African Journal of Agricultural Research

Volume 11 Number 31 4 August 2016
ISSN 1991-637X

Academic Journals

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African Journal of Agricultural Research

Table of Contents: Volume 11 Number 31, 4 August, 2016

ARTICLES

African Journal of Agricultural Research

Table of Contents: Volume 11 Number 31, 4 August, 2016

ARTICLES

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Vol. 11(31), pp. 2833-2840, 4 August, 2016
DOI: 10.5897/AJAR2016.11291
Article Number: 73AB72459807
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Efficiency of commercial products for the control of *Sclerotinia sclerotiorum* (Lib.) de Bary in soybean cultivar NS 5909 RG

Alfredo José Alves Neto[1], Álvaro Guilherme Alves[2], José Renato Stangarlin[1], Jéssica Caroline Coppo[1*], Leandro Rampim[1] Bruna Broti Rissato[1] Diego Augusto Fois Fatecha[1], Eloisa Lorenzetti[1], Paulo Sérgio Giacomelli[1] and Cristiane Belmonte[1].

[1]State University of West Paraná - UNIOESTE, Marechal Cândido Rondon – Paraná – Brazil, street Pernambuco, Number 1777, Box: 91, Center, Brazil.
[2]União Educacional de Cascavel-Paraná- Brazil, street Paraná, Number 2767, Corbélia - Box: 91, Center, Brazil.

Received 2 June, 2016; Accepted 8 July, 2016

The constant assessment of methods of disease control is essential in achieving production regularity and for ongoing increments in soybean productivity. Among the various diseases, the intensity of the white mold has been increasing, caused especially by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. The present work has the objective to assess different seed treatments in association with fungicide application to control the white mold in soybean. The experimental design used was complete randomized blocks design with split plots. In the plots, the treatments were: control - no seed treatment; Certeza® (thiophanate methyl + fluazinam 45 + 60 g ha^{-1}) and Trichodermil® (*Trichodermaharzianum* '1306' 1.250 mL 100 kg^{-1} - 10^9 viable spores mL^{-1}). In each seed treatment, the application was done on aerial part of subplots.The treatmentsin aerial part were: control (with no fungicides application); three applications of Derosal® (carbendazim) 1.000 gi.a./ha^{-1} in R1, R2 and R5.1; three applications of Cercobin 700 WP® (thiophanate methyl) 600 gi.a./ha^{-1} in R1, R2 and R5.1;Cercobin 700 WP® (thiophanate methyl) in R1, Sumilex (procimidone) 750 gi.a./ha^{-1} in R2 and Frowncide (fluazinam) 750 gi.a./ha^{-1} in R5.1.There were assessments on the emergence and height of the plants in V3 and the severity of the disease at the phenological stages R1, R3, R5.5 and R7. The variables thousand-grain weight, productivity and the economic analysis were determined after the harvesting of the plots. The treatments of seeds with thiophanate methyl+ fluazinam and *Trichoderma harzianum* and the applications of thiophanate methyl, procimidone and fluazinam on the aerial part stood out in the control of the disease. The net income showed that despite the high cost of the plant protection products, there were increases in the profitability of the crop.

Key words: White mold, *Glycine max*, Economic analysis.

INTRODUCTION

The agricultural crop of greater growth in the Brazilian market is the soybean, which corresponds to 49% of cultivated area with grains, especially in the Midwest and South Regions. Currently, one of the barriers to the increase of its productivity in South America is the disease known as white mold, incited by the fungus *Sclerotiniasclerotiorum* (Lib.) de Bary. This disease has reported losses ranging from 10 to 20% of productivity, on average, in Brazil (Almeida et al., 2005), and losses ranging from 11.5 to 96% of productivity in an estimate made only in the Southern region of Brazil (Silva et al., 2011).

The control of white mold is difficult due to the permanence of viable *S. sclerotiorum* for a long time on the soil, which can remain on the soil for up to 11 years, keeping its pathogenic power intact. Similarly, the inefficiency of the chemical control, coupled with the high susceptibility of cultivars, causes facilitated contamination in cultivated areas (Leite, 2005). Among the control measures of the disease, the ones which stand out as the most efficient ones are, when integrated, the crop rotation, the cultivation of non-host crops to the fungus allied to the formation of straw to the no-till farming system and the biological control with living beings antagonistic to the fungus that causes the white mold (Bolton et al., 2006). The presence of straw results in lower rates of incidence of the white mold, since the soil cover establishes a physical barrier increasing microbial activity in the soil of antagonistic fungus, in addition to restricting the ascospores dispersion in the air and inhibiting the formation of apothecia (Paula Junior et al., 2007). However, the most widely adopted crop succession system in the Southern region of Brazil is the soybean system (first harvest/summer) corn (second harvest/winter), the areas of crop rotation being sporadic, which results in a decrease, over time, of the soil cover (Alves Neto et al., 2016). As consequence, the aggressiveness, the incidence and the severity of the disease have increased in the soybean areas, due to the characteristics of the *S. sclerotiorum*, which has wide adaptability (Ito and Parisi, 2010).

The main measure used aiming the control of disease is the usage of resistant cultivar, however, there have not been reports about the discovery of genotypes resistant to the white mold of the soybean yet, as the genetic resistance regarding the host in complex and of low heritability, being limited to genotypes that present only partial resistance (Guo et al., 2008; Vuong et al., 2008). Accordingly, the usage of chemical fungicides becomes an alternative to the control of the white mold. However, the difficulty in obtaining complete coverage of the plant by the fungicides during the pulverization, besides the total prices of the products, which have high cost, are challenges to obtain a proper and effective application (Görgen et al., 2009).

In addition to the fungicides for the application in post-emergence of the crop, there are chemical fungicides and antagonistic microorganisms for the seed treatment, given that they might come from the field with mycelia of *S. sclerotiorum* (Maude, 1996). Thus, both methods are designed to ensure proper initial population, with more vigorous and resistant plants, delaying the onset of the disease.

The seed is the most efficient means of dissemination and survival of the pathogen. Under favorable conditions, it only takes 0.5% of infected seeds to produce an epidemic on the field, in addition to increasing the inoculum potential of each crop cycle (Halfeld-Vieira and Sousa, 2000). Some practices have been adopted without the technical recommendation of research institutions, expanding unbalanced use of crop protection products, as the application of carbendazim in dose 1.000 gi.a./ha^{-1}, in an attempt to achieve suppression over *S. sclerotiorum*, mainly for being a fungicide with a relatively low cost when compared to fungicides specific to the white mold. This attempt to suppress *S. sclerotiorum*, may increase its resistance, besides causing unsatisfactory results in the control of the pathogen.

In this context, the objective of this work was to assess different seed treatments in association with the application of fungicides of aerial on the control of white mold in the soybean crop, with the determination of the economic analysis of the treatments.

MATERIALS AND METHODS

The experiment was conducted at the Cristavel Farm, in the City of Cascavel, Paraná State, Brazil, located at an average altitude of 735 m, latitude 25° 02'00,35" South and longitude 53° 10'32,09" West, in glebe with slope of 1.5%, in the months of October of 2015 to February of 2016. The soil is classified as typical Dystrophic Red Latosol (EMBRAPA, 2013), with 3.60% of organic matter and the weather of the region, according to Köppen, is type subtropical Cfa, with a hot summer and a tendency towards the concentration of rainfall, winter of not frequent frosts without a defined season. The annual average rainfall stands around 1.500 mm, presenting average temperatures in the summer exceeding 20°C and in the winter average temperatures below 18°C (IAPAR, 2016). The area has been conducted in no-till farming system for over 10 years, with crop rotation, soybean being cultivated in the summer: wheat, black oats, second harvest corn and canola in the winter, all crops fertilized exclusively with mineral fertilizers and according to the needs of the crop. The soil cover contained wheat straw (*Triticum aestivum*).

Before the installation of the experiment it was collected, at random, sclerotia in 5 points of 0,25 m^2 and 0,05 m of depth, aiming to determine the amount of sclerotia in the area, which was of 21 m^{-2} in the glebe, according to the methodology described by Sartori et al. (2011). The cultivar used in the experiment was the NS 5909 RG ®, this is the most used cultivar in southern Brazil, with super-young cycle and maturity group 5.9, presenting indeterminate growth, purple flower, pubescence of grayish color and brown hilum. The

*Corresponding author. E-mail: coppojessica0@gmail.com. Tel: +55(45) 32847878, Fax: +55(45) 32847879.

sowing was held on the 17 of October of 2015 with a population of 355.000 viable seeds per hectare, with spacing of 0, 45 m. The cultural practices were performed with chemical control during the crop cycle, taking into account the levels of economic damage for the control of pests, diseases and weeds.

Three applications were made of Fox® (trifloxystrobin + protioconazol 45 + 60 g ha^{-1}) in all treatments on the stages of R1, R3 and R5.5 aiming for rust control (*Phakopsora pachyrhizi* Syd. and P. Syd.) and target spot (*Corynespora cassicola*). The experimental design used was the randomized complete block design, with split plots, consisting of three seed treatments with four applications of fungicides on the aerial part of the plant, with five repetitions. In the plots the seed treatment factor was allocated, and in the subplots the factor was fungicide on aerial part. The main plots were allocated in the glebe measuring 18 m long x 5,5 m wide, with subplots allocated within the main plot with 4,5 m long x 5,5 m wide. The plots were spaced one meter apart, for uniformity in the application and, also, to facilitate the access and the severity assessments. The following seed treatments were tested (TS): TS 1: control, no seed treatment: TS 2: Certeza® thiophanate methyl + fluazinam 45 + 60 g ha^{-1} and TS 3: Trichodermil® *Trichoderma harzianum* '1306' 1.250 mL 100 kg^{-1} - 10^9 viable spores mL^{-1}.

On the subplots the different managements of fungicides (MF) were applied on the aerial part: MF 1: control no fungicide application: MF 2: three applications of Derosal® (carbendazim) 1.000 g i.a. ha^{-1} on R1, R2 and R5.1: MF 3: three applications of Cercobin 700 WP® (thiophanate methyl) 600 g i.a. ha^{-1} on R1, R2 and R5.1 and MF 4: Cercobin 700 WP® (thiophanate methyl) 600 g i.a. ha^{-1} on R1, Sumilex (procimidone) 750 g i.a. ha^{-1} on R2 and Frowncide (fluazinam) 750 g i.a./ha^{-1} on R5.1.

The applications were performed with a battery powered knapsack sprayer with a 20 liters capacity, PJB to 20c to Jacto® model, with a 1.5 meters long bar adapted with 3 nozzles distance of 0.5 meters between nozzles, with bar pressure regulated at 350 kPa and tank volume regulated to apply a flow of 150 L ha^{-1}, with flat spray nozzle (fan) 110 02. The assessments of emergence and plant height were performed in V3, on 11/11/2015, 33 days after the sowing. The assessments of severity of the disease white mold *S. sclerotiorum*, were based on the observation of the percentage of symptoms on the plant and were conducted at the phenological stages R1 (09/12/2016), R3 (16/12/2015), R5.5 (04/01/2016) and R7 (26/01/2016) and based on diagrammatic scale with ratings ranging from 5 to 90% of severity (Juliatti, 2010).

The thousand grain weight of soybean was determined from the weighing, on a precision balance, of 1000 grains. As for the productivity of the crop, it was determined from the manual harvest of five 4.5 m long lines, spaced in 0.45 m, representing 10.12 m^2 of useful plot. The material collected and threshed and the mass determined on precision scale, standardized to 13% of moisture and extrapolating to productivity per hectare. The economic analysis included the net income to each applied treatment, discounting the cost of each application for each treatment of gross revenues. It was considered the cost in dollars per hectare of US$ 0.00 for plot without seed treatment: US$ 13.82 for seed treatment with thiophanate methyl + fluazinam: US$ 7.00 for seed treatment with *Trichoderma harzianum*.

For the aerial applications with fungicide handling, there was a cost of US$ 11.50 of carbendazim for each application, the total cost of three applications being US$ 34.65 ha^{-1}: the application of thiophanate methyl costed US$ 8.15 the application, the total cost being of US$ 24.50 ha^{-1}: the application of thiophanate methyl, procimidone and fluazinam resulted in cost of US$ 8.15, US$ 39.00, US$ 41.96 respectively, for a total cost of US$ 89,11 ha^{-1}. These were the prices offered in the Western region of Paraná to soybean producers in the agricultural year 2015/2016, the average of quoted prices being made on 04/01/2016, in three suppliers of crop protection products. With the multiplication of the crop productivity (kg ha^{-1}), considering the prices in dollar of US$ 13,90 for soybean, considering the average of the month of February of 2016 (obtained CBOT, Chicago Board of Trade), the gross revenue of each treatment was obtained. The results of the collected data were submitted to the variance analysis and the averages of severity and productivity were submitted to the analysis of variance (ANOVA) by the F-test using the SISVAR statistical analysis software.

RESULTS AND DISCUSSION

In Figure 1, it is possible to verify on the meteorological data, which show that the climatic conditions were favorable to the occurrence of the white mold, given that the *S. sclerotiorum* fungus develops at temperatures ranging from 11 to 25°C, with high relative humidity of the air and the soil, with reports of developments of the disease on the soybean crop at temperatures ranging from 5 to 30 °C (Saharan and Mehta, 2008).

Juliatti et al. (2011) stress that the biggest impacts of white mold are reported in the highland areas, above 700 m high, where night time temperatures are milder, especially in areas with no crop rotation, with host plants and with a reduced amount of straw. There were similar conditions in the experimental area, which had mild temperatures and an area with 735 m of altitude. Following the sowing, there were rainfall and temperature conditions favorable to germination, very common in the Southern Region of Brazil (Mertz et al., 2009). The plants emergence was not influenced by the seed treatment used, however, the height of seedlings and the field emergence were lower for the treatment with Certeza® - (thiophanate methyl + fluazinam), being below the average in both assessments performed (Table 1).

Sid-Ahmed et al. (2003), in assessing the influence of peat and chitin in combination and not combined with biological agents, concluded that the association of microorganisms, as *Trichoderma harzianum*, enhanced the germination levels, due to the increase in antagonistic potential against pathogens. However, in the current experiment, the treatment of seeds with *Trichoderma harzianum*, did not promote significant increases on the germination levels and the plants height, being considered similar to the other treatments. It was none the less the treatment which presented the highest germination rate among all treatments. For the disease severity variable, there was no detection of white mold incidence in any of the treatments in the first assessment (R1). In fact, the infection of *S. sclerotiorum* on the plant starts on the R1 stage, where the crop is more susceptible to diseases, as the ascospores need an exogenous source of energy, originating from the senescing flowers of the plant, which, in favorable meteorological conditions, start the infection process of the plants (Boland and Hall, 1994).

The severity assessments in the subsequent phases, R3 and R5.5, showed that there was an increase in severity over time (Figure 2), with differences for the fungicides application on the R3 stage (Figure 2A) and

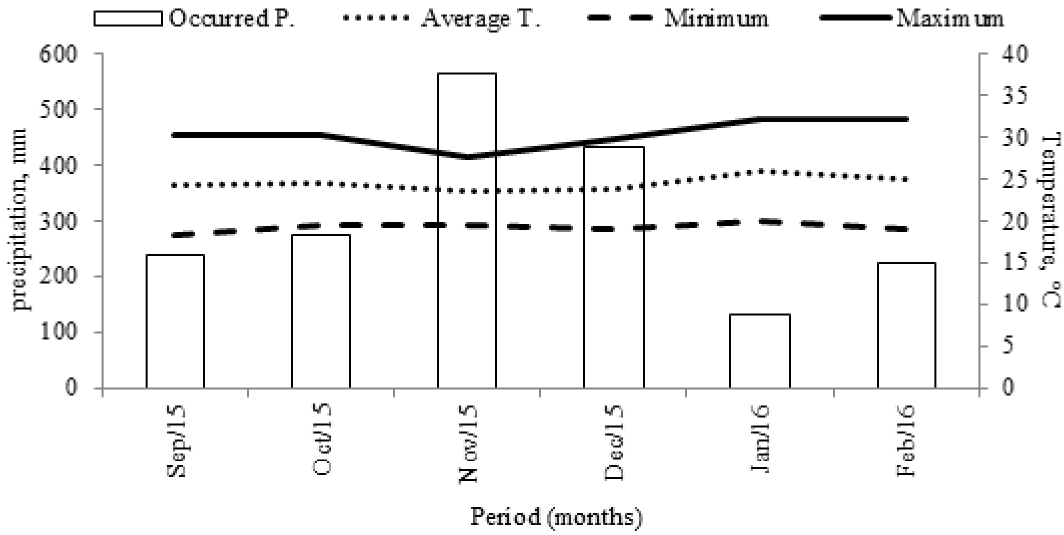

Figure 1. Data collected at the IAPAR (Agronomic Institute of Paraná State) meteorological station, during soybean cultivation, from September of 2015 to February of 2016.

Table 1. Emergence and height of plants, according to seeds treatment, 33 after sowing. Paraná State, 2015/16.

Treatment	Field emergence (%)	Plant height (m)
Thiophanate methyl + fluazinam	93.75[ns]	0.1398[b]
Trichoderma harzianum	94.56	0.1471[ab]
Control	93.81	0.1482[a]
Average	94.04	0.1450
CV (%)	2.09	5.20

* ns: non-significant values.* Averages followed by the same letter do not differ at level (α = 0.05) of significance.

for the seeds treatments on the R5.5 stage (Figure 2B). According to Almeida et al. (2005), the most vulnerable stage of the soybean plants to white mold incidence comprises R2 to R3. For a greater efficiency in the chemical control of the white mold, the authors recommend the first application to be made at R1, as at this stage there is still a gap between lines, which allows a bigger coverage and a greater quality on the fungicide application. Both on the assessment in R3, as on the assessment in R5.5, the active ingredient thiophanate methyl showed potential in the decrease of the severity of *S. sclerotiorum*, either on the seed treatment or on the aerial application. The assessment in R.3 determined that the lower severities happened with two applications of thiophanate methyl (R1 and R2 stages) with severity level of 2, 92%, being superior to carbendazim fungicide in 70, 02%, which presented severity of 4,17% at the same stage. The application of thiophanate methyl (R1) and procimidone (R2) resulted in a level severity of 1, 67%, value 57, 19% superior to the 2 applications of thiophanate methyl.

Thiophanate methyl belongs to the chemical group of benzimidazoles, which act on the cellular division, disrupting the mitotic cycle, preventing the formation of the metaphase plate, leading to the collapse of mycelium cells, which terminates the mycelium growth (Juliatti, 2007). However, this specificity of the benzimidazoles causes this fungicide to present high risk of acquired resistance by the pathogen. Due to these characteristics, the isolates resistant to benzimidazoles are generally as adapted as sensitive. Therefore, the high selection pressure caused by intensive usage of benzimidazoles may result in the selection of resistant isolates in a short period of time (Rodrigues et al., 2007). This information is important in the taking up of anti-resistance strategy. Procimidone belongs to the chemical group of dicarboximide, which inhibits the phosphorylation of the respiratory chain, disrupting the transport of electrons, being active from the oxidative phosphorylation (Encinas, 2004).

Meneghetti et al. (2010) stress the importance of using different active ingredients with different mechanisms of action for more effective disease control on the crop, noting that the combination of products increases the

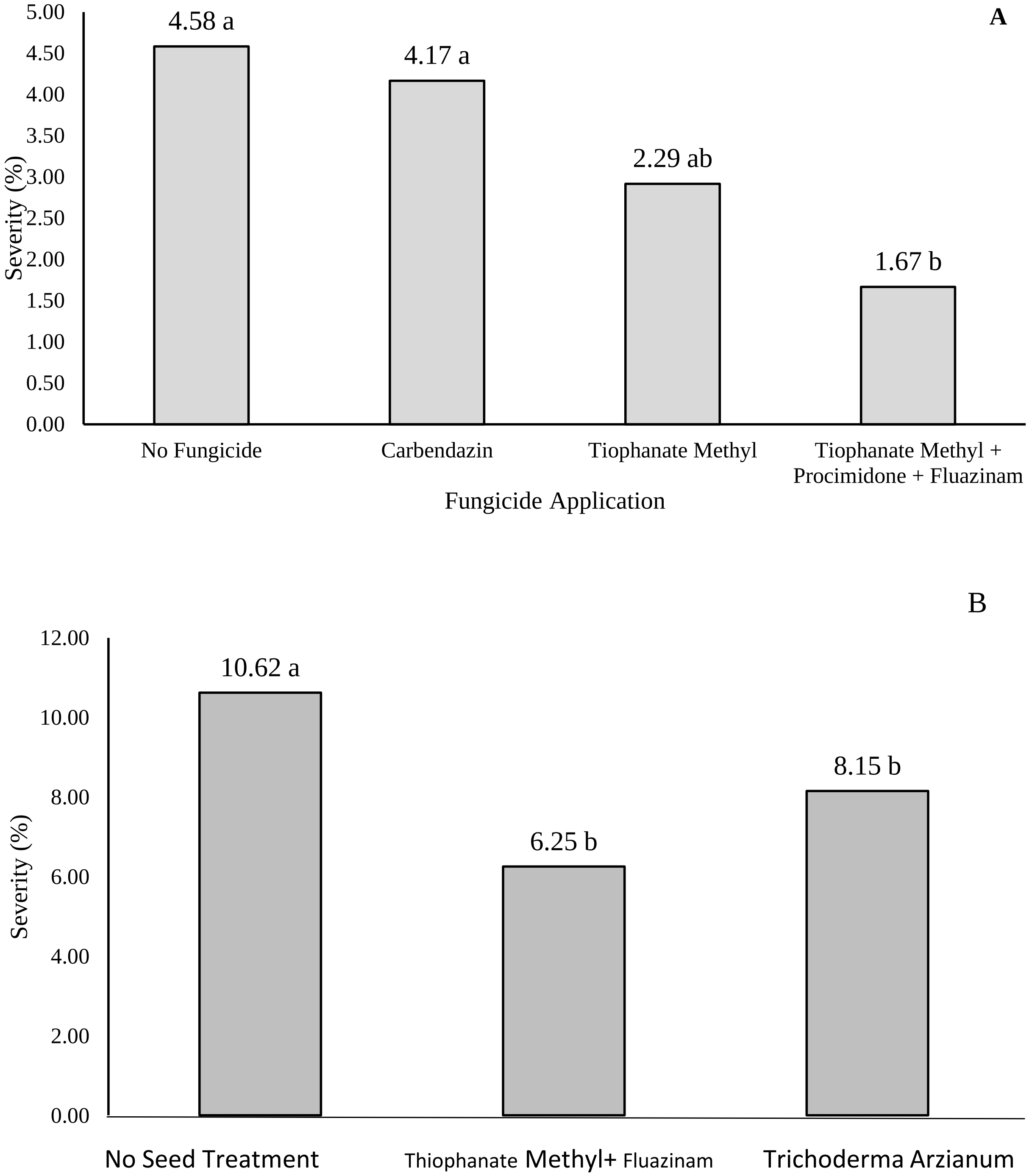

Figure 2. Severity of white mold (*S. sclerotiorum*) in R3 (A) and R5.5 (B), according to different fungicides applied on the aerial part and seed treatment on the soybean crop.* Averages followed by the same letter do not differ at level (α = 0, 05) of significance.

action spectrum, providing greater residual, besides decreasing the risk of arising resistant populations. On the assessment of R5.5, the importance of seed treatment in the soybean crop is evident, where it is observed that even under the different active ingredients on the aerial application, the treatments with thiophanate methyl + fluazinam and *Trichodermaharzianum*, providing control of *S. sclerotiorum* in soybean crop. Pereira et al. (2009) point out that the seeds are the main transmission vehicle of fungi, which are responsible for the reduction in germination and in vigor, originating the primary outbreaks of the disease.

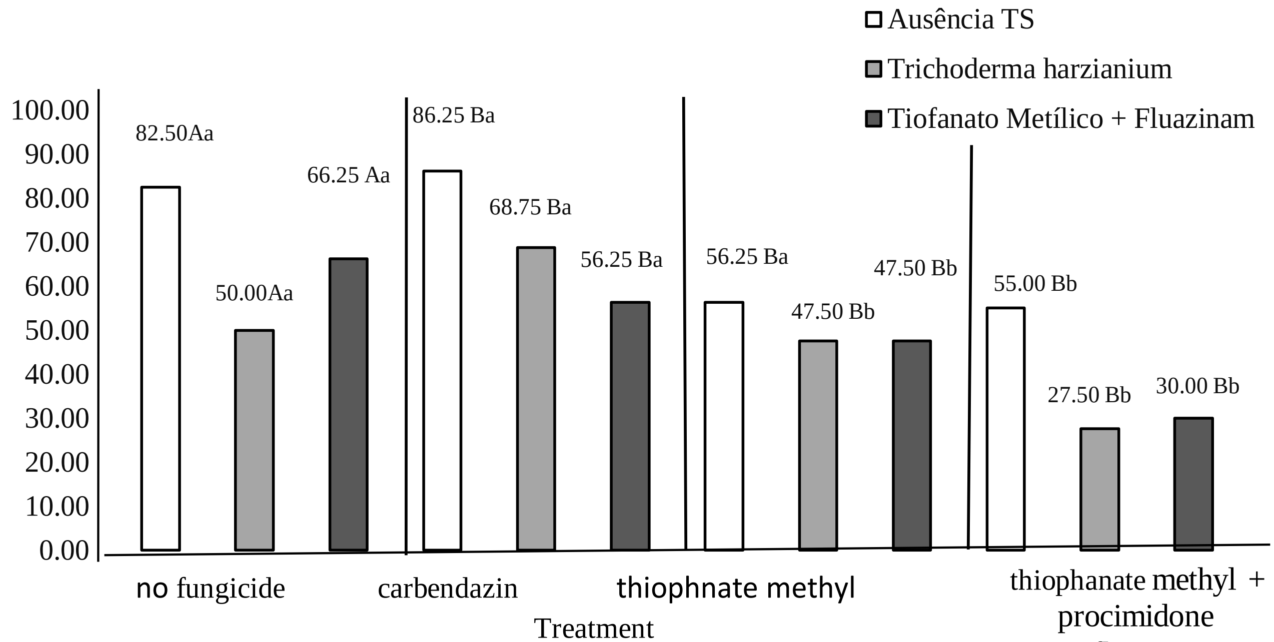

Figure 3. * Averages followed by the same letter do not differ for seed treatment and fungicide application, at level (α = 0,05) of significance. Severity of white mold (*S. sclerotiorum*) in R7, according to different seed treatment and fungicides applied on the aerial part and seed treatment on the soybean crop.

On studying the incidence of white mold on the soybean crop, Henneberg et al. (2012) used soybean seeds artificially and naturally contaminated during three crops. They concluded that the methods being currently used (filter paper, paper roll and medium Neon-S) for the detection of white mold had not been capable of efficiently exposing the pathogen on naturally infected soybean seeds, highlighting the importance of seed treatment on the crop. On the fourth severity assessment, at the R7 stage, there was a difference between treatments, being that the treatments with thiophanatemethyl + fluazinam and *Trichodermaharzianum* and fungicide applications on the aerial part thiophanatemethyl, procimidone and fluazinam presented greater efficiency in disease control (Figure 3). On testing the fungicide efficiency in the control of white mold on the area infested by *S. sclerotiorum,* Vieira et al. (2001) determined that fluazinam was the fungicide which presented the best results for controlling the disease, reducing its severity and the number of *S. sclerotiorum* produced per plot, corroborating the results below Figure 3.

As for the income components, thousand grain weight and productivity, the treatments which presented the lowest levels of severity at the R7 stage, consequently presented the highest levels on the thousand grain weight and productivity (Table 2). The seeds treatment did not provide significant levels for the thousand grain weight and productivity, however, the fungicide applications differed from each other, the superior ones being the treatments with 3 applications of thiophanate methyl and applications of thiophanate methyl, procimidone and fluazinam.

Similarly, Meyer et al. (2013) note better levels of *S. sclerotiorum* control in the soybean crop with applications ranging from two to four sprayings at 10-day intervals, starting at the R1 stage, with the use of fluazinam (chemical group of fenilpiridinilamin) with procimidone, obtained 67 to 85% of control. Besides this result, another tested result was fluazinam + thiophanate methyl, with 76% of *S. sclerotiorum*. However, according to the authors, the plots which carbendazim applications presented satisfactory levels. On the plots of the experiment, at the moment of harvesting the blocks with absence of seed treatment presented susceptibility of the crop towards the *S. sclerotiorum* (Figure 4). As for the economic analysis, despite the treatments which showed the best results in the control of *S. sclerotiorum* were the treatments with the highest costs, the net income revealed that there were increases in the profitability of the crop, emphasizing the potential of the white mold disease as a cause of losses to the soybean crop. This fact is alarming given the fact that there are estimates that 23% of the area cultivated with soybean in Brazil deals with the presence of *S. sclerotiorum*, which represents 6.8 million hectares (Meyer et al., 2014). Especially, it was evident that the use of fungicides for seed treatment and together with aerial applications reduces the severity of diseases, providing greater economic return, even with the cost of fungicides.

Table 2. Income components and net income due to seed treatments and fungicides applied on the aerial part of soybean crop.

Treatments	Income Components		Economic analysis	
	Thousand grain weight (g)	Productivity (kg ha^{-1})	Cost ha^{-1} (US$)	Net income ha^{-1} (US$)
00	138.07^{Ab}	3273.82^{Ab}	0.00	1106.69
10	152.25^{Ab}	3435.77^{Ab}	13.82	1147.62
20	153.36^{Ab}	3351.54^{Ab}	7.00	1125.96
01	143.10^{Ab}	3486.92^{Ab}	34.65	114408
11	141.17^{Ab}	3334.07^{Ab}	48.47	1078.59
21	158.37^{Ab}	2848.23^{Ab}	48.47	914.35
02	155.63^{Aab}	4273.46^{Aa}	24.45	1420.16
12	163.61^{Aab}	4128.08^{Aa}	38.27	1357.20
22	152.13^{Aab}	4086.54^{Aa}	31.45	1349.97
03	156.89^{Aa}	4298.85^{Aa}	89.11	1364.08
13	162.43^{Aa}	4241.15^{Aa}	10293	1330.76
23	166.98^{Aa}	4354.62^{Aa}	96.11	1375.94
Average	153.66	3759.42		
C.V., %	8.11	15.83		

* Averages followed by the same letter do not differ for seed treatment and fungicide application, at level (α = 0,05) of significance. T0-0: absence of seed treatment and absence of application of fungicide on the aerial part. T0-1: absence of seed treatment and aerial application of carbendazim. T0-2: absence of seed treatment and aerial application of thiophanate methyl. T0-3: absence of seed treatment and aerial application of thiophanate methyl, procymidone and fluazinam. T1-0: thiophanate methyl+fluazinam in seed treatment and absence of fungicide application on aerial part. T1-1: thiophanate methyl+fluazinam in seed treatment and aerial application of carbendazim. T1-2: thiophanate methyl+fluazinam in seed treatment and aerial application of thiophanate methyl. T1-3: thiophanate methyl+fluazinamin seed treatment and aerial application of thiophanate methyl, procimidone and fluazinam. T2-0: *Trichodermaharzianum* in seed treatment and absence of fungicide application on aerial part. T2-1: *Trichodermaharzianum* in seed treatment and aerial appication of carbendazim. T2-2: *Trichodermaharzianum* in seed treatment and aerial application of thiophanate methyl. T2-3: *Trichodermaharzianum* in seed treatment and aerial application of thiophanate methyl, procimidone and fluazinam.

Figure 4. Plot with seed treatment with *Trichoderma harzianum,* (left) and plot with no seed treatment (right) with incidence of white mold (*S. sclerotiorum*) at the moment of harvesting, in soybean crop.

Conclusion

The seeds treatments with thiophanate methyl+fluazinam and with *Trichoderma harzianum* and the applications of thiophanate methyl, procimidone and fluazinam on the aerial part stood out in the control of the white mold disease. The fluazinam fungicide resulted in the lowest values of severity of the disease and number of *Sclerotinia sclerotiorum*. Despite the high cost of fungicides, the income is higher when there is a control of the white mold disease. The efficiency of the control of the white mold is greater when there is an association between the seed treatment and the fungicide application on the aerial part.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Authors acknowledge the coordinators of improvement of higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq) and Araucaria Foundation for Scientific and Technological Development of Paraná (Araucaria Foundation) for their support.

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academicJournals

Vol. 11(31), pp. 2841-2848, 4 August, 2016
DOI: 10.5897/AJAR2016.11106
Article Number: D1C9F4B59809
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Growth and production of common bean in direct seeding under irrigated deficit condition

José Joaquim Carvalho[1], João Carlos Cury Saad[2], Marconi Batista Teixeira[3], José Alves Júnior[4], Melissa Selaysim Di Campos[4], Ana Carolina Oliveira Horschutz[5] and Fernando Nobre Cunha[5]*

[1]UNESP, Botucatu – SP, Brazil.
[2]Depto. de Engenharia Rural, Faculdade de Ciências Agronômicas, UNESP, Botucatu – SP, Brazil.
[3]IF Goiano - Câmpus Rio Verde, Zona Rural, Km 01, Caixa Postal 66, Rio Verde (GO), Brazil.
[4]Escola de Agronomia, Universidade Federal de Goiás - UFG, Goiânia-GO, Campus 2, Rod. Goiânia Nova Veneza, Km 0, caixa postal 131, Brazil.
[5]Ciências Agrárias – Agronomia. IFGoiano – Câmpus Rio Verde, Zona Rural, Km 01, Caixa Postal 66, Rio Verde (GO), Brazil.

Received 8 April, 2016; Accepted 2 June, 2016

Plant growth is one of the most sensitive physiological processes to water deficiency. The objective of this study was to evaluate different levels of water replacement combined in two periods of the bean cycle, cv. IAC Alvorada, with respect to the biometric parameters. The study was carried out for two years (2010 and 2011) in a Red Latosol, distroferric, of clay texture, in Botucatu, SP. The experimental design was a randomized block in factorial 4×4, with four replications. The treatments consisted of four water replacement levels of 40, 60, 80 and 100% of ETc, applied in two phases in crop cycle, with phase I - beginning after emergence to flowering, and phase II from flowering to physiological grain maturity. The treatment with 100% ETc consisted of increasing the soil water content at field capacity, with a deficit in percentage for the other treatments. The variables evaluated were: Leaf area index, number of nodes, and plant height. Reducing the water applied in the vegetative or reproductive phases significantly affected leaf area index and plant height. Plant height was the most sensitive component to water deficit. It was concluded that different combinations of irrigation regimes provided different responses in the development parameters of common bean.

Key words: *Phaseolus vulgaris* L., growth analysis, yield components, irrigation.

INTRODUCTION

Brazil is the largest producer and consumer of common bean *Phaseolus vulgaris* L., and the main producers belong to the states of Paraná, Minas Gerais, Mato Grosso, Sao Paulo, Goiás and Bahia, which account for

*Corresponding author. E-mail: fernandopanison@hotmail.com. Tel: (55)4932899294.

more than 67% of national production. Conab (2014) show that 2.832 million tons year^{-1} were produced in the 2012/2013 harvest in a cultivated area of 3.111 million hectares, which implies an average productivity of 910 kg ha^{-1}, considered low. However, productivity up to 3 t ha^{-1} can be achieved at irrigated crops and high technological levels (Lopes, 2011). Of the total of 3111.0 ha planted in the 2012/2013 harvest, 558.3 ha were irrigated, accounting for 28.8% of production (Conab, 2014).

Winter cultivation enables five times higher yields than conventional seasons. Silveira et al. (2001) reported that spray-irrigated beans are economically viable, with rates of return higher than 70%. Water deficit reduces the size of leaves and branches (Taiz and Zieger, 2009). In the vegetative phase (from V2 to V4), it reduces the size and plant development with indirect effect on grain yield. Water stress from pre-flowering to flowering shortens ripening period and prolongs the bean cycle (Oliveira and Kluthcouski, 2009). At flowering, it may reduce plant height and the number of seeds per pod (Silva and Ribeiro, 2009). During the pod formation (R7), water deficit causes ovules abortion, producing empty pods, and in the pod filling stage (R8), it causes abortion of young pods and production of empty pods.

The withholding irrigation on grain filling phases reduces grain yield, and the number of grains per pod (Miorini et al., 2011). At physiological maturity (R9), it reduces the grain mass (Oliveira and Kluthcouski, 2009). The reproductive phase is the phase of higher water demand and the most sensitive to water deficit.

Guimarães et al. (2011) found yields of 863 and 2084 kg ha^{-1} for growing conditions with and without water deficit, respectively, for two years, with a 58.6% yield reduction due to water deficit.

For proper irrigation management, two intrinsic aspects should be considered. The first is the natural conditions of water supplier due to the high cost of capture and distribution. The second is based on crop response to the water applied. In addition to compromising the production costs, excessive irrigation is also harmful because it reduces crop yield. On the other hand, insufficient irrigation exposes the crop to water stress conditions, reducing its productive potential.

Bean irrigation allows significant productivity gains, while high water deficit or excess water due to rainfall at harvest time causes losses (Aguiar et al., 2008).

This study aimed to evaluate different levels of water replacement combined in two periods of bean cv. IAC Alvorada cycle, in winter cultivation, in the first and second year of direct seeding.

MATERIALS AND METHODS

The experiment was carried out in two consecutive years (2010 and 2011), in direct seeding system during winter (April-September), in an experimental area of Lageado Farm, Botucatu, center west region of São Paulo (22º51 'south latitude, 48º26' west longitude) and altitude of 786 m. According to Cepagri (2010), the climate is classified as Cwa by Koeppen classification, characterized as warm temperate mesothermal, with rains in summer and dry in winter.

The soil of the area is classified as Red Latosol (LVdf), distroferric, and of clay texture (Embrapa, 2006). The chemical and physical characteristics were evaluated for four months prior to the beginning of the experiment in the field, obtaining twenty-four trenches. Soil samples were collected in layers from 0 to 10, and 10 to 20 cm deep.

The chemical analyses of soil were performed as described by Raji et al. (2001), and the physical analyses were carried out according to Embrapa (2009). The area was fallow, on braquiaria residue, and corn planting was carried out before the experiment in order to raise the straw content and organic matter of the soil.

The chemical characteristics of the soil at 0-0 to 0.2 m depth prior to the experiment were: pH 4.7 in $CaCl_2$; 21.0 g/dm^3 organic matter; 4.7 mg/dm^3 P_{resin}; 1.7; 13; 7; 30; and 1 mmol$_c$/dm^3 K, Ca, Mg, H + Al, and Al, respectively, and 41.5% base saturation (V%), 0.16; 11.55; 38.5; 13.85; 1.15 mg/dm^3 B, Cu, Fe, Mn and Zinc, respectively. In the second year prior to the experiments, the same layer of 0-0 to 0.2 m exhibited pH 4.85 in $CaCl_2$; 24.0 g/dm^3 organic matter; 24 mg/dm^3 P_{resin}; 1.9; 29; 14; 40 and 1 mmol$_c$/dm^3 K, Ca, Mg, H + Al, and Al, respectively, and 51% base saturation (V%); 0.28; 11.9; 43.5; 16.5; 1.2 mg/dm^3 B, Cu, Fe, Mn and Zinc, respectively. The soil presented clay texture, according to the particle size analysis (423.1, 444.7, and 132.1 g kg^{-1} sand, silt and clay, respectively).

In the first and the second growing seasons, the bulk density varied from 1.35 to 1.38 g cm^{-3} and 1.39 to 1.41 g cm^{-3} for the layers of 0 to 15 cm and 15 to 30 cm depth, respectively. Liming was performed before sowing in order to raise the base saturation (V%) to 70%, adequate to the bean crop. Lime was manually distributed on the soil surface.

Direct sowing of cv. IAC Alvorada was held on 04/09/2010 and 05/10/2011, respectively, with 0.45 cm spacing between lines, and 13 seeds per meter to obtain a final density of 200000 to 240000 plants ha^{-1}. A seeder exact model air JM 2980 PD Jumil was used, following the ground contour.

Fertilization was based on the soil chemical composition, in which 321 and 145 kg ha^{-1} of fertilizer was added at concentrations of 8-28-16 + zinc, and 70 kg of N applied in coverage and divided into two applications, for an expected productivity from 2.5 to 3.5 t. Cultural and phytosanitary treatments were performed according to the recommended for bean crop, when necessary.

The experimental design was a randomized complete block design with four replications in a factorial 4 × 4. The factors used were 4 levels of water replacement, 100, 80, 60 and 40% of crop evapotranspiration (ETc) applied in two phases during the cycle (vegetative - I, reproductive -II). Similar levels were applied in both phase I and phase II.

The treatments consisted of water replacement combinations in two application times. Each plot had 7.2 m^2, and 4 m x 1.8 m, consisting of four bean lines, with three lateral irrigation lines each. Spacing between blocks was 2 m, and 1.5 m between plots. To evaluate the experiment, external lines in each plot were considered as surround, plus one meter by adding the two ends of each plot.

The differentiation between treatments and the drip irrigation began the ninth day after sowing. Previously different irrigation treatments were carried out, using the irrigation sprinkler system.

The side lines, composed of dripper hose with wall thickness of 625 µm, and 20 cm spaced emitters, with a flow rate of 7.5 L/h/m at a pressure of 100 kPa, were distributed at a spacing of 0.45 m between the bean lines, forming a continuous wet track in the floor area.

Soil water storage capacity was 18.9 mm for an effective soil depth of 30 cm. Under the experimental conditions, a period of 1.15 h was required to reach the irrigation field capacity, with 90%

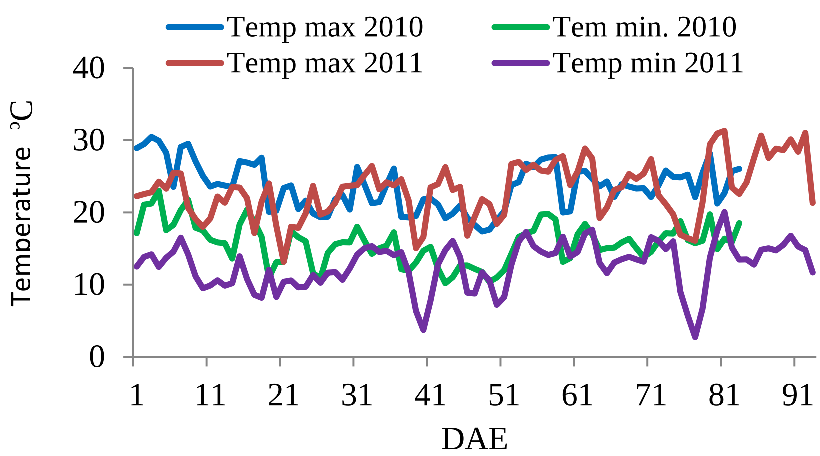

Figure 1. Variation of maximum (Tmax) and minimum (Tmin) temperatures during the bean cycle, Botucatu-SP, 2010 and 2011.

efficiency and effective depth of bean roots of 0.3 m, for the treatment with 100% ETc in both stages studied.

Water management was based on the reference evapotranspiration (ETo) from the evaporation of a Class A tank, located at 150 m away from the experimental site. Data were collected daily and corrected by the correction coefficient (Kp) as reported by Allen et al. (1998), Equations 1 and 2.

$$ETo_{TCA} = Kp \times ECA \quad (1)$$

Where:

$$Kp = 0.108 - 0.0286U + 0.0422Ln\,(F) + 0.1134Ln\,(F) - 0.0006331[Ln(F)]^2\,Ln\,(H) \quad (2)$$

F = Distance from the border area (10 m); U -= wind speed at 2 m height (km d^{-1}); RH = daily relative humidity (%) Kc values were 0.4, 1.15 and 0.35 for initial, middle and end, respectively, with a maximum crop height of 0.4 m, according to Allen et al. (1998).

The treatment T_{16} was kept as reference without water restriction, both in the initial phase (phase - I) as the final phase (phase - II). For the other treatments, water restriction was performed in one of the phases (I and II) after germination. Phase I began in the V2 stage (22 DAE) to flowering (40 DAE), with an average period of 18 days. Phase II began in flowering to physiological grain maturity (40-60 DAE), with an average period of 20 days.

In the field, irrigation was controlled as a function of time by the flow emitters of each plot (ratio between the discharge portion and plot area). Irrigation was performed daily, and a register for each treatment was installed, and a timer measured the daily irrigation time for the reference plot (100%). Then, the time corresponding to the 80 60 and 40% ETc relative to the phases I and II was determined.

At flowering, three plants were sampled at random from the working area of each plot, in order to estimate the effect of water deficit on plant height characteristics (AP), number of nodes on the main stem (NN), and leaf area index (LAI). Subsequently, the (NN) in the main stem was estimated, after the removal of plants in the field. Leaf area (cm^2) was estimated by weighing method (Benincasa, 2003).

The results were subjected to analysis of variance by the F test, and the means of treatments were compared by Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The minimum and maximum air temperature during the phenological cycle of the bean for the years 2010 and 2011 are shown in Figure 1. In the first cycle, in 2010, the minimum temperature was not less than 15°C in both phases I and II. In general, the values are within the range recommended by Silva and Ribeiro (2009), for the bean crop, who found ideal values of minimum, mean and maximum air temperature of 12, 21 and 29°C, respectively. In the second cycle, in 2011, mean values of minimum temperatures below 15°C were observed. In the vegetative phase, the crop was for about 20 days with minimum temperature close to 11°C. These temperature variations occurred during flowering and grain filling phases, interfering with the crop cycle.

Table 1 show the water applied per treatment for the first and second years (2010 and 2011). For the treatment 16, with 100% ETc, the total water applied was 191.22 mm in 2010 and 218 mm in phases I and II, in 2011. It has been found that water applied in Phase I and II was higher in the agricultural year of 2011 when compared to 2010. The effective rainfall during Phases I and II corresponded to 5.26 and 9.12 mm for the year 2010, totaling 14.38 mm. In 2011, in the second experiment, in Phases I and II, the effective rainfall was 5.19 and 9.64 mm, respectively, totaling 14.83 mm. Rainfall in the first experiment took place during the vegetative and late flowering phase. In contrast, in the second experiment, the rainfall concentrated mainly in the vegetative phase, on the third trifoliate, and at the end of the grain filling in the reproductive stage.

In the first year, the bean harvest occurred in July, while the one corresponding to the second year occurred in September. The cumulative number of day-degrees for each treatment is shown in Table 2 for the first and second years (2010 and 2011), respectively.

Table 1. Irrigation water and effective rainfall (mm), corresponding to phases I and II during the bean cycle in the years 2010 and 2011.

Treatment	Water levels in mm in phases I, II, and total water level (I + II + rainfall)[1]							
	Phase I I (2010)	Phase II I (2010)	I + II I (2010)	I + II + Pe I (2010)	Phase I I (2010)	Phase II I (2010)	I + II I (2010)	I + II + Pe I (2010)
	2010				2011			
T1	26.42	44.31	70.73	85.11	32.02	49.24	81.23	96.06
T2	26.42	66.47	92.89	107.27	32.02	73.86	105.88	120.71
T3	26.42	88.63	115.05	129.43	32.02	98.40	130.42	145.25
T4	26.42	110.79	137.21	151.59	32.02	123.10	155.12	169.95
T5	39.63	44.31	83.94	98.32	48.08	49.24	97.32	112.15
T6	39.63	66.47	106.10	120.48	48.08	73.86	121.94	136.77
T7	39.63	88.63	128.26	142.64	48.08	98.40	146.48	161.31
T8	39.63	110.79	150.42	164.80	48.08	123.10	171.18	186.01
T9	52.84	44.31	97.15	111.53	64.05	49.24	113.29	128.12
T10	52.84	66.47	119.31	133.69	64.05	73.86	137.91	152.74
T11	52.84	88.63	141.47	155.85	64.05	98.40	162.45	177.28
T12	52.84	110.79	163.63	178.01	64.05	123.10	187.15	201.98
T13	66.05	44.31	110.36	124.74	80.06	49.24	129.30	144.13
T14	66.05	66.47	132.52	146.90	80.06	73.86	153.92	168.75
T15	66.05	88.63	154.68	169.06	80.06	90.40	170.46	185.29
T16	66.05	110.79	176.84	191.22	80.06	123.10	203.16	217.99

[1]Pe, Effective rainfall: 14.38 mm in 2010, and 14. 83 mm in 2011.

Table 2. Year of cultivation, cycle time, cumulative number of day-degrees, grain yield and reduced bean yield, Botucatu - SP, 2010 and 2011.

Treatment	Cycle/days	day-degrees	Cycle/days	day-degrees
	2010		2011	
T1	93	882.20	104	795.65
T2	93	882.20	105	798.92
T3	95	907.00	106	800.68
T4	95	907.00	107	806.35
T5	93	882.20	104	795.65
T6	93	882.20	105	798.92
T7	95	907.00	106	800.68
T8	95	907.00	107	806.35
T9	93	882.20	104	795.65
T10	93	882.20	105	798.92
T11	95	907.00	106	800.68
T12	95	907.00	107	806.35
T13	93	882.20	104	795.65
T14	93	882.20	105	798.92
T15	95	907.00	106	800.68
T16	95	907.00	107	806.35

In the experiment in 2010, the cycle lasted 93 days for the treatments subjected to water replacement of 40 and 60% of ETc in Phase II, regardless of Phase I. In the Experiment 2, there was an increase in the number of days, ranging from 104 to 107 days, due to the low air temperature and accumulation of day-degrees during the bean development in the year 2011.

In the second experiment, the combinations of water

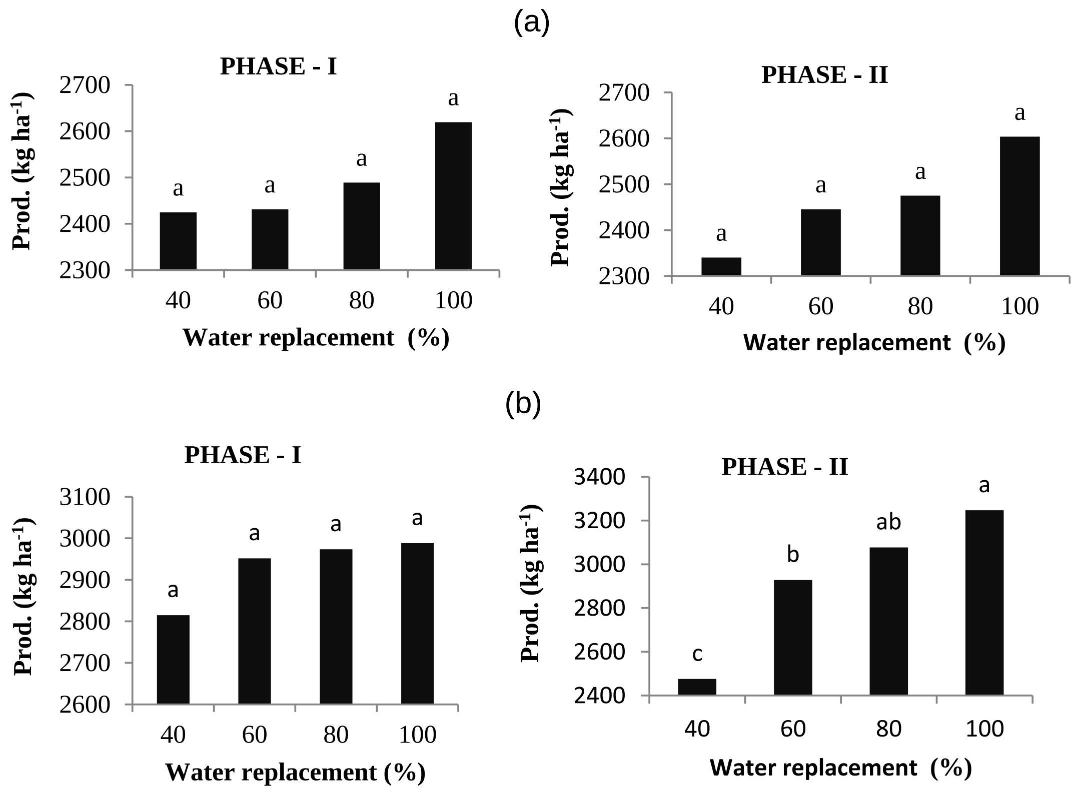

Figure 2. Average productivity in different irrigation combinations on bean crop cycle, 2010 (a) and 2011 (b).

Table 3. F Values and significance level obtained in the analysis of variance in bean crop under different irrigation combinations.

Source of variation[1]	I	II	IxII	Treat/test	Years	CV (%)
Number of nodes per plant						
Flowering 2010	9.22*	2.04*	2.04*	6.86*	4.45^{ns}	4.70
Flowering 2011	4.32*	6.53*	4.44*	4.16^{ns}	4.45^{ns}	5.48
Plant height						
Flowering 2010	141.79*	87.86*	33.50*	94.88*	6.11^{ns}	1.93
Flowering 2011	34.16*	27.00*	0.82*	24.74*	6.11^{ns}	6.17
Leaf area index						
Flowering 2010	0.43^{ns}	2.13^{ns}	0.98^{ns}	1.26^{ns}	0.77^{ns}	23.35
Flowering 2011	5.32*	5.12*	0.77^{ns}	2.87*	0.77^{ns}	22.20
Seed yield						
Flowering 2010	1.39^{ns}	1.51^{ns}	0.47^{ns}	1.98^{ns}	3.01*	14.28
Flowering 2011	0.98^{ns}	17.06*	1.08^{ns}	7.77*	3.01*	10.93

[1]* and ^{ns} are significant at 5% and not significant, respectively by F test. The letter (I) corresponds to the factor first phase, while (II) corresponds to the second phase. Thus 11, corresponds to 40% ETc; I2, 60%; I3, 80%, and I4, 100%. In turn, B1 corresponds to 40% ETc at phase II; II2, 60%; II3, 80% and II4, 100%, respectively.

deficit in Phases I and II reflected in seed yield, which was not observed in the first experiment. In general, it was found that lower water levels in Phase II, combined with Phase I, reflected in lower seed yield when compared to the treatment without water restriction in all phases (Figure 2). Cunha et al. (2015) verified an average production of dry beans was 1339.15 kg ha^{-1}, with a water consumption of 215 L for plant.

Table 3 summarizes the analysis of variance for the number of nodes, plant height, leaf area index, and yield

Table 4. Number of nodes per plant in different combinations of irrigation water on bean crop cycle.

2010		Number of nods per plant[1]			
Phases (I*II)		**Phase - II**			
	(%)	**40**	**60**	**80**	**100**
Phase - I	40	10.58^{aA}	10.25^{abA}	10.17^{bA}	11.08^{bA}
	60	10.75^{aA}	10.67^{abA}	10.66^{abA}	11.50^{abA}
	80	10.50^{AB}	9.92^{bB}	11.58^{aA}	11.33^{abAB}
	100	11.08^{aB}	11.08^{aB}	11.41^{aAB}	12.25^{aA}
2011		**Number of nods per plant**			
Phases (I*II)		**Phase - II**			
	(%)	40	60	80	100
Phase - I	40	8.58^{bB}	9.75^{aA}	9.75^{aA}	9.83^{bA}
	60	10.42^{aA}	9.92^{aAB}	9.25^{aB}	8.91^{abB}
	80	10.08^{aAB}	10.67^{aA}	9.41^{aB}	10.33^{aAB}
	100	9.75^{aAB}	10.50^{aA}	9.16^{aB}	9.50^{abAB}

[1]Means followed by different lowercase letters in the columns, or uppercase letters in the lines differ by Tukey's test (P≤0.05). Periods 2010 and 2011.

Table 5. Plant height in different irrigation combinations on bean crop cycle.

2010		Plant height[1] (cm)			
Phases (a*b)		**Phase - II**			
	(%)	**40**	**60**	**80**	**100**
Phase - I	40	41.58^{Bb}	43.70^{Ac}	45.05^{Ab}	45.25^{Ac}
	60	41.58^{Cb}	47.60^{BAa}	49.08^{Aa}	46.54^{Bbc}
	80	42.33^{Bb}	47.66^{Aa}	46.62^{Ab}	48.13^{Ab}
	100	50.44^{Ba}	45.45^{bC}	49.75^{aB}	55.62^{aA}
2011		**Plant height (cm)**			
Phases (a*b)		**Phase - II**			
	(%)	**40**	**60**	**80**	**100**
Phase - I	40	36.15^{Bc}	39.08^{ABb}	42.47^{Ab}	43.77^{Ac}
	60	38.52^{Cbc}	43.63^{CBb}	49.67^{Aa}	46.80^{BAcb}
	80	42.32^{Cba}	44.33^{CBb}	49.18^{BAa}	49.90^{Aba}
	100	46.25^{Ba}	49.81^{ABa}	51.20^{ABa}	53.69^{Aa}

[1]Means followed by different lowercase letters in the columns, or uppercase letters in the lines differ by Tukey's test (P≤0.05). Periods 2010 and 2011.

for the first and second experiments. The interaction treatments x irrigation 40, 60, 80 and 100% of ETc presented significance for the number of nodes and plant height in the first and second years, but it was not significant for leaf area index and yield, where the collection was carried out during flowering.

The results indicated that the water deficit observed during Phases I and II decreased the number of nodes per plant (N) (Table 4), compared to the treatment without water restriction in all phases of the bean cycle.
In the first cycle, the lowest NN values were obtained with the largest reductions in water applied in Phases I and II, which was also observed in the second year. These values are similar to those obtained by Moraes et al. (2010) in a study conducted in a greenhouse in Alegre, ES, with and without water deficit applied in the pre-flowering stages and formation of floral buds, with NN values of 12.85 and 14.84, respectively. Aguiar et al. (2008) in a study with and without water stress, found a small reduction of NN per plant, ranging from 1.8 to 5.3%, except for the lineage LP 99-79.

The results presented in Table 5 indicated that water deficit during Phases I and II led to a reduction in plant height. The water reduction in Phases I and II resulted in the lowest plant height when compared to the treatment without water restriction in all phases. Moraes et al. (2010) investigated water deficit interrupted for 15 days during both the pre-flowering and formation of flower

Table 6. Leaf area index in different irrigation combinations on bean crop cycle.

Water (mm)	Leaf area index ($cm^2\ cm^{-2}$)	
	2010	2011
	Phase - I (emergence to full flowering)	
40%	5.80^a	4.95^b
60%	6.09^a	4.93^b
80%	6.30^a	6.28^a
100%	6.30^a	5.98^{ab}
	Phase - II (flowering to physiological maturity)	
40%	6.08^a	4.79^b
60%	5.57^a	5.18^b
80%	6.01^a	5.76^{ab}
100%	6.83^a	6.42^a

Table 7. Comparison among number of nodes, plant height and leaf area index during flowering.

Treatment	Nodes per plant		Plant height (cm)		LAI ($cm^2\ cm^{-2}$)	
	Evaluation during flowering[1]					
	2010	2011	2010	2011	2010	2011
T01	10.58^A	8.58^B	41.58^A	36.15^B	5.95^A	3.95^B
T02	10.25^A	9.75^A	43.65^A	39.08^B	5.74^A	4.46^A
T03	10.16^A	9.75^A	45.05^A	42.47^A	6.12^A	5.55^A
T04	11.08^A	9.83^B	45.24^A	43.77^A	5.40^A	5.20^A
T05	10.75^A	10.41^A	41.57^A	38.52^B	5.92^A	5.00^A
T06	10.66^A	9.91^B	47.59^A	43.63^B	5.73^A	4.64^A
T07	10.66^A	9.25^B	49.08^A	49.67^A	5.18^A	5.52^A
T08	11.50^A	8.91^B	46.54^A	46.80^A	7.53^A	5.58^B
T09	10.50^A	10.08^A	42.33^A	42.32^A	6.90^A	5.92^A
T10	9.91^B	10.66^A	47.90^A	44.33^B	5.22^A	4.99^A
T11	11.58^A	9.41^B	46.62^A	49.18^A	6.14^A	6.23^A
T12	11.33^A	10.33^B	48.13^A	49.90^A	6.95^A	5.89^A
T13	11.08^A	9.75^B	50.44^A	46.25^B	5.53^A	4.95^A
T14	11.08^A	10.50^A	45.46^B	49.81^A	5.60^A	5.64^A
T15	11.41^A	9.16^B	49.75^A	51.20^A	6.62^A	7.82^A
T16	12.25^A	9.49^B	55.62^A	53.69^A	7.44^A	7.27^A

[1]Means followed by capital letters on the lines differ by Tukey's test ($p \leq 0.05$).

buds phases, and did not find differences on plant height under water deficit or not, obtaining values of 100.71 and 105.08 cm respectively, in a study conducted in the greenhouse. Aguiar et al. (2008) in a study performed with and without water stress found that the common bean genotypes of the carioca group showed an increase in plant height under water stress conditions. This result may be a result of shading the plants when subjected to these conditions.

The leaf area index (LAI) of IAC Alvorada subjected to water deficit in Phases I and II, in the first and second experiments, was influenced by the water applied, resulting in lower means when compared to the treatment without water deficit (Table 6). Aguiar et al. (2008) studied common beans, and found significant lower leaf area index for the carioca group when the genotypes were subjected to water stress. Sousa and Lima (2010) evaluated the effect of water deficit in the LAI characteristic of common bean, and found values of 0.88, 0.72, 0.35, 0.32 and 0.69 m^2m^{-2} with deficit irrigation in vegetative phases, pre-flowering full flowering, grain filling and ripening, respectively.

The NN of the plants from the first and second years was significant for the treatments T_1, T_4, T_6, T_7, T_8, T_{11}, T_{12}, T_{13}, T_{15} and T_{16} (Table 7), and the averages of NN in the first year were higher when compared to the second year, except for the T_{10}.

The average plant height between the first and second cycles was significant for the treatments T_1, T_2, T_5, T_6, T_{10}, T_{13}, T_{14}, T_0, which was higher in the first year when compared to the second year, except for the T_{14}, probably due to higher rainfall, reducing water scarcity. A significant difference only between T_1 and T_8 (Table 7) was observed between the LAI of the first and second experiments. Jauer et al. (2003) observed that the leaf area index reached the maximum closed to the beginning of grain filling period.

Conclusions

The characteristics, number of nodes per plant, plant height, leaf area index, and seed yield were sensitive to water stress. The largest water reductions of the bean cycle in Phase I (early after emergence to flowering) combined with Phase II (from full flowering to physiological maturity) resulted in greater effects on plant height and leaf area index. The higher the water deficit in the reproductive phase (Phase II beginning of full flowering to physiological grain maturity), the smaller the grain yield in kg ha^{-1} is.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES), National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq), Science and Technology Ministry (MCT), FAPEG and Instituto Federal Goiano – Câmpus Rio Verde, for their financial support.

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academicJournals

Vol. 11(31), pp. 2849-2859, 4 August, 2016
DOI: 10.5897/AJAR2016.11011
Article Number: F938E5E59811
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Study of the genetic variability, correlation and importance of phenotypic characteristics in cactus pear (*Opuntia* and *Nopalea*)

Francisco Abel Lemos Alves[1]*, Albericio Pereira de Andrade[2], Riselane de Lucena Alcântara Bruno[3], Djalma Cordeiro dos Santos[1] and Vanda Lucia Arcanjo Pereira[1]

[1]Agronomic Institute of Pernambuco, Av. General San Martin, 1371, Bongi, 50761-000, Recife-PE, Brazil.
[2]Federal University of Paraíba, Centre of Agricultural Sciences, Campus II, 58397-000, Areia-PB, Brazil.

Received 17 March, 2016; Accepted 2 June, 2016

The cactus pear is a widely cultivated plant in the Northeast of Brazil, contributing significantly to the feeding of livestock, especially in times of drought. Because of the high variation between phenotypic varieties grown in this region, it is essential to study the genetic diversity. The objectives of this study were to characterize the genetic diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 19 morphological and behavioral characteristics, and to determine the phenotypic correlation and importance of these characteristics to the variability among genotypes, using multivariate analysis techniques. The study was conducted at the experimental station of Agronomic Institute of Pernambuco (IPA), located in city of Arcoverde, State of Pernambuco, Brazil using randomized block design with three replications. The materials IPA-100003, IPA-200016, IPA-200008, IPA-100004, IPA-200021, IPA-200205 and IPA-200149 were evaluated for 19 quantitative characteristics of the plants. The collected data were analyzed by analysis of variance by F test, and the means grouped by the Scott-Knott test (p<0.05). The broad-sense heritability and phenotypic correlation characteristics were estimated. The genetic diversity was estimated by multivariate methods (unweighted pair group method with arithmetic mean-UPGMA, Tocher, principal component and canonical variables). Analyses of variance and genetic diversity revealed significant differences among genotypes, with the possible formation of two, three or four genetically distinct groups. The heritability values ranged from 79.6 to 97.0% for all 19 quantitative characteristics. The water content and cladode fresh matter are the characteristics that contributed most to the genetic divergence among the materials. Moreover, these characteristics are significantly and positively correlated with dry matter, width, length and cladode area. Thus, the genetic variability among the studied varieties of cactus pear and their potential use in breeding programs are confirmed. The uni and multivariate methods used for the genetic divergence differ and gather genotypes in two, three or four groups.

Key words: Brazilian semiarid, forage, genetic distance, grouping, multivariate analysis.

INTRODUCTION

The cactus pear (*Opuntia* spp. and *Nopalea* spp.) is a cactaceae originally from Mexico, which is exploited since the pre-Hispanic period, holding the greatest genetic diversity and one of the largest cultivated areas in the world with over 28.3 million hectares. The current distribution of these plants in the world includes different

Table 1. Cactus pear varieties, genera *Opuntia* and *Nopalea*, used in the study and grown in the state of Pernambuco, Brazil.

Number	Varieties	Species	Common name
1	IPA-100003	*Opuntia fícus indica*	IPA-20
2	IPA-200016	*Opuntia stricta*	Elephant Ear Mexican
3	IPA-200008	*Opuntia atropes*	F-08
4	IPA-100004	*Nopalea cochenillifera*	Little
5	IPA-200021	*Nopalea cochenillifera*	F-21
6	IPA-200205	*Nopalea cochenillifera*	IPA-Sertânia
7	IPA-200149	*Opuntia larreri*	-

environments and a wide range of species, which explains the high genetic variation that originates from the great ecological diversity of the areas where they are native (Barrios and Muñoz-Urías, 2001; López-García et al., 2001).

These plants are used for various purposes: human and animal food, energy production, medicine, cosmetics, chemical, and food industry. It is difficult to find a plant as distributed and exploited, especially in arid and semiarid areas, or as subsistence economy by producers of small animals, or as a culture focused on the industrial market (Barbera et al., 2001).

For their physiological, morphological, and chemical characteristics that enable these plants to tolerate arid and semiarid environments, especially with regard to absorption, recovery and use of water, they have adapted very well in Brazilian northeast. This region is characterized by having a high index of annual evaporation, greater than 2.000 mm, and average rainfall of less than 750 mm, concentrated in a single period of 3 to 5 months; in addition, in some years, the lack of rain is prolonged, resulting in the phenomenon of droughts (Araújo et al., 2005).

The species *Opuntia ficus indica* Mill. and *Nopalea cochenillifera* Salm Dyck. are widely cultivated, especially in the states of Pernambuco, Alagoas, Paraiba, Sergipe, Bahia and Ceará. It is estimated that the area cultivated in Brazil reaches about 550,000 ha. The main use of these plants in this region is based on food support for ruminants, mainly in the dry season, considering that this segment is strongly affected by the lack of forage plants during this period (Santos et al., 2006).

Most of the work involving selection and breeding of cactus pear uses statistical methods of univariate type, since they are focused on the analysis of the variation in a single random variable. However, the selection of plants based on various important variables may be more advantageous, especially when performed on a set of quantitative characteristics (Ferreira et al., 2003).

In the simultaneous handling of several characteristics, the multivariate techniques consider simultaneously a set of random variables, each of which has the same degree of importance. The multivariate techniques for diversity studies are applied from dissimilarity measures among the genotypes, such as the Mahalanobis distance. In addition, grouping analysis brings together individuals with similar characteristics in relation to the observed variables. Among the methods, we highlight the Tocher; the hierarchical, such as the unweighted average linkage (UPGMA), and principal component analysis and canonical variables (Cruz et al., 2012).

The objectives of this study were to characterize the genetic diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 19 morphological and behavioral characteristics, and to determine the phenotypic correlation and importance of these characteristics in the variability among genotypes.

MATERIALS AND METHODS

Location of the experiment

The study was conducted at the Experimental Station Arcoverde, from the Agronomic Institute of Pernambuco (IPA), located in city of Arcoverde, State of Pernambuco, Brazil (8°25'S, 37°05' W), 680.7 m altitude, average annual temperature 22.9±1.7°C, average annual relative humidity 69.6±5.3%, wind speed (annual average 3.9±0.5 m s^{-1}), accumulated evaporation (average 1700.4 mm), average annual accumulated rainfall of 798.1 mm, microregion of the sertão of Moxotó (Inmet, 2015).

Plant material and conducting the experiment

The materials used are listed in Table 1. The cladodes of the clones were planted on April 22 and 23, 2010, spaced 1.0 x 0.5 m; using one cladode per hole. The experimental design was a randomized complete block design represented by seven treatments and three

*Corresponding author. E-mail: abel.alves@ipa.br. Tel: +55 081 31847200.

replications. Each block consisted of three rows planted with eight plants of each variety, totalizing twenty four plants of each treatment. The experimental plot was composed by the middle row using with six plants and covering 3.0 m^2 of area. The soil was fertilized 30 days after planting, with 20 t ha^{-1} of manure spread between the lines. Periodically, cultural practices were carried out in the form of weeding with hoe in all the cultivated area. The measurements in the plant, and collection secondary and tertiary cladodes were held at 8:00 am on 19 February, 2013 (dry season). After the measurements, the cladodes was cleaned, weighed, cut into small pieces (2 to 3 cm in length) and dried in a forced-air oven at 55°C, where it remained for 72 h until reaching constant weight, by which the dry matter (DM) of cladodes was obtained.

Determination of morphological characteristics and production

The evaluated variables were: the width (CW), length (CL), thickness (CT), area (CA), fresh matter (CFM), dry matter (CDM) and water content (H_2O) and total number of cladodes (NC); width (PW), height (PH), total photosynthetic area (TPA), cladode area index (CAI), fresh (FM), and dry mass production (DM) of plants. CW, CL, CT, PW and PH were measured with a caliper and measuring tape. The CFM and CDM were obtained with a precision scale (0.01 g). The H_2O was determined as described by Guimarães and Stone (2008) using the following formula: H_2O = [(CFM – CDM) / CDM] x 100. PH was estimated as described by Sales et al. (2013), using the following formula: CA = CL x CW x 0.632. The TPA was estimated by multiplying CA with NC. The cladode area index (CAI) was estimated according to Sales et al. (2013), by the ratio between TPA and the soil area occupied by the plant. The FM and DM was estimated by the NC multiplied by CFM and CDM.

Determination of behavioral characteristics

The varieties was visually assessed by the characteristics of Desirability (DESIR) - general appearance of the genotype, in which are considered: budding, productive aspect and plant health, infestation by the carmine cochineal (*Dactylopius opuntiae*) (CAR), infestation by cactus scale (*Diaspis echinocacti*) (SCA), disease incidence (DIS), and wilt index (WIL).

We used for DESIR, scores: 1 (high), 2 (average), 3 (low). For CAR, SCA, DIS and WIL, scores: 0 (absence), 1 (low), 2 (average), 3 (high) and 4 (highest). The observations were made in the twenty four plants of the three rows of each variety (Pereira et al., 2014).

Statistical analysis

The data were initially evaluated by analysis of variance (ANOVA), and the means were compared by the Scott and Knott's test at 5% (Scott and Knott, 1974).

The broad-sense heritability was calculated by the estimator: $h^2 = \sigma^2_g / \sigma^2_p \times 100$, where: σ^2_g is the genetic variance and σ^2_p is the phenotypic variance. The genetic variance was calculated by the estimator: σ^2_g = SMTreat – SMRes / J, where SMTreat is the square mean of the treatment, SMRes is the square mean of the residue, and J is the number of replicates. The phenotypic variance was calculated by the estimator: σ^2_p = SMTreat / J. The environmental variance was calculated by the estimator: σ^2_e = SMRes / J. The genetic variation coefficient was calculated by the estimator: (GV) = (σ_g / M) x 100, where M is the average of the characteristic. The environmental variation coefficient was calculated by the estimator: (EV) = (σ_r / M) x 100 (Alves et al., 2006; Rufino et al., 2010; Rêgo et al., 2011).

The genetic diversity among varieties was estimated using a measure of dissimilarities expressed by the Mahalanobis distance (D^2) according to Cruz et al. (2012). Grouping was performed by hierarchical method unweighted pair group method with arithmetic mean (UPGMA), Tocher optimization method (Rao, 1952), principal component and canonical variables analysis methods (Cruz et al., 2012).

The relative importance of characters in relation to genetic diversity was studied according to Singh (1981), canonical variable and principal components analysis (Cruz et al., 2012). The correlations of Pearson among the characteristics were obtained as described by Rêgo et al. (2011), and the probability of 1 and 5% by t-test.

Data analyzes were performed in the statistical software GENES®- Computer Application in Genetics and Statistics (Cruz, 2001) and Assistat® 7.7 (Silva and Azevedo, 2006).

RESULTS AND DISCUSSION

The analysis of variance by F test ($p \leq 0.01$) showed significant differences among cactus pear varieties for width (CW), length (CL), area (CA), dry matter (CDM) of cladodes; number of cladodes (NC), infestation by *D. opuntiae* (CAR), infestation by *D. echinocacti* (SCA), wilt (WIL) of plants and ($p \leq 0.05$) for total photosynthetic area (TPA), cladode area index (CAI) and dry mass (DW) of plants. There were no significant differences among cactus pear varieties for thickness (CT), fresh matter (CFM), water content (H_2O) of the cladodes, width (PW), height (PH), fresh mass (FM), desirability (DESIR), and incidence of diseases (DIS) in the plants (Table 2).

Ferreira et al. (2003) and Neder et al. (2013), studying the genetic diversity of cactus pear (*Opuntia ficus indica* Mill.), reported significant differences among 38 accesses studied in relation to CT, CL, CW, NC, FM, DM, PH and PW. The failure to detect differences among the characteristics CT, CFM, H_2O, PW, PH, FM and DESIR among varieties of cactus pear, is probably related to the time of data collection (dry season) after a large water deficit suffered by plants, approximately 13 months. This may have affected the results of the measurements, since the plants were not in their full turgor, besides, some genotypes lost some cladodes and these could not be accounted for in the measurements. The phenotypic difference among varieties in the rainy season, when the plants are fully turgid is clear (Alves et al., 2013).

The genotypes showed an average variation in CW (8.50 to 22.33 cm), CL (19.90 to 32.33 cm), CT (0.36 to 0.76 cm), CA (107.40 to 434 , 90 cm^2), CFM (59.46 to 401.66 g), CDM (20.20 to 81.06 g), H_2O (39.26 to 320.60 g), NC (12.16 to 79.83 units), PW (65.66 to 114.11 cm), PH (52.33 to 80.33 cm), TPA (2,949.90 to 9,898.06 cm^2), CAI (0.59 to 1.97), FM (2164.58 to 8356.25 g), DM (611.55 to 2293.86 g), DESIR (2.33 to 3.00), CAR (0.00 to 1.66), SCA (0.00 to 2.00), DIS (1.00 to 2.00) and WIL (1.66 to 3.00) (Table 3). These values are consistent with found in the genera *Opuntia* and *Nopalea*. Researchers relate this variation to genotypic variability, plant age, soil, management, crop treatment and environmental factors (Paixão, 2012; Amorim, 2011; Ferreira et al., 2003;

Table 2. Analysis of variance and estimates of the environmental variation coefficient (EV), ratio of genetic (GV) and environmental variation (EV) coefficients, broad-sense heritability (h^2), morphological and behavioral characteristics of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Mean squares												
S.V	D.F	CW	CL	CT	CA	CFM	CDM	H_2O	NC	PW	PH	TPA
Block	2	16.10	44.08	0.02	18493.82	41172.76	1082.57	28904.64	346.30	280.61	228.00	35062515.45
Varieties	6	71.47**	66.54**	0.07ns	42112.81**	47023.10ns	2014.03**	30611.97ns	1609.96**	773.35ns	222.41ns	16513675.79*
Residues	12	2.13	2.96	0.04	1456.89	20190.56	411.08	15291.76	80.30	288.31	149.39	5385859.75
EV (%)	-	9.99	6.67	32.92	15.17	75.92	41.66	89.29	23.30	19.38	18.93	29.52
GV/EV	-	3.30	2.67	0.54	3.05	0.67	1.14	0.58	2.52	0.75	0.40	0.83
h^2 (%)	-	97.02	95.55	46.77	96.54	57.06	79.59	50.05	95.01	62.72	32.83	67.39

Mean squares									
S.V	D.F	CAI	FM	DM	DESIR	CAR	SCA	DIS	WIL
Blocks	2	1.40	26736681.39	1067008.28	0.33	0.05	0.00	0.90	0.76
Varieties	6	0.66*	14134953.97ns	836550.51*	0.22ns	1.19**	1.97**	0.38ns	0.75**
Residues	12	0.22	7906517.59	266899.74	0.22	0.05	0.11	0.40	0.15
EV (%)	-	29.52	52.93	35.24	0.00	91.65	58.33	0.00	18.53
GV/EV	-	0.83	0.5124	0.84	0.00	2.83	2.36	0.00	1.15
h^2 (%)	-	67.39	44.06	68.10	0.00	96.00	94.35	0.00	79.79

CW Cladode width, CL cladode length, CT cladode thickness, CA cladode area, CFM cladode fresh matter, CDM cladode dry matter, H_2O water content of cladode, NC total number of cladodes, PW plant width, PH plant height, TPA total photosynthetic area of the plant, CAI cladode area index, FM fresh matter production of the plant, DM dry matter production of the plant, DESIR Desirability, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), SCA infestation by cactus scale (*Diaspis echinocacti*), DIS Incidence of Diseases and WIL wilt index. *, ** significant at 5% and 1%, respectively or ns not significant, by Fisher's test.

Sales et al., 2003).

The ratio between the coefficient of genetic (GV) and environmental variation (EV), was above one for the characteristics CW, CL, CA, CDM, NC, CAR, SCA, WIL, with heritability values (h^2) between 79.6 to 97.0%, indicating high genetic control among these characteristics. For the other characteristics CT, CFM, H_2O, PW, PH, TPA, CAI, FM, DM, DESIR and DIS, the (GV)/(EV) indices were below one, indicating the dominance of the environment on these characteristics (Table 2).

Neder et al. (2013) report that CW, CL, CT, PW, PH, FM and DM are controlled by genetic factors and that NC is controlled by environmental factors. However, as the researchers reported that the genetic correlations among these characteristics were higher than the phenotypic and environmental, but not significant, it may indicate possibly the effect of the environment on the association with the genetic characteristics (Gonçalves et al., 1996). Paixão (2012) studying variance components and genetic parameters for the variables PH, PW, NC, CW and CL on cactus pear progenies, genera *Opuntia* and *Nopalea*, reported that environmental variance is greater than genotypic variance for these characteristics, indicating that the variability was biased, and that it could be overestimating the genetic variance. In general, the variance caused by the environment is an important source of error, capable of reducing experimental precision (Falconer, 1987; Paixão, 2012). However, those same researchers reported that the estimates of broad-sense heritability coefficients were of high magnitude to PH, CW and CL, demonstrating good genetic control and the possibility of genetic advances (Paixão, 2012).

Given the existence of genetic variability among genotypes, we proceeded to the study of genetic divergence among the materials. For the characteristics in which the analysis of variance was significant, differences were identified ($p \leq 0.05$) by the Scott-Knott's test, and observed the formation of two (CL, CDM, TPA, CAI, CAR and WIL), three (CA and NC) and four (CW and ESC) groups of means (Table 3). The Tocher grouping method gathered the seven varieties into two distinct groups. The group I was represented by genotypes 1, 2, 3, 4, 5 and 6. Group II consists of the genotype 7 (Table 4).

According to the dendrogram obtained by hierarchical clustering method UPGMA, the cactus pear varieties were gathered into three groups, considering the cut of 38% of relative genetic distance, according to the criterion mentioned by Arriel et al. (2006) and Cruz et al. (2012), in which the high-level change points are considered delimiters of the number of genotypes for a certain group. Group I was composed by genotypes 1, 3, 4, 5 and 6; II group by 2; and group III by 7 (Figure 1).

The principal component analysis demonstrated that the use of the first three variables was sufficient to account for almost 85% of the total variation obtained in the seven genotypes (Table 5). Thus, a reasonable description of the genetic diversity of genotypes can be made by these components, since, according to Cruz et

Table 3. Mean of morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Variable	Varieties							C.V
	1	2	3	4	5	6	7	
CW	18.16^b	22.33^a	11.06^c	8.50^d	11.10^c	13.60^c	17.33^b	9.99
CL	32.33^a	30.50^a	21.90^b	19.90^b	25.00^b	28.40^a	22.50^b	6.67
CT	0.70^a	0.60^a	0.50^a	0.43^a	0.36^a	0.76^a	0.70^a	32.92
CA	372.56^a	434.90^a	153.46^c	107.40^c	176.76^c	248.46^b	250.80^b	15.32
CFM	268.33^a	401.66^a	88.33^a	59.46^a	71.66^a	232.23^a	188.33^a	75.93
CDM	65.06^a	81.06^a	25.60^b	20.20^b	21.90^b	73.8^a	53.00^a	41.67
H_2O	309.72^a	333.12^a	245.20^a	197.40^a	228.60^a	215.71^a	257.92^a	25.91
NC	24.66^c	21.66^c	46.83^b	79.83^a	53.16^b	30.83^c	12.16^c	23.31
PW	114.11^a	87.66^a	84.00^a	90.00^a	72.88^a	98.89^a	65.66^a	19.38
PH	80.33^a	68.66^a	52.33^a	61.33^a	63.66^a	65.33^a	60.33^a	18.93
TPA	9157.06^a	9356.16^a	7292.75^a	8579.86^a	9898.06^a	7799.16^a	2949.90^b	29.52
CAI	1.83^a	1.87^a	1.45^a	1.71^a	1.97^a	1.55^a	0.59^b	29.52
FM	6680.00^a	8356.25^a	4279.48^a	4753.83^a	3776.93^a	7180.35^a	2164.58^a	52.92
DM	1628.35^a	1718.91^a	1235.51^a	1611.43^a	1160.95^a	2293.86^a	611.55^a	35.25
DESIR	2.33^a	2.66^a	2.66^a	2.66^a	3.00^a	2.33^a	3.00^a	17.68
CAR	1.66^a	0.00^b	0.00^b	0.00^b	0.00^b	0.00^b	0.00^b	91.65
SCA	0.00^d	2.00^a	0.66^c	0.00^d	0.00^d	1.33^b	0.00^d	58.33
DIS	1.33^a	1.00^a	1.66^a	1.00^a	2.00^a	1.33^a	1.33^a	46.07
WIL	1.66^b	1.66^b	2.33^a	2.33^a	3.00^a	1.66^b	2.00^b	18.53

CW Cladode width, (cm) CL cladode length (cm), CT cladode thickness (cm), CA cladode area (cm^2), CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O cladode water content (g), NC total number of cladodes, PW plant width (cm), PH plant height (cm), TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, FM fresh matter production of the plant (g), DM dry matter production of the plant (g), DESIR Desirability, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), SCA infestation by cactus scale (*Diaspis echinocacti*), DIS Incidence of Diseases and WIL wilt Index. The varieties followed by the same letter belongs to the same group by Scott-Knott criterium ($P \leq 0.05$).

Table 4. Grouping of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, based on morphological and behavioral characteristics, the Mahalanobis distance and the optimization method of Tocher.

Group	Varieties
I	1, 3, 5, 4, 6, 2
II	7

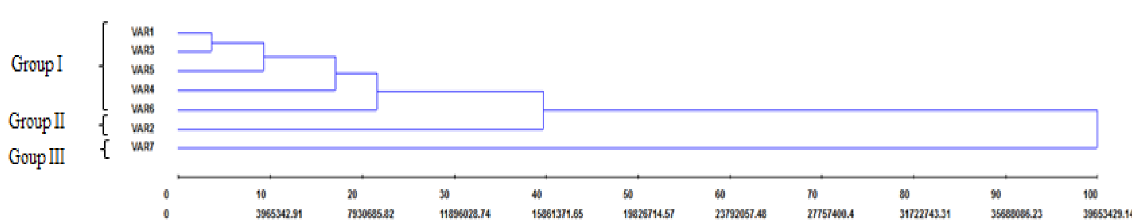

Figure 1. Representative dendrogram of the grouping by UPGMA of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, based on morphological and behavioral characteristics.

Table 5. Estimates of the eigenvalues associated to the principal components and relative importance (eigenvectors) for 19 morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Components	Root (eigenvalue)	Root %	Accumulated %	Relative Importance (eigen vectors)										
				CFM	CDM	H_2O	CT	CW	CL	CA	NC	TPA	CAI	PW
1	10.05	52.87	52.87	0.30	0.30	0.29	0.23	0.25	0.28	0.29	-0.20	0.07	0.07	0.21
2	4.06	21.38	74.26	-0.11	-0.13	-0.11	-0.22	-0.23	0.07	-0.10	0.34	0.44	0.44	0.29
3	2.03	10.71	84.96	-0.10	-0.07	-0.11	0.11	0.01	0.14	0.04	-0.11	-0.06	-0.06	0.20
4	1.81	9.50	94.46	0.14	-0.01	0.17	-0.32	0.25	0.21	0.25	-0.12	0.28	0.28	-0.26
5	0.82	4.31	98.77	-0.08	0.11	-0.14	0.27	-0.14	0.21	-0.09	-0.29	-0.03	-0.03	0.01
6	0.23	1.23	100.00	-0.02	0.28	-0.10	0.24	-0.15	0.13	-0.10	0.056	-0.001	-0.001	-0.26

Components	Raiz (eigenvalue)	Root %	Accumulated %	Relative Importance (eigenvectors)							
				PH	FM	DM	WIL	DIS	CAR	DESIR	SCA
1	10.05	52.87	52.87	0.23	0.26	0.18	-0.27	-0.16	0.15	-0.21	0.19
2	4.06	21.38	74.26	0.12	0.21	0.30	0.11	0.04	0.11	-0.23	-0.007
3	2.03	10.71	84.96	0.35	-0.21	-0.22	0.02	0.20	0.57	-0.06	-0.52
4	1.81	9.50	94.46	0.12	0.006	-0.24	0.30	0.32	-0.01	0.38	0.06
5	0.82	4.31	98.77	-0.19	0.007	0.20	0.073	0.74	-0.09	-0.19	0.20
6	0.23	1.23	100.00	0.53	-0.07	0.37	0.15	-0.09	-0.34	0.26	-0.25

CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O cladode water content (g), CT cladode thickness (cm), CW cladode width (cm), CL cladode length (cm), CA cladode area (cm^2), NC total number of cladodes, TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, PW plant width (cm), PH plant height (cm), FM fresh matter production of the plant (g), DM dry matter production of the plant (g), WIL wilt Index, DIS Disease Incidence, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), DESIR Desirability and SCA Infestation by cochineal Scale (*Diaspis echinocacti*).

al. (2012), it is necessary that the first principal components exceed 80% of the accumulated value to account for the variability manifested among individuals, leading the interpretation of the phenomenon with considerable simplification of characters. By analyzing the chart of the score dispersions of the principal component analysis, the formation of four groups was observed. The genotypes were divided into group I (3, 4 and 5), group II (1 and 6), group III (2) and Group IV (7) (Figure 2).

In the analysis of canonical variables, the first two variables explained 96.75% of the total variation among the varieties of cactus pear, providing good reliability of the variability among genotypes in the two-dimensional plane (Table 2). When analyzing the chart of the score dispersion of canonical variables, the formation of three groups was observed. Group I, represented by genotypes 1, 3, 4, 5 and 6, group II by genotype 7 and group III by genotype 2 (Figure 3).

The grouping methods of the genotypes were similar to each other. However, the grouping order of genotypes among groups was different. For example, in UPGMA methods, principal components and canonical variables, the genotype 2 was classified into one group, diverging from the Tocher method that classified this genotype as similar to genotypes 1, 3, 4, 5 and 6. The method of principal components classified genotypes 1 and 6 in different groups, differing from other methods that classified the genotypes 1, 3, 4, 5 and 6 as similar (Figure 1, 2, 3 and Table 4).

The grouping analysis (cluster analysis) identifies groups of similar individuals after estimation of a dissimilarity matrix. There are several grouping methods that differ by type of result and by the different ways to define the closeness between individuals or groups formed. In all cases, it is not known a priori, the number of groups to be established and different methods give different results (Cruz et al., 2012).

The grouping methods are mainly based on hierarchical and optimization methods. In the hierarchical methods, there is the method of the average distance among groups (UPGMA), in which the groups are identified in the form of dendrograms, arranged on multiple levels and

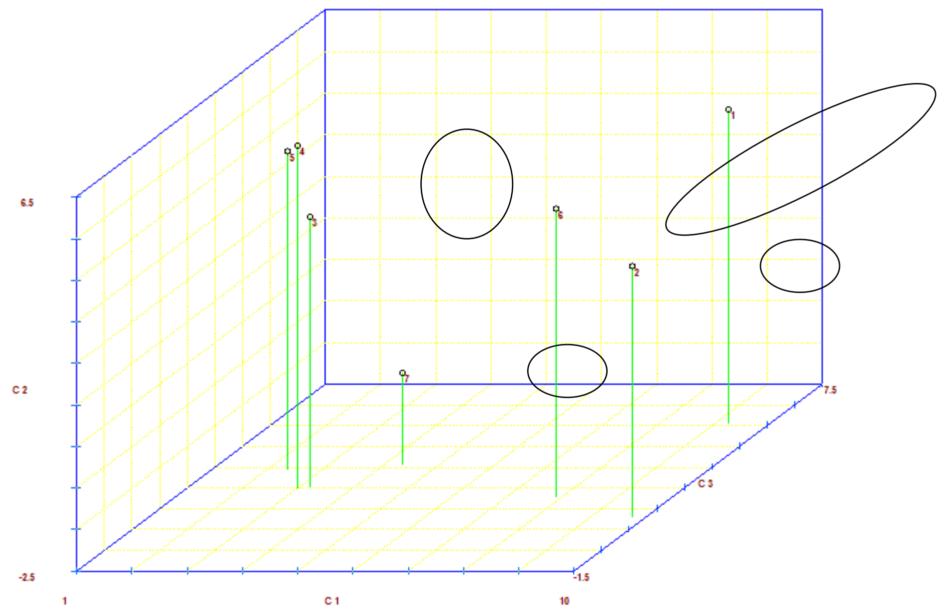

Figure 2. Graphic dispersion of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, in relation to the first, second and third principal component (C1, C2, C3), based on six morphological and behavioral characteristics.

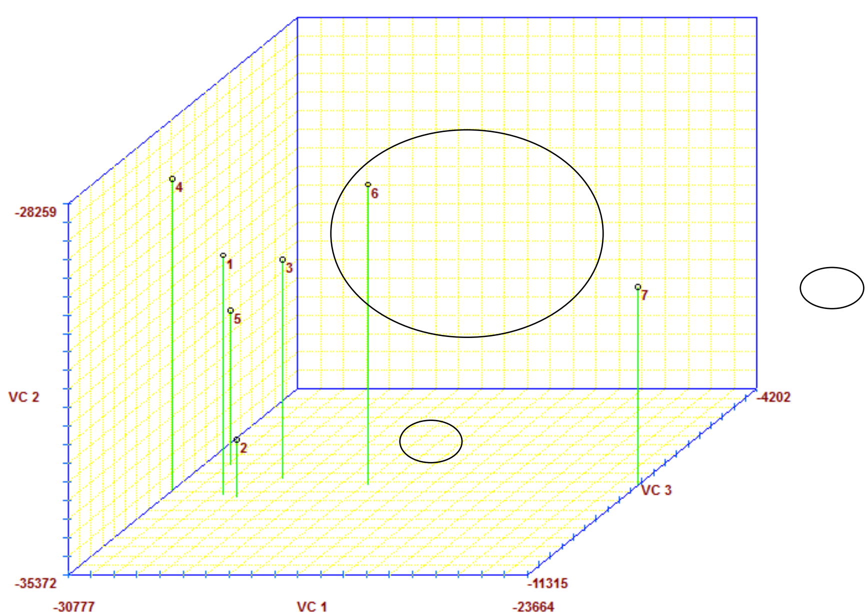

Figure 3. Graphic dispersion of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, in relation to the first, second and third canonical variable (CV1, CV2 and CV3) based on six morphological and behavioral characteristics

does not take into account the optimal number of groups. In optimization methods, there is the Tocher algorithm, in which the goal is to achieve a partition of individuals that optimize (maximize or minimize) some predefined measure. It is based on the formation of groups in which the distances within the groups are smaller than the distances among the groups obtaining the optimal number of groups (Cruz et al., 2012).

Table 6. Estimate of the eigenvalues associated to canonical variables and relative importance (eigenvectors) for 19 morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Canonical variable	Root (eigenvalue)	Root %	Accumulated %	Relative importance (eigenvectors)									
				CFM	CDM	H_2O	CT	CW	CL	CA	NC	TPA	CAI
1	5950441.88635	65.76975	65.76975	0.00005	0.00027	0.0	0.00039	0.00069	-0.0006	-0.00223	-0.00299	0.00043	-0.00009
2	2803191.65355	30.98345	96.75320	-0.00027	0.00009	0.0	0.00084	-0.0022	-0.0002	0.00154	0.00309	-0.0008	0.0
3	172614.96673	1.90789	98.66110	-0.00148	-0.0015	0.0	0.00074	-0.0068	-0.0007	0.00131	0.00603	0.00051	0.00007
4	120244.63357	1.32905	99.9901	0.0006	0.00135	0.0	0.00098	0.00485	0.00399	-0.00211	-0.01036	0.00329	-0.00014
5	886.82921	0.00980	99.99996	0.0099	0.02711	0.0	0.00552	0.00003	0.01874	-0.00932	-0.4237	0.03919	-0.00011
6	3.19831	0.00003	100.0000	0.12444	0.36019	0.00001	-0.0743	0.20859	0.40727	0.02923	-0.2794	0.16108	0.00692

Canonical variable	Root (eigenvalue)	Root %	Accumulated %	Relative Importance (eignevectors)								
				PW	PH	FM	DM	WIL	DIS	CAR	DESIR	SCA
1	5950441.88635	65.76975	65.76975	0.00502	0.00072	0.00303	0.00285	-0.3576	-0.3529	0.58204	0.07891	0.6344
2	2803191.65355	30.98345	96.75320	0.00582	-0.0008	-0.0042	-0.0015	0.31095	0.29452	0.05789	0.88437	0.17614
3	172614.96673	1.90789	98.66110	0.00474	-0.0039	-0.0072	0.00092	0.25413	0.70908	0.11707	-0.4296	0.48388
4	120244.63357	1.32905	99.9901	-0.0099	0.00595	0.01342	-0.0054	0.12349	0.17886	0.79893	-0.4384	-0.5586
5	886.82921	0.00980	99.99996	-0.02294	-0.0354	0.0601	-0.0846	0.82691	-0.4990	0.07197	-0.1570	0.14183
6	3.19831	0.00003	100.0000	-0.05287	0.601	0.38999	-0.1260	-0.0397	0.03136	-0.0169	0.00784	0.00594

CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O cladode water content (g), CT cladode thickness (cm), CW cladode width (cm), CL cladode length (cm), CA cladode area (cm^2), NC total number of cladodes, TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, PW plant width (cm), PH plant height (cm), FM fresh matter production of the plant (g), DM dry matter production of the plant (g), WIL wilt Index, DIS Disease Incidence, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), DESIR Desirability and SCA Infestation by cochineal Scale (*Diaspis echinocacti*).

The techniques of principal component analysis and canonical variables aims to reduce the dimensionality of the variables so that the new combination of resulting uncorrelated linear variables explains the structure of variance and covariance of the set of original variables(Cruz et al., 2012).

The use of multivariate techniques in the detection of genetic diversity requires a certain degree of structure in the data. It is important that different grouping criteria be used and that the consensus structure of most of them be considered as correct, so as to assure that the result is not an artifact of the technique used (Arriel et al, 2006; Viana, 2013).

The formation of two groups of cactus pear by the Tocher method, three by UPGMA methods and canonical variables and four groups by the method of principal components, provides relevant information to the conservation of genetic material as a source for breeding programs. According to Silva et al. (2011), crossbreeding between genotypes from different groups provide superior lines for the improvement of characteristics of interest. Obtaining lineages from commercial varieties is a viable alternative because they represent improved and tested genotypes in various cultivation environments. Considering the hybrids, it is also possible to have a high proportion of fixed different loci, facilitating the selection and recombination of favorable alleles (Amorim and Souza, 2005).

Thus, as a suggestion for the breeding program with genera *Opuntia* and *Nopalea*, the breeder must consider not only the distance between groups as a criterion to guide the crossings, but also the individual performance of the genotype for each characteristic of agronomic and zootechnical interest (Ferreira et al., 2003), besides the possibility and ease to have crossings between individuals of different genera (Paixão, 2012).

In the principal component analysis, the characteristics that contributed most to the total variance of the first component were CFM, CDM, H_2O and CA. In the second component, TPA, CAI and NC stood out; in the third component, CAR and PH (Table 5); in the analysis of canonical variables, the characteristics that contributed most to the total variance in the first variable were SCA and CAR, and in the second variable DESIR and WIL (Table 6). The most important characteristics are those whose weighting coefficients (eigenvectors) are of greater magnitude, in absolute value, in the first principal components or canonical variables (Cruz et al., 2012). Therefore, these would be the most responsive characteristics in the selection process among cactus pear populations.

In the relative contribution analysis of the characteristics for the genetic diversity among the seven varieties of cactus pear by Singh methodology (1981), H_2O contributed with 43.29% and CFM with 39.63% for the variability among genotypes. These two characteristics contributed with 82.92% of the total variability among the materials. The characteristics that contributed least to the divergence were CT, DESIR, SCA and DIS (Table 7). The lower contribution variables are little informative in the characterization of genetic variability, and can be discarded in genetic diversity studies (Rêgo et al., 2011).

Table 7. Relative contribution of 19 morphological and behavioral characteristics for the genetic diversity of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, through Singh's methodology (1981).

Variable	Relative contribution (%)
CW	2.69
CL	2.86
CT	0.01
CA	0.13
CFM	39.63
CDM	0.89
H_2O	43.29
NC	0.23
PW	0,15
PH	0.23
TPA	2.66
CAI	0.37
FM	5.51
DM	0.83
DESIR	0.02
CAR	0.26
SCA	0.04
DIS	0.05
WIL	0.12

CW Cladode width, (cm) CL cladode length (cm), CT cladode thickness (cm), CA cladode area (cm^2), CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O water content of cladode (g), NC total number of cladodes, PW plant width (cm), PH plant height (cm), TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, FM fresh matter production of the plant (g), DM dry matter production of the plant (g), DESIR Desirability, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), SCA infestation by cactus scale (*Diaspis echinocacti*), DIS Incidence of Diseases and WIL wilt Index.

Paixão (2012) and Ferreira et al. (2003) cite as most important characteristics for the divergence in genera *Opuntia* and *Nopalea*: CW, CL, CT, NC and CFM. Although this study present CFM and H_2O as the main characteristics of diversity, they are positively and significantly correlated in the ($p \leq 0.01$) and corelated to CDM, CW, CA, and in the ($p \leq 0.05$) with CL. These results indicate the importance of the morphological characteristics related to cladodes for the study of genetic diversity in cactus pear (Table 8).

The CDM was correlated positively and significantly with H_2O, CA, CT, CW, CL. The CW was positively correlated with CA. The CL was positively correlated with CA, PH and FM. The TPA was positively correlated with CAI. The PW was positively correlated with DESIR. The PH was positively correlated with CAR. FM was positively correlated with DM and SCA. The DM was positively correlated with DESIR. The NC was negatively correlated with CDM, CT, CW and AC. The WIL was negatively correlated with CDM, CT, CFM and H_2O (Table 8).

Most of the morphological characteristics of cladodes evaluated in this study correlated positively and significantly with each other. This is expected because they are characteristics related to agronomic production. Positive correlations among agronomic characteristics of these pests with the plant. An important aspect to consider is the CA of the genotype: the larger the area, the greater the H_2O and more resistant is the plant to water deficit and WIL.

This characteristic is of interest to the breeding program of cactus pear aimed at the selection of genotypes more tolerant to water deficit. In addition, the CA was positively correlated with the CFM and CDM, relevant agronomic characteristics for the production of forage for food and animal nutrition.

A priori, the selection of genotypes with increased production of CDM should be preferred, since variation in water content harms the nutritional calculations. The selection by CFM or CA can be used, since these characteristics are significantly and positively correlated with each other and would not require the determination of the CDM (Neder et al., 2013). According to Cruz et al. (2012), the existence of significant correlations among characteristics indicates the feasibility of indirect selection in order to obtain gains in the characteristic of great importance. The CFM and CA are very important characteristics in terms of technical and economic aspects of a rural property. For being of easy viewing and production measurement in the Brazilian semiarid region, it is used as calculation basis for sizing the number and flow of animals on farms (Amorim, 2011). The genotypes that obtained the highest means for production characteristics, water retention, and resistance to pests and diseases were 2 and 6 (Table 3). Then, crossings that involve these genotypes could generate superior progenies in characteristics of agronomic, zootechnical or physiological interests. (NC, CT, CL, CW, PH, PW, FM and DM) were also reported by Neder et al. (2013), who studied 19 accesses of cactus pear (*Opuntia ficus indica*) at 30 months of age, except for the NC, which was negatively correlated with CT and CW, corroborating the results of our work.

The positive correlations of PH and FM with CAR and SCA, respectively, are given by the greater contact area Thus, the results suggest future work aimed to explore the variability found among the cactus pear genotypes studied and the possibility of using other methods such as protein molecular markers, physiological, biochemical and chemical characteristics, and chromosomal variation for the determination of the genetic variability; thereby providing a complementary analysis to studies through morphological and behavioral characteristics. Additional studies must be performed in other locations and in several years.

Table 8. Correlations among morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco

Variable	CFM	CDM	H_2O	CT	CW	CL	CA	NC	TPA	CAI	PW	PH	FM	DM	WIL	DIS	CAR	DESIR	SCA
CFM	1	0.94**	0.99**	0.64Ns	0.93**	0.81*	0.96**	-0.74ns	0.08ns	0.08ns	0.36ns	0.60Ns	0.73ns	0.36ns	-0.81*	-0.51ns	0.28ns	0.39ns	0.71ns
CDM	-	1	0.91**	0.83*	0.84*	0.80*	0.88**	-0.78*	-0.04ns	-0.04ns	0.41ns	0.59Ns	0.69ns	0.45ns	-0.88**	-0.48ns	0.27ns	0.50ns	0.66ns
H_2O	-	-	1	0.59Ns	0.94**	0.80*	0.96**	-0.71ns	0.11ns	0.11ns	0.34ns	0.59Ns	0.72ns	0.33ns	-0.78*	-0.51ns	0.28ns	0.35ns	0.70ns
CT	-	-	-	1	0.59ns	0.54ns	0.58Ns	-0.78*	-0.42ns	-0.42ns	0.39ns	0.41Ns	0.34ns	0.28ns	-0.87**	-0.39ns	0.34ns	0.53ns	0.31ns
CW	-	-	-	-	1	0.72ns	0.96**	-0.85*	-0.09ns	-0.09ns	-0.15ns	0.56Ns	0.47ns	0.02ns	-0.69ns	-0.38ns	0.32ns	0.13ns	0.51ns
CL	-	-	-	-	-	1	0.87**	-0.59ns	0.42ns	0.42ns	0.63ns	0.84*	0.76*	0.50ns	-0.61ns	-0.14ns	0.61ns	0.57ns	0.46ns
CA	-	-	-	-	-	-	1	-0.76*	0.14ns	0.14ns	0.37ns	0.71Ns	0.65ns	0.23ns	-0.71ns	-0.37ns	0.45ns	0.33ns	0.54ns
NC	-	-	-	-	-	-	-	1	0.43ns	0.43ns	-0.01ns	-0.35Ns	-0.18ns	0.12ns	0.63ns	0.08ns	-0.26ns	-0.08ns	-0.34ns
TPA	-	-	-	-	-	-	-	-	1	1.00**	0.51ns	0.41Ns	0.61ns	0.58ns	0.16ns	0.08ns	0.24ns	0.35ns	0.22ns
CAI	-	-	-	-	-	-	-	-	-	1	0.51ns	0.41Ns	0.61ns	0.58ns	0.16ns	0.08ns	0.24ns	0.35ns	0.22ns
PW	-	-	-	-	-	-	-	-	-	-	1	0.68Ns	0.72ns	0.74ns	-0.57ns	-0.34ns	0.72ns	0.94**	0.16ns
PH	-	-	-	-	-	-	-	-	-	-	-	1	0.57ns	0.38ns	-0.50ns	-0.26ns	0.80*	0.51ns	0.04ns
FM	-	-	-	-	-	-	-	-	-	-	-	-	1	0.85*	-0.64ns	-0.47ns	0.27ns	0.74ns	0.75*
DM	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.47ns	-0.36ns	0.13ns	0.83*	0.56ns
WIL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.69ns	-0.37ns	-0.68ns	-0.52ns
DIS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.05ns	-0.38ns	-0.38ns
CAR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.54ns	-0.30ns
DESIR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.32ns
SCA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

* and ** significant at 5 and 1% probability, respectively, by the t test; ns: not significant.

Conclusion

The varieties studied of cactus pear, genera *Opuntia* and *Nopalea*, present genetic divergence. The uni and multivariate methods used for the divergence differ and gather genotypes in two, three or four groups.

Conflict of interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 11(31), pp. 2860-2870, 4 August, 2016
DOI: 10.5897/AJAR2016.10913
Article Number: EDBA77559812
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Challenges facing agricultural cooperative system: Analysing participation using a discrete choice model for the southern communal area of Namibia

Uchezuba, D. I.[1]*, Mbai, S.[2] and Laubscher, K.[3]

[1]Department of Agriculture and Natural Resource Sciences, Namibian University of Science and Technology, Namibia.
[2]Department of Agriculture and Natural Resource Sciences, University of Science and Technology, Namibia.
[3]World Academy of Productivity Science.

Received 18 February, 2016; Accepted 19 April, 2016

The problem of trust, non-transparency, and dysfunctionality has been ascribed to the character and the nature of the agricultural cooperative system in Namibia. Perhaps the true characterisation of the problem is not known with certainty albeit, the concern about the role of the institution, government intervention and member laxity. Further insight from the institutional economics suggests that organisational behaviour has a much larger role to play. Using a survey of 340 livestock farmers in six regions of the Southern Communal Areas of Namibia and adopting a logistic probability outcome model, the study examines the relevance of agricultural cooperatives with regards to the extent members are willing to participate with due cognisance to these concerns. The result shows that the probability that a farmer will join a cooperative is 29.5%. Education and technical constraints such as lack of adequate market information and training negatively affect willingness to participate. Participation is region specific, the odds of participation increases by 65, 91 and 14% if they are from Hardap, Kunene South/Erongo and Omaheke respectively. Increases in farm credit increase the odds of participation by 34%. The study also found that younger and inexperienced farmers are more likely to join cooperatives than older and experienced ones. The results highlight a general lack of knowledge about the cooperative system which calls for the strengthening of the policy framework to incorporate the concerns raised by new institutional economics.

Key words: Cooperative, institution, livestock, probability, participation.

INTRODUCTION

The importance of cooperative system as a means of promoting economic and social development thus strengthening human capital has been overemphasized in literature (Cook, 1995; Royers, 1995; Ortmann, 2007; Thomas and Hangula, 2011). It is a key development priority such that, it is encouraged and facilitated by government, stakeholders and non-governmental organisations (NGO). Fundamentally, in the agricultural

*Corresponding author. E-mail: stickydamap@gmail.com. Tel: +264810425716.

sector, farmers (especially the small-scale farmers in the rural communal areas) are encouraged to form or join agricultural cooperatives because it is an important organisational instrument for farmers' collective bargaining, growth and the development of the rural economy. To enhance this objective, section 3 under the Namibian National Cooperative Policy (1992) states the need to create an economic, legal and institutional environment which is conducive for the development and growth of all cooperatives in Namibia. With the collaboration of the public service, NGOs and major stakeholders, the institutional framework of cooperative is enhanced. Institutions under the public service, for example, the Ministry of Agriculture, Water and Forestry (MAWF) govern agricultural cooperatives with the sole aim of developing entrepreneurial, organisational and managerial skills for members through extension services, training, and mentorship programmes. Amongst all, create an adequate framework for improved knowledge of the benefit of cooperative especially amongst the communal farmers'. But to all effects, there is a major concern that the cooperative institution in Namibia (especially livestock cooperative) seem to be dysfunctional. This has been a major concern to policy makers and major stakeholders which have left a big question as to whether the agricultural cooperative system has lost its credibility hence in a state of institutional degeneration.

The credibility of livestock cooperatives was examined in this study with regards to the extent of the lack of trust and reliability amongst the communal livestock farmers in the South Communal Areas (SCA) by investigating the factors that influence the farmers' willingness to participate in livestock cooperatives, albeit allegation of mistrust and lack of transparency. The focus of the study is on cattle, fat-tailed sheep and goat livestock cooperatives in the SCA comprising: Kunene South, Otjozondjupa, Omaheke, Erongo, Hardap and Karas. It is part of the study conducted by the National Namibian Farmers' Union (NNFU) (2015), which investigated the alternative scenario to improve communal livestock marketing position in the southern communal area of Namibia. The Southern region was chosen for this study because of the outbreak of Foot and Mouth Disease (FMD) in the Northern region during the early part of 2015 when the study was on-going. Livestock agricultural enterprise was chosen for this study because the rural economy is mainly agro-pastoral and the majority of the communal dwellers derive their livelihood from livestock farming. According to the Namibian National Development Path 4 (NDP4) 2012/13 to 2016/17, priority 2, an increase in the livestock production will stimulate growth in the rural economy which constitutes 62% of the Namibian population (National Household Income and Expenditure Survey (NHIES), 2009/10), hence the justification for this study. Thomas and Hangula (2011) reviewed the principle of cooperative and highlighted some of the challenges facing agricultural cooperative in Namibia without empirically investigating any of the parameters. This study explores the theoretical underpinnings and investigates some of the key challenges that influence cooperatives participation.

The role of government in cooperative development in Namibia

The government and other institutions such as financial institutions, non-governmental organisation, international agencies as well as cooperative movements support cooperative development through amongst others, financial incentives, training and logistics. For instance, a large amount of financial resources is committed since independence by the Namibian government to give financial support to cooperatives. However, the extent to which this support culminated into the transformation of cooperative has much to be desired. In the contrary, it was observed that the support actually contributed to the demise of most cooperative organisations in Namibia. This can be attributed to a lot of factors some of which are stressed in the Namibian National Cooperative Policy (1992).

Of paramount importance is the fact that cooperatives became an instrument of the state instead of a member-owned, member-run and member-serving business organisation. As a result, they become politicised, members no longer identify themselves with cooperatives as a result they lost interest in them. The nurture and care given to them sooner resulted in dependency syndrome, albeit incompetence and economically unviable. In addition, preferential marketing and supply transactions were given to them, yet to no avail, as these did not improve the situation; instead, most cooperatives became inefficient and costly to manage. In spite of this, an increasing number of public expenditure is often allocated to cooperative administration without adequate recourse as to the macroeconomic implications. The implication of this is more impactful on the future of cooperative than on government fiscal as cooperative dwindle into degeneration and less relevant. Evidence can be drawn from the existing documented records about cooperatives. The record shows that the oldest cooperatives in Namibia are Alfa cooperative formed in 1964 and Agra cooperative Pty formed in 1980 (Thomas and Hangul, 2011). Alfa cooperative became defunct for lack of viability, while Agra on the other hand, restructured into Investor-Oriented Firm (IOF) or public liability company. The aim of the restructuring is to adopt a strategic shift in the organisational structure, management and corporate governance - a feature that characterizes the IOFs. Since the restructuring, Agra has stepped up its operation in terms of horizontal and vertical integration and the investments in offshore markets through improved quality management, logistics, and corporate development. The same cannot be said of the vast majority of cooperatives who are in various stages of development.

According to the registrar of cooperatives in Windhoek (2016), there are only seven existing fully registered cooperatives between 1998 and 2015 out of which three are multipurpose agricultural cooperatives. About a hundred and twenty-eight documented cooperatives are provisionally registered (Registrar of Cooperative, 2016). Provisional registration accords them the opportunity to operate only temporarily while waiting to complete the process of registration. They will become a legal entity upon full registration, then they can enter into contracts and own property. Out of the provisionally registered cooperatives, about sixty-two are agricultural cooperatives; nine are livestock cooperative whereas, fifty-three are non-agricultural, majority of which fall into the category of services cooperatives as compared to workers cooperative. Despite the records, there is the concern that the available number of existing, unregistered and undocumented cooperative abound. This has damning consequences on the understanding of, and the adherence to the principle, rules and regulations governing cooperatives by members.

The role of institutions in cooperative development

Cooperatives operate within sets of principles and rules centred on the interest of the members, voluntary participation, democracy, autonomy, equity and service (Ortmann and King, 2007). Yet there are legal frameworks that define how they are created, their *modus operandi* and their termination. The Namibian cooperative Act (No 23 of 1996), the Namibian National Cooperative Policy (1992), the Namibian National Agricultural Policy (revised and adopted in 2015) and the General Model By-Laws for the Namibian Cooperatives (in accordance with the Cooperative Act (No 23 of 1996), recognizes the importance of creating an economic, legal and institutional environment conducive enough for the growth and the development of cooperatives in Namibia. This is because institutional development plays a key role in the formation, organisation and development of cooperatives. It provides the foundation upon which cooperative principle and governance are built. It is said that, if institutions are the rules then the cooperatives are the players while the members are the goal. In the current status quo of cooperatives in Namibia, the question is which of the three is in the spotlight: The institution, the cooperatives or the members? The question can be answered by investigating the type of relationship among the three (this is outside the scope of this study). Exercising intuitive judgement one can infer an unidirectional cause-and-effect relationship flowing from institution to cooperative and to the members, on the premise that, weak institutions set pace for market failure, whereby physical, legal and logistic infrastructural development are lacking, therefore, it is difficult to enforce contracts and anti-competitive laws, control prices and reduce transaction cost thus, resulting in high operational cost, non-compliance and poor performance of cooperatives. The poor performance of cooperatives discourages members from playing an active role and further engagement.

Within this descriptive paradigm, it will be of utmost importance to also consider the impacts of the oligopoly conglomerates to the development of local rivalry. In other words, concentration, vertical integration and anti-competitive behaviours may result in foreclosures at the farmers ‘and cooperative level of market operation. Perhaps one would not understand the role of the institution in organisational design without understanding the true behavioural attributes of cooperatives and its members. Insight about this can be drawn from institutional economics discussed subsequently.

The institutional economics

Helmberger and Hoos (1962) used the neo-classical theory of the firm to describe the behavioural attribute of cooperatives, leading to one of the first organisational theory of cooperatives. According to Helmberger and Hoos (1962), cooperatives maximise benefits to members by maximising the per unit value (volume) of patronage or average price paid by members (in terms of service cooperative) for the commodities purchased, which they, by the provisions of most cooperative Acts, ought to fulfil (Ortmann and King, 2007:50; Torgerson et al., 1998:5). Accordingly, the Namibian cooperative Act (No. 23 of 1996), section 58, subsections (a) to (h) clearly stipulates the distribution of net surpluses to members. In other words, the theory is based on optimisation and profit maximisation behaviour by a firm under the assumptions of zero transaction and adjustment costs with full employment of resources (Royer, 1999:45; Ortmann, 2007).

Critiques argue that in the wake of the new institutional economics, the neoclassical paradigm for cooperatives operation leaves much to be desired (Staatz, 1994; Royer, 1999; Torgerson et al., 1998). This could perhaps be why the organisational form of most cooperatives self-destruct leading to higher rates of closures, mergers and conversions to IOFs. The new institutional economics (NIE) explain the shortfall in the neoclassical paradigm and possibly, the cause of the rise and fall of the cooperative organisations by considering the theory of transaction cost economics, the principal-agency relations and the property rights. According to the NIE, an economic transaction involving the exchange of goods and services is not costless. It involves the cost of search, transportation, information, bargaining and the cost of contracting and enforcement. These costs are influenced by institutions - be it legal, political or economic (Ortmann, 2007). The failure of one or more of these institutions, results in the failure of service unit of the cooperatives - the market institution, leading to high transaction cost, asymmetric information, incomplete and unenforceable contracting. These often depict the market institution of a developing economy such as Namibia - a precarious situation for agricultural cooperative governance.

In the light of agency relations agreement, the situation is even a lot worst. Agency relationship occurs when an individual or more (principal) employs the cooperative organisation (agent) to perform a service on behalf of the principal. The principal-agent problem arises because there might be divergent objectives between the principal and the agent (Alchian and Demsetz, 1972; Royer, 1999; Sykuta and Chaddad, 1999). For example, while the principal might want to maximise returns, the agent might aim to maximise goodwill. Aligning the two interests have cost implications hinging on market efficiency and the competitiveness of the organisation. Property right also play a role in defining the existence of cooperatives. Like transaction cost, property right has significant consequences on the economic organisation, behaviour and performance (Sykuta and Chaddad, 199:73). According to Ortmann (2007:56), a well-defined property right is vital for cooperative performance. This has a strong bearing on the institutional obligations discussed earlier. Other problems include free-rider; the horizon, portfolio, control and influence cost problems discussed fully in Cook (1995), Ortmann (2007) and Thomas and Hangula (2011).

MATERIALS AND METHODS

Sample and sampling

The sample population comprises the livestock region in the SCA. Six representative regions in SCA were selected. The selected regions are as mentioned previously. Livestock farmers were randomly sampled from each region. A semi-structured questionnaire containing both open and closed-ended questions was used to gather information from farmers. The questionnaire was first pre-tested on selected farmers and later modified to include additional opinions. Farmers' were asked whether they belong to livestock cooperative group, if the answer is no, a follow-up open-ended question would be why?

A total of three hundred and forty respondents were interviewed in the survey. The total number of respondents in each region is shown in Table 1. The samples for Kunene South and Erongo were combined due to a small sample collected in each region. A total of 109 farmers were sampled in Otjozondjupa, Omaheke (96), Southern Kunene and Erongo (50), Hardap (51) and Karas (34). Note that the sample size per region is, however, not equal, so is the livestock production and marketing potentials in these regions. For example, according to the Namibian Statistical Agency (2012) livestock census, Otjozondjupa cattle population represented 16.6% of the national cattle herd, Kunene South (2.7%), Omaheke (15.6%), Erongo (2.8%). Hardap and Karas had 3 and 2.1% of the national herd size respectively. On the other hand, sheep production in Hardap and Karas alone constitute 74.3% of the sheep herd, others are, Omaheke (11.4%), Otjozondjupa (2.6%), Erongo (2.8%), and Kunene South (1.7%).

Model specification

The concept investigated is that the structure, conduct and performance of cooperatives system have been influenced by a lot of factors, culminating into the belief that it has perhaps affected livestock farmers marketing positions in terms of price discovery, contracting and profitability albeit, with uncertainty. Therefore, a probability outcome decision model is needed to determine the probability that the envisaged event, that is, the probability that, given the circumstances discussed previously the livestock farmers in the study area would have a higher probability of belonging to a cooperative or not. The modelling framework for the outcome decision model is given subsequently.

The logistic model

A probability outcome decision model was used to determine the probability that farmers are willing to join a cooperative. The response variable for the model estimation is membership of a cooperative denoted MEMCOP. MEMCOP is a dichotomous variable taking the value of one if a farmer is a member of a cooperative, zero otherwise. This is an example of a binary decision outcome variable whereby the code 1 represents a positive outcome indicating that an event occurred, whereas, zero is a negative outcome whereby an event did not occur. The aim is to estimate the relationship between MEMCOP and a set of independent variables namely; FARMER EXPERIENCE (Continuous variable), GENDER (male = 1, female = 0), AGE (Continuous variable), farmer's indebtedness (whether farmer has a loan = 1, 0 otherwise), EDUCATION (which includes, no education = 1, 0 otherwise, secondary education = 1, 0 otherwise and tertiary education = 1, 0 otherwise), FARMERS CONSTRAINTS (No cooperatives = 1, 0 otherwise; lack of information = 1, 0 otherwise and No benefit = 1, 0 otherwise) and lastly, set of regional dummies comprising; one for a region, zero otherwise.

Modelling the above hypothesized discrete choice relationship requires the assumption of utility maximization. The farmers i are faced with alternatives j whereby decisions are made based on the alternatives that maximize their utility subject to observed deterministic and unobserved random components, given as; $U_{ij} = V_{ij} + \varepsilon_{ij}$. The utility maximization of this form is additive and random, consisting of observed farmer characteristic and technical constraint listed above plus unobserved idiosyncrasies. Therefore,

$$\begin{aligned}\Pr(y_i = j) &= \Pr(U_{ij} \geq U_{ik}),\ for\ all\ k \\ &= \Pr(U_{ik} - U_{ij} \leq 0),\ for\ all\ k \\ &= \Pr(\varepsilon_{ik} - \varepsilon_{ij} \leq V_{ij} - V_{ik}),\ for\ all\ k\end{aligned} \quad (1)$$

According to the specification of the response variable, MEMCOP, a qualitative dichotomous regression analysis was applied assuming utility function Equation 1. A model of dichotomous nature has mutually exclusive outcomes (Cameron and Trivedi, 2010:459). It is either that an outcome is observed or not observed, therefore, the aim is to determine the probability (p) of the occurrence of one outcome rather than the alternative that occurs with a probability of ($1-p$). Suppose y represent the outcome

Table 1. Farm and farmer characteristics.

Farm and farmer characteristics	Hardap	Karas	South Kunene and Erongo	Omaheke	Otjozondjupa
Average farmers age	52	48	50	40	45
Average farming experience	19	19	27	21	23
Average herd size cattle	22	19	46	67	81
Average herd size sheep	49	99	80	44	40
Average herd size goat	66	77	69	52	46
Total herd size cattle	809	427	2182	6139	8646
Total herd size sheep	1772	2376	2390	2319	3433
Total herd size goat	3151	2553	2914	3873	4053
Average number of secondary education (%)	53	65	46	59	59
Average number of tertiary education (%)	6	15	26	18	17
Number of farmers with No Education (%)	41	21	28	23	24
Number of male farmers (%)	53	65	92	82	94
Number of female farmers (%)	47	35	8	17	6
Number of respondents-farmers	51	34	50	96	109

variable, an outcome is observed for $(y=1)$ with probability p or not observed $(y=0)$ with probability ($1-p$). According to the specification of the discrete model, the nature of the observed data dictates the special treatment of a binary dependent variable model (Greene, 2012:724). The interest is to model a positive outcome of p as a function of a set of covariates, x. The probability mass function for the observed outcome, y is $p^y(1-p)^{1-y}$, with $E(y)=p$ and Var$(y)=p(1-p)$ (Cameron and Trivedi, 20105:460). The conditional probability takes the form:

$$p_i \equiv (\Pr(y_i = 1 \mid x) = F(x_i^{'}\beta) \quad (2)$$

Or simply

$$Logit\ (PX) = \alpha + \sum \beta_i x_i + \mu_i \quad (3)$$

Where

$$(PX) = \frac{1}{1+e^{-(\alpha + \sum \beta_i x_i)}} \quad (4)$$

Where x is a vector of regressors, α, β_i are vectors of unknown parameters to be estimated, μ_i is a random disturbance term. The set of parameters β_i reflects the impact of changes in x on probability of y (Greene, 2003:665). It represents the change in the log odds that will result from a one unit change in x while other variables in the model remain constant (Kleinbaum and Klein, 2010:21). Another interpretation of logistics coefficients is in terms of odds ratio obtained by exponentiation of the log odds. The odds ratio represents the number of times or percentage points the outcome variable will change given a one unit change in x. The function $F(.)$ is the cumulative distribution function which ensures that $0 \le p \le 1$ is satisfied (Cameron and Trivedi, 2010:460). This specification is applied to the data described above using logistic binary outcome model. This was preferred to other binary outcome models such as probit model because of its mathematical simplicity. The major difference between logit and probit is in their distributional assumption about error variance. The probit model assumes error variance of 1 for a standard normal distribution whereas, it is $\pi^2/3$ for a logistic distribution (Long and Freese, 2001), this notwithstanding, the results under both models do not differ greatly (Greene, 2003:667; Gujarati and Porter, 2009:571; Cameron and Trivedi, 2010:472).

In estimating the probability of an outcome as shown in Equations (2) to (4), it should be noted that the probability p_i is non-linearly related to β and x. Therefore, ordinary least square (OLS) estimator cannot be used to estimate the parameters. As a result, the logit model is evaluated through an iteration process by using a non-linear maximum likelihood estimation technique. The likelihood function for a logit model is:

$$\ln L = \sum_{i \in s} \omega_i \ln F(x_i \beta) + \sum_{i \notin s} \omega_i \ln\{1 - F(x_i \beta)\} \quad (5)$$

Where s is a set of all observation i, such that $y_i \neq 0$. $F(z) = e^z/(1+e^z)$, ω_i is an optional weight (Stata 13 documentation, 2014). The model was estimated assuming heteroscedastic error variance; robust standard errors are calculated instead of the usual standard error. Using the logistic model Equation 3, the following model was specified:

$$\begin{aligned}\ln(p_i/1-p_i) = {} & \beta_0 + \beta_1 Age + \beta_2 Gender + \beta_3 Farm\exp \\ & + \beta_4 Credit + \beta_5 Hardap + \beta_6 Karas \\ & + \beta_7 Kserongo + \beta_8 Omaheke + \beta_9 Sec + \beta_{10} Tert + \\ & \beta_{11} lack\inf + \beta_{12} Nobenefit + \mu_t \end{aligned} \quad (6)$$

Equation (6) was fit to estimate the probability of farmer participation in a cooperative. The assumption is that AGE, EXPERIENCE, and the level of EDUCATION have a positive relationship with willingness to participate. Three levels of education dummies were considered; SECONDARY, TERTIARY and NO EDUCATION, with NO EDUCATION serving as the base category. Regional dummies were included by considering the location of the decision maker. Four regional dummies excluding the reference category OTJOZONDJUPA were included. Technical constraints such as INFORMATION and the perception of the farmers towards the BENEFIT they can derive from cooperative membership are other variable included in the model. The third category for technical constraints, NOCOP, was omitted.

EMPIRICAL RESULTS AND DISCUSSION

Descriptive statistics

The descriptive statistics for the variables are given in Appendix Table A1. The mean of the MEMCOP variable is 0.2941 with a standard deviation of 0.4563. The result shows that on average, 29.41% are willing to participate. Average age and farm experiences are 46 and 22 years respectively. The most experienced farmer in the sample is 65 years. The youngest farmer is 18 years of age whereas, the oldest is 83 years - indication that the communal livestock farming community in the study area is made up of an aging population.

Farm and farmer characteristics

The farm and farmer characteristics are shown in Table 1. The average farmers' age ranges from 40 years for farmers in Omaheke to 52 years for those in Hardap. The least experienced farmer on average in Hardap is 19 years old whereas the most experienced farmer has farmed for at least 27 years in South Kunene and Erongo regions. Education is important in the farming enterprise because educated farmers are likely to have more technical knowledge on farm management than non-educated ones. They are better disposed of in terms of decisions making and price discovery, therefore, the literacy level of farmers in the sampled regions was determined. On average 33 and 30% of the respondents in Otjozondjupa and Omaheke respectively have secondary and tertiary educations, whereas 29 and 24% in these regions respectively do not have formal education. About 23% in Hardap and 16% in Southern Kunene and Erongo have no education. Gender demography shows that 81% of the total numbers of farmers sampled are men while 19% are women. The ratio of men to female in the five regions is Otjozondjupa (94%), Southern Kunene and Erongo (92%), Omaheke (82%), Karas 65% and Hardap 53%.

Membership of a cooperative

The study investigated the number, the level of participation and reasons for participation by these farmers. The number of cooperatives was found to be 47 in all the sampled regions. About (19) of these are in Omaheke, Southern Kunene and Erongo have (10), Otjozondjupa (8), whereas Hardap and Karas have (5) each. According to the Registrar of cooperatives (2016), there are only three multipurpose fully registered and about nine provisionally registered agricultural co-operatives. The number of forty-seven obtained in the survey is an indication that there are some cooperatives that are not on record. Perhaps these are the unregistered and undocumented multi-purpose cooperatives.

Out of the 340 respondents, 41% are members of various cooperatives. The largest number of participation was in Southern Kunene and Erongo region, with 38% participation, others are Omaheke (28%), Hardap (20%), Otjozondjupa (9%) and Karas (7%). The farmers were further asked to state reasons for not joining a cooperative. This question helps to capture the farmers' perception about the cooperative system of marketing. Forty-three percent of the respondents said they have no knowledge of the existence of cooperatives in their area. This is an indication that information dissemination is a major challenge. Thirty-one percent have no interest in becoming a member in the future because of the perception that cooperative system is dysfunctional and does not offer many benefits to its members. Other concerns are about trust and non-existence of livestock cooperatives in their area. Nine percent of the respondents do not trust livestock cooperatives, due to lack of transparency, they would rather operate alone than join a cooperative. About 11% claim cooperative system is good but it is difficult to organise farmers into the cooperative group.

Using Equation 6, some of the factors that influence farmers' decision to participation were investigated. The hypothesized relationship between farmers' participation in cooperatives and sets of covariates are shown in Table 2. The sign of the coefficients for the Secondary and Tertiary education variables are both negative and statistically significant. The result shows that the education level of the farmers' negatively and significantly influences the log odds of the decision to join a cooperative. Contrary to expectation, farmers seem to become more independent as their education level increases. With more skill and technical knowledge about market price discovery mechanism they seem to become averse to cooperative governance because according to them, they rather operate alone than join dysfunctional farmers' cooperatives system. The odd that farmers will join cooperatives with respect to their academic level is 0.0003 times smaller if they had a secondary education or a decrease of 0.03%.

Technical constraints such as lack of information and the perception of "no benefit" with coefficients (-2.7757) and (-2.2926) respectively also have a negatively and

Table 2. Parameter estimates of farmers' membership of a cooperative.

Variables	Coefficients	z-statistics	P-value	Odds ratio	z-statistics	P-value
Age	-0.0198	-1.2900	0.1950	0.9804	-1.2600	0.2080
Gender	-0.3639	-1.0100	0.3130	0.6950	-1.0000	0.3170
Farming experience	0.0066	0.4400	0.6590	1.0066	0.4300	0.6650
Loan	1.2082***	3.1600	0.0020	3.3474***	3.0400	0.0020
Hardap	2.1584***	4.6900	0.0000	8.6573***	4.3700	0.0000
Karas	0.8581	1.4900	0.1350	2.3587	1.5900	0.1110
Kunene South and Erongo	1.0711**	2.3300	0.0200	2.9187*	2.3400	0.0190
Omaheke	1.9668***	4.8300	0.0000	7.1477***	4.9000	0.0000
Secondary	-7.9658***	-15.4000	0.0000	0.0003	-0.0100	0.9900
Tertiary	-15.8545***	-19.9800	0.0000	0.0000	-0.0100	0.9900
Lackinfo	-2.7757***	-3.1200	0.0020	0.0623***	-3.5600	0.0000
Nobenefit	-2.2926***	-3.0100	0.0030	0.1010***	-2.8500	0.0040
Constant	-8.6399***	-9.3400	0.0000	0.0002	-0.0100	0.9890
Diagnostic tests						
Wald / LR (χ^2)	521.9			83.05		
Probability > χ^2	0.0000			0.0000		
Pseudo R^2	0.202			0.202		
Log-Likelihood	-164.096			-164.096		
Number of observation	339			339		

The signs ***, ** and * Signifies statistical significance at 1, 5 and 10% levels, respectively.

statistically significant influence on participation given other variables are constant. This implies that an increase in the availability of information about the benefit of cooperatives results in an increase in the log odds of joining a cooperative. If there is an increase in the deterioration of the information system the odds that farmers' will participate will decrease by 6.23%. The lack of adequate information dissemination framework about cooperative system results in increased perception that it is not beneficial. A one unit increase in farmers' perception results in the decrease in the odds of their participation by 10.10%. A positive influence of information dissemination was also found in Jari and Frasers (2012:80).

It was found that an increase in the availability of farm credits statistically and significantly increases the likelihood that farmers will join a cooperative. This result lends credence to the influence of farmer support programmes that increases the sense of belonging to farmers thus increasing their enthusiasm and confidence. Increasing the level of farm credit increases the odds of their participation 3.34 times or by 34%. The result also shows that the odds of participation increase by 65, 91 and 14% if farmers were from Hardap, Kunene South / Erongo and Omaheke, respectively.

The diagnostic tests for the model estimated via a logistic distribution are shown in the lower panel of Table 2. The first row shows the chi-square statistics for the Wald test of the joint statistical significance of the estimated coefficients. The null hypothesis of the test is that the coefficients of the estimated model are all zero. The null hypothesis is rejected at one percent level of significance, signifying that at least, one of the regressors is different from zero. The test of joint statistical significance of the proportional odds ratio is a Likelihood ratio (LR) test which is similar to Wald test. The null hypothesis of zero coefficients is rejected as in the Wald test. The McFadden's (1974.) pseudo R^2 is estimated as measures of goodness of fit of the model. It is not equivalent to the R^2 obtained in linear ordinary least square models but mimics it. The pseudo R^2 value of 0.2020 calculated for the model is high, an indication of the goodness of model fit.

Marginal effects of regressors on the farmers' membership of a cooperative

In the linear regression models, the coefficients are interpreted as having marginal effects on the conditional mean of a one unit change in the relevant regressor(s), but in a non-linear model such as logistic regression, the coefficients are interpreted as the marginal effects on the conditional probability of an event happening, such as the probability of joining a cooperative. Three types of marginal effects are usually estimated namely; (a)

Table 3. Marginal effects of the explanatory variables on the likelihood of farmers' membership of a cooperative.

Variables	AME			MEM		
	Margin	Z-stats	P-value	Margin	Z-stats	P-value
Age	-0.0032	-1.31	0.192	-0.0034	-1.3	0.194
Gender	-0.0581	-1.02	0.306	-0.0617	-1.02	0.31
Farmexpr	0.0011	0.44	0.658	0.0011	0.44	0.658
Credit	0.193***	3.39	0.001	0.2048***	3.23	0.001
Hardap	0.3448***	5.18	0.000	0.3659***	4.87	0.000
Karas	0.1371	1.49	0.137	0.1455	1.49	0.137
Kunene South and Erongo	0.1711*	2.38	0.017	0.1816*	2.4	0.017
Omaheke	0.3142***	5.56	0.000	0.3334***	5.06	0.000
Secondary	-1.272***	-11.23	0.000	-1.3504***	-9.38	0.000
Tertiary	-2.533***	-12.43	0.000	-2.6876***	-9.88	0.000
Lackinfo	-0.443***	-3.4	0.001	-0.4705***	-3.39	0.001
Nobenefit	-0.366***	-3.1	0.002	-0.3886***	-3.08	0.002

***, ** and * Signifies statistical significance at 1, 5 and 10% levels respectively.

Average marginal effects (AME) that is, evaluation is at the sample value and then averaged, (b) Marginal effects at the mean - evaluation is at the sample means of the regressors and (c) Marginal Effects at a Representative value (MER) - evaluation is at a representative value. Two of these are estimated in this study; the AME and MEM. The parameters of the marginal effects for the logistic model are shown in Table 3. It can be seen that the results for AME and MEM are almost the same. This implies that either method can be used to explain the marginal effects of the coefficients on the conditional probability. On average, a unit increase in the level of secondary and tertiary education decreases the probability to join farmers' cooperative by 1.2720 and 2.533 units respectively. This is because, as previously discussed, when farmers gain knowledge about the industry through experience or by learning, they seem to be independent especially when they do not have the trust that their interest will be adequately protected by a cooperative. A unit increase in the technical constraints such as the lack of information results in a 0.443 and 0.366 unit decreases in probability to join cooperative. In contrast, improved infrastructural development increases the likelihood that a farmer will join a cooperative by the same amount. This is because; infrastructural development plays a key role in the development of efficient production and marketing system. For instance, lack of access to information results in the production and supply of livestock to the market when price signal is not favourable. Members of a farmer cooperative are less likely to experience this compared to non-participants. This is because cooperative gather and disseminate information about production inputs, market price and other logistics to their members such as (a) increased capacity and bargaining power, (b) access to new markets and marketing channels, (c) access to credit and support programmes, (d) access to better technical and market information, (e) more opportunities for exchanging experiences, and (f) greater access to training programmes (Santacoloma et al., 2009).

Regional effects were found to increase the conditional probability of participation. The marginal effects of joining a cooperative will increase by 34, 17.1 and 31.42% if the respondents are from Hardap, Kunene South and Erongo and Omaheke.

Predicted probabilities of joining of a cooperative

Here, the conditional probabilities that the outcome variable occurred as opposed to not occurred are estimated. This is equivalent to estimating the probability that the outcome variable is equivalent to one. The estimation is evaluated at the mean and at the individual representative sample. According to the result in Table 4, on average, the probability that farmers will join a cooperative, that is, the outcome variable Memcop = 1, is 29.50% given that all predictors are set at their mean. The reason for this low probability can be attributed to the challenges discussed previously; some of these were investigated with findings that are consistent with expectation. The average predicted probability by the logit model is 0.2950 compared to the average sample mean of 0.2941 shown in Table A1. There is actually no difference between the two implying that the model has a good predictive property and fits the data well.

Further in the analysis, the probabilities at a representative sample were also estimated. The aim was to investigate the cumulative impacts of variables of interest on the probability to join cooperative. The result in Table 5 shows that the young and inexperienced male farmers with secondary education from all the selected

Table 4. Average predicted probabilities

Variable	Margin	Std.Error	Z-stat	P-value	[95% Conf. interval]	
Membership of a cooperative	0.2950***	0.0203	14.5100	0.0000	0.2551	0.3348

***, ** and * signifies statistical significance at 1, 5 and 10% levels respectively.

Table 5. Predicted probabilities for farmers' willingness to participation in cooperatives.

Sensitivity	Farm EXP	Gender	Age	Hardap	Karas	Kunene South and Erongo	Omaheke	Secondary	Tertiary	Margin	Z-stat	P-value
Young and inexperience	10	1	25	1	0	0	0	1	0	0.3156***	3.0700	0.0020
	10	1	25	0	1	0	0	1	0	0.1116*	1.9100	0.0570
	10	1	25	0	0	1	0	1	0	0.1346**	2.4300	0.0150
	10	1	25	0	0	0	1	1	0	0.2757***	4.1800	0.0000
Old and experienced	35	1	62	1	0	0	0	1	0	0.2075***	3.0400	0.0020
	35	1	62	0	1	0	0	1	0	0.0666**	1.9700	0.0490
	35	1	62	0	0	1	0	1	0	0.0811***	2.6400	0.0080
	35	1	62	0	0	0	1	1	0	0.1777***	3.2500	0.0010

***, ** and * signifies statistical significance at 1, 5 and 10% levels respectively.

regions are more likely to join cooperatives than the elderly and experienced male farmers with secondary education. For example, a 25 years old male livestock farmer from Hardap region with ten years farming experience who have secondary education has 31.56% probability of joining a cooperative compared to the probability of 20.75% for an old and experienced male farmer of 62 years from the same region who have secondary education and have farmed for 35 years. The result shows that new entrants in the livestock business are likely to strive to capture favourable market position and price discovery mechanism through liaising and partnerships with stakeholders or through cooperative system. This association becomes a less popular option as the farmer becomes establish in the business, therefