K as compared to *Mucuna*. It is important for the improvement of soil fertility and may provide essential nutrient for crops. On the other hands, *Mucuna* presented high Ca content in its composition and it contributes for the increase in soil Ca in soil with this legume specie. These findings are in agreement with Adediran et al. (2004) which evaluated the effect of *Mucuna* intercropped with maize (*Zea mays* L.) on soil fertility and observed, after two months of *Mucuna* application, the increasing in the content of exchangeable Ca by 81% when compared with the chemically fertilized soil.

Our results showed that soil organic C did not change, in short-term, with the addition of green manure indicating that soil organic matter is not sensitive to short-term changes of soil quality with different soil or crop management practices (Liu et al., 2013). Usually, soil organic C increases in medium- to long-term as reported in previous studies (Dou et al., 2006; Huang et al., 2010).

On contrary, soil microbial biomass is a sensitive indicator of soil quality and responds quickly to changes in soil management. Several studies reported that soil microbial biomass improved significantly by using of legumes as green manure (Biederbeck et al., 2005; Liu et al., 2006; Shah et al., 2010). The results showed that soil microbial biomass and showed different values according to legume specie as also reported by Shah et al. (2010) who evaluated six types of legumes as green manure and observed that soil microbial biomass varied with the type of legume, where the highest microbial biomass was found in soil with sesbania and lowest in plot with guar. In our study, the higher soil MBC found in plots with *Mucuna* occurred due to its high input of dry mass with low C/N ratio which increases the availability of C to soil microorganisms (Franchini et al., 2007). It may suggest that this legume specie presents high potential to improve soil biological properties as reported by Blanchart et al. (2006) who evaluated the use of *Mucuna* as cover crop and found a positive modification in diversity and composition of soil biota.

On the other hands, the increase in soil microbial biomass from 30th to 60th day suggests that the all green manure maintained C source to soil microbial biomass. It may have occurred since the legumes are applied on soil surface and releases slowly the C for soil microbial consumption. Therefore, there is an increasing in soil microbial biomass over-time. The highest microbial quotient observed in plot with *Mucuna* is due the higher soil microbial biomass found in this plot. As the microbial

quotient is an indicator of availability of organic C for soil microorganism (Anderson and Domsch, 1989), this result means that in plot with *Mucuna* the organic C is more available for soil microbial biomass (Steiner et al., 2008).

Soil management practices which modify the soil microbial biomass also affect the enzyme activities (Dick et al., 1996). The FDA hydrolysis is directly proportional to the microbial growth (Swisher and Carroll, 1980) and is involved in the transformation of soil organic matter (Sicardi et al., 2004). Also, FDA hydrolysis is a good indicator of soil microbial activity and reflects the activity of several enzymes, including lipases, esterases and proteases, and its activity increases with SMB (Schnurer and Rosswall, 1982). Our results showed that microbial metabolism was positively influenced by *Mucuna* and *Canavalia*, suggesting these legume species may increase the soil microbial activity.

The NMS analyses of the soil biological and chemical properties revealed distinct trends for the legume species used as green manure. The plot with *Mucuna* clustered, at 30th and 60th days, and was characterized by higher microbial biomass C and microbial activity. This may suggest that this legume species has a potential to improve soil biological properties. On the other hand, *Crotalaria*, *Guandu* and *Canavalia* clustered together and were characterized by basal respiration and microbial quotient, which can be indicative of higher decomposition and availability of C and others nutrients.

Conclusion

Our results supported the hypothesis that different types of legume used as green manure affect differently the biological and chemical properties of soil. It occurs mainly due the different chemical composition of each legume species used. In this case, *Mucuna* was more effective to improve soil biological properties, while *Crotalaria* seems to be more efficient in the improvement of chemical properties.

Conflict of Interests

The authors have not declared any conflict of interests.

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Effect of resistant and susceptible soybean cultivars on the development of male and female *Heterodera glycines* Ichinohe

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Soybean cyst nematode, *Heterodera glycines* Ichinohe, is one of the major phytopathological problems affecting soybeans, *Glycine max* (L.) Merr., in the major producing countries and the use of resistant cultivars and crop rotation have been the main methods of control adopted to reduce the nematode population in infested soils. Purpose of this study was to evaluate the effect of resistant (BRSGO Ipameri and BRSGO Chapadões) and susceptible (BRSGO Araçu, BRSGO Jataí, BRSGO Luziânia, BRS Favorita RR, BRS Valiosa RR, BRS Silvânia RR) soybean cultivars on the development of *H. glycines* males and females during two successive years (2007 and 2008). In the trial of 2008 the plants were divided in three plots, with the last one having the roots stained to count the juveniles and to evaluate survival rate. Resistant cultivars always maintained a small number of females and males, except for cultivar BRSGO Ipameri that had a high count of males. Only cultivars BRS Favorita RR and BRS Silvânia RR had a sex ratio of 1:1. All other susceptible cultivars had, in general, greater number of males than females. Survival rate was nil on both resistant cultivars, and varied from 6.75 to 35.00% on the susceptible cultivars.

Key words: *Glycine max*, cyst nematode, sex ratio, hydroponics.

INTRODUCTION

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is a major disease of soybean, *Glycine max* (L.) Merr. in the main producing countries of this legume, such as the United States, Brazil and Argentina (Wrather et al., 1997).In Brazil, *H. glycines* was first identified in the cropping season 1991 /1992 (Lima et al., 1992; Lordello et al., 1992; Monteiro and Morais, 1992), and now occurs in ten states with the races 1, 2, 3, 4 (Dias et

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al., 2009), 4+ (Dias et al., 1998) 5, 6, 9, 10, 14 (Dias et al., 2009) and 14+ (Dias et al., 1999) been identified.

Losses caused by *H. glycines*, depending on its incidence, can be greater than those caused by any other disease affecting soybeans (Wrather et al., 1997). Yield decreases caused by *H. glycines* in the United States, the years 2003, 2004 and 2005 were 2.9, 3.47 and 1.93 million tons of the grain, respectively, highlighting the disease severity (Wrather and Koenning, 2006).

Genetic resistance is one of the major management strategies for the control of soybean cyst nematode. In general, on resistant cultivars, *H. glycines* juveniles are incapable of establishing feeding sites, due to the deterioration of syncytium, which occurs soon after infection, leading to nematode death in the root tissue (Kim et al., 1987; Kim and Riggs, 1992). Nematode females are responsible for most damage due to the formation of syncytium and by feeding from J2 to adulthood, while the males feed only as J2 and J3 (Endo, 1964, 1965). Therefore, sex determination in *H. glycines* is fundamental, since females cause most damage to soybeans.

In ideal conditions, the sex ratio of H. glycines is 1:1 (Endo, 1965; Koliopanos and Triantaphyllou, 1972; Luedders, 1987; Halbrendt et al., 1992). However, several factors can affect this proportion, such as inoculum density (Koliopanos and Triantaphyllou, 1972; Stelle, 1975; Evans and Fox, 1977), temperature (Melton et al., 1986), host nutritional status (Grundler et al., 1991) and genetic resistance (Endo, 1965; Luedders, 1987; Halbrendt et al., 1992; Colgrove and Niblack, 2005). Sex determination in this species is under strong genetic control, and is determined in the zygote instead of by environmental conditions during juvenile development. Stress conditions can reflect in greater mortality of a given sex and, consequently, in a sex ratio other than 1:1 (Koliopanos and Triantaphyllou, 1972). Most reports of unbalanced sex ratio are related to differential female death (Evans and Fox, 1977; Colgrove and Niblack, 2005).

Soybean cultivars with syncytium degeneration occurring four to five days after infection, present a small number of males (Endo, 1965). Luedders (1987) found that resistance genes against this nematode can affect males and females differentially, as occurs with PI 88788, but does not seem to affect male development. Halbrendt et al. (1992) also did not find changes in male development in PI 209332. However, in the same study, they observed that male development is severely affected in PI 89772 and in cultivar Pickett. Those authors stated that resistance of PI 209332 affects the development of J_3 and J_4, resistance of cultivar Pickett affects the development of J_2 and J_3, while resistance of PI 89772 affects all developmental stages. Since *H. glycines* males feed only during the stages J_2 and J_3, the genotypes with resistance affecting the end of the nematode cycle (J_3 and J_4), exert lesser influence on male development.

Colgrove and Niblack (2005) found sex ratio 1:1 for PIs 548402, 90763, 437654 and 89772, and increased male number for PIs 88788, 209332 and 547316. Those authors found that the greater proportion of males occurred due to differential death of males and females, contributing to the hypothesis that sex determination are a genetic characteristic of nematode *H. glycines* unaffected by environmental factors. Thus, this study evaluated the development of *H. glycines* males and females on resistant and susceptible soybean cultivars recommended for the Cerrados region in Brazil.

MATERIALS AND METHODS

The population of *H. glycines* race 14 used in the experiments was collected from a naturally infested field in the county of Campo Alegre (GO), and sequentially multiplied in a greenhouse, using the susceptible cultivar BRSGO Luziânia. *H. glycines* race 14 was chosen due to its wide spread in Brazil. Two experiments were conducted under greenhouse conditions, at Universidade Federal de Goiás (16°35'47.36"S; 49°16'48.01"W; 726 m above sea level), using six commercial soybean cultivars susceptible to *H. glycines*: BRSGO Araçu, BRSGO Jataí, BRSGO Luziânia, BRS Favorita RR, BRS Valiosa RR, BRS Silvânia RR and two resistant cultivars: BRSGO Ipameri and BRSGO Chapadões. These soybean cultivars are recommended for planting at the central part of Brazil (Cerrados region).

Experiment 1

The experiment was conducted from March to April 2007, in a completely randomized design with eight treatments and five replications. The eight soybean cultivars were germinated in a germination chamber, at Universidade Federal de Goiás (16°35'47.36"S; 49°16'48.01"W; 726 m above sea level), and four seedlings were transplanted to 1,400-cm^3 plastic pots, containing naturally infested soil, with an average initial population of 157 cysts of the nematode per 100 cm^3, with a mean of 225 eggs per cyst. The pots containing the seedlings were maintained over a wet sand bed on the benches. Two seedlings were removed from the pots ten days after transplanting, and transferred to a hydroponics system, and maintained there for 19 days, to collect *H. glycines* males. The other plants remained in the pots until completing 30 days, when they were removed to evaluate the number of females present in the root system and the number of eggs per female.

Experiment 2

The experiment was done in January and February 2008, in a completely randomized design, with eight treatments and four replications. The eight soybean cultivars were germinated in sand in the greenhouse and six seedlings were transplanted to 1,400 cm^3 clay pots, containing naturally infested soil, with an average initial population of 118 cysts of the nematode per 100 cm^3, with a mean of 150 eggs per cyst. The pots containing the seedlings were maintained over a wet sand bed on the benches. Two seedlings were removed from the pots ten days after transplanting, and transferred to a hydroponics system to collect the males. On the following day, two other seedlings were removed and the root system stained, using the clearing technique with NaOCl and staining with acid fuchsin (Byrd et al., 1983), to quantify the juveniles and determine the survival rate. The other two plants

Table 1. Number of male and female individuals, number of eggs per female and sex ratio of *H. glycines*, in a trial conducted in 2007.

Soybean cultivars	2007			
	Number females	Number eggs/female	Number males	Sex ratio
BRSGO Jataí	467[a]	217[a]	1.003[a]	2,15*
BRSGO Luziânia	528[a]	285[a]	818[ab]	1,55*
BRSGO Araçu	304[a]	178[ab]	879[abc]	2,88*
BRS Favorita RR	270[a]	248[a]	212[bc]	0,78
BRS Valiosa RR	1.184[a]	183[ab]	839[ab]	0,71*
BRS Silvânia RR	401[a]	231[a]	334[abc]	0,83
BRSGO Ipameri	3[c]	15[c]	201[c]	62,98*
BRSGO Chapadões	19[b]	36[b]	51[d]	2,74*
CV (%)[1]	10.01	12.43	7.77	

Averages in a column followed by the same letter do not differ significantly (Tukey P < 0.05). Data were transformed into log (x+1) for statistical analysis purposes.*Sex ratio different from 1:1 (χ^2 test at α=0.05).[1]Coefficient of variation.

remained in the pots until completing 32 days, when the number of females and the number of eggs per female were evaluated.

The hydroponics system was set with 500 cm^3 PET flasks and an air compressor adjusted to 40 PSI, with fine hoses connected to the flasks to maintain constant airing of the water. Nematode collection started 10 days after transferring the seedlings, and was done every three days. The water removed from the flasks was transferred to glass bottles and taken to the laboratory for decanting for two hours. Subsequently, the water volume was reduced, with the aid of a vacuum pump, to 20 m*l* and then homogenized, and the males present in the suspension counted, using the Peter's slide. Each sample was counted three times under an optical microscope (magnification of 50 *x*).

The number of females was evaluated by removing the plants from the pots and rinsing the root system under running water over a set of 20 and 60 mesh sieves. The material retained in the 20 mesh sieve was discarded and that retained in the 60 mesh screen was filtered with filter paper over a plastic screen (Andrade et al., 1995) and counted under the stereoscope (magnification of 15 *x*). Ten females were arbitrarily picked and broken over a set of 100 mesh and 400 mesh sieves, and the eggs recovered in water on the 400 mesh sieve and quantified under the stereoscope, using Peters' slide (magnification of 50 *x*).

The stained roots were placed in Petri dishes and the number of juveniles was quantified under the stereoscope (magnification of 15 *x*). The number of juveniles in the stained roots and the number of females present in the root system of the plants maintained in the pots were used to determine the survival rate [(females present in the root system / number of juveniles in the stained roots) *x* 100] (Congrove and Niblack, 2005).

The chi-square (χ^2) test was used to confirm the hypothesis that the males of *H. glycines* comprise 50 % of the adult population (sex ratio 1:1). The data were transformed into logx + 1 and submitted to the analysis of variance. The averages were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Significant differences for the number of *H. glycines* females in the roots among the cultivars were observed for both experiments, in 2007 and 2008 (Tables 1 and 2). The commercial cultivars resistant to *H. glycines*, race 14, BRSGO Ipameri and BRSGO Chapadões, confirmed the expected performance in both experiments, presenting smaller numbers of females (Tables 1 and 2). All other cultivars performed as susceptible to the nematode.

In 2007, the number of eggs per female on the cultivar BRSGO Ipameri differed statistically from the other cultivars presenting the lowest number. The cultivar BRSGO Chapadões had low number of eggs per female but did not differ statistically from BRS Araçu and BRS Valiosa RR. In 2008 both resistant cultivars, BRSGO Ipameri and BRSGO Chapadoes, had low development of eggs per female differing from the susceptible cultivars (Tables 1 and 2).

A high number of *H. glycines* males were found in the roots of plants from the hydroponic system in both experiments (Tables 1 and 2). In 2007, the cultivar BRSGO Chapadões differed from all the other cultivars presenting the lowest number of males. Cultivar BRSGO Ipameri only differed from BRS Valiosa RR, BRSGO Luziânia and BRSGO Jataí (Table 1). In 2008 the cultivar BRSGO Chapadões presented the lowest number of males differing from cultivars BRS Favorita RR, BRS Valiosa RR and BRS Silvânia RR (Table 2).

The sex ratio between males and females in 2007 (Table 1), is near 50% only for cultivars BRS Favorita RR and BRS Silvânia RR, by the χ^2 test (5%). In 2008 all cultivars had the sex ratio different from 1:1. The resistant cultivars had sex ratios extremely high due to the absence or very low development of females. The survival rate, done only for the 2008 experiment, varied from 0% to 35.00 % (Table 2). All root systems were colonized by the nematode however, females developed only in the root system of susceptible cultivars.

The greater number of females found in 2007 than in 2008 may be explained by the greater initial inoculum concentration in the first experiment and, also, by the inoculum condition, which had been collected from the field, presenting greater virulence in 2007 than the population maintained in the greenhouse for several

Table 2. Number of male and female individuals, number of eggs per female, sex ratio and survival rate of *H. glycines*, in a trial conducted in 2008.

Soybean cultivars	2008				
	Number females	Number eggs/female	Number males	Sex ratio	Survival rate (%)[1]
BRSGO Jataí	118[a]	195[a]	627[ab]	5.32*	8.17[b]
BRSGO Luziânia	231[a]	261[a]	435[ab]	1.88*	35.00[a]
BRSGO Araçu	76[a]	163[a]	840[ab]	11.06*	6.75[b]
BRS Favorita RR	213[a]	264[a]	1048[a]	4.91*	26.25[a]
BRS Valiosa RR	132[a]	250[a]	942[a]	7.14*	10.25[ab]
BRS Silvânia RR	116[a]	262[a]	866[a]	7.46*	12.25[ab]
BRSGO Ipameri	1[b]	14[b]	970[ab]	970.75*	0.00[c]
BRSGO Chapadões	0[b]	0[b]	248[b]	248.5/0*	0.00[c]
CV (%)[2]	13.4	20.3	6.38		12.74

Averages in a column followed by the same letter do not differ significantly (Tukey P < 0.05). Data were transformed into log (x+1) for statistical analysis purposes; [1]Survival percentage (number of females present in the root system / number of juveniles in the stained roots) *x* 100. Averages followed by the same letter do not differ significantly (Tukey P < 0.05); [2]Coefficient of variation;*Sex proportion different from 1:1 (χ^2 test at α=0.05).

generations until the next year. Brito et al. (1999) and Koenning (2000) also observed that the increase in the initial concentration of inoculum of *H. glycines*, tends to increase the number of females present in the root of soybean system.

Genetic resistance to the soybean cyst nematode affects the number of male individuals, as highlighted by the evaluations of these trials. Cultivar BRSGO Chapadões, presented the smallest number of males in both experiments. However, cultivar BRSGO Ipameri, which is also resistant to *H. glycines*, race 14, had greater number of males than BRSGO Chapadões. This difference in values is probably due to the source of resistance of each of these cultivars. Cultivar BRSGO Chapadões had PI 437654 as genetic background, while cultivar BRSGO Ipameri had PI 88788 (Dias et al., 2007). Studies done by Luedders (1987) and Colgrove and Niblack (2005) demonstrated that greater proportions of males are found in PI 88788 than in PI 437654, as a function of differential mortality of males and females.

Halbrendt et al. (1992) also found effect of the source of resistance on the development of *H. glycines* male individuals which confirms the results found in this study for both resistant cultivars. These authors confirmed that the resistance that affects the development of J3 and J4, as the resistance from PI 209332, provides higher male development than the sources of resistance that affects the stages J2 and J3, like the resistance from cultivar Pickett. This occurs due to the feeding period of the males. *H. glycines* males only feed during the stages J2 and J3 while females feed from J2 until adult (Endo, 1964, 1965). Therefore, the sources of resistance that are not effective during the early stages of development result in lower mortality of males.

It was expected to find a sex ratio of 1:1 in all susceptible soybean cultivars, since, according to Luedders (1987), Halbrendt et al. (1992) and Colgrove and Niblack (2005), resistance is one of the stress factors that inhibits *H. glycines* female development and thus causes a differential death among male and female. In general, the number of males found, especially in the resistant cultivars, was greater than the number of females. Koliopanos and Triantaphylou (1972) found that under greater population densities, a trend of forming more male than female individuals existed, especially with the inoculation of 5,000 eggs and J_2. Considering that the initial population in the substrate used was greater than 5,000 eggs and J_2, it may explain the greater proportion of *H. glycines* males found.Survival rate was 0% for both resistant cultivars, which is near the values found by Colgrove and Niblack (2005) for the plant introductions PI 88788 and PI 437654. In contrast, the susceptible cultivars had survival rates varying from 6.75 to 35.00%. These values corroborate those found by Evans and Fox (1977) and Colgrove and Niblack (2005) for the soybean cultivar used as susceptibility standard, Lee. Colgrove and Niblack (2005) found survival rates varying from 41.00 to 112.00% for several resistance sources evaluated in their experiments. Halbrendt et al. (1992) reported mortality rate varying from 23.00 to 51.00% and stated that high mortality percentage is normal among juveniles.

The developments of *H. glycines* males do not get the same proportion as the development of females in the same soybean cultivar. The results presented in this study suggest that the differential development of males and females occur as a function of resistance sources of soybean cultivars. The Brazilian resistant cultivars BRSGO Ipameri and BRSGO Chapadoes are effective on controlling *H. glycines* by the reduction of female development although allowing high male development.

Conflict of Interests

The authors have not declared any conflict of interests.

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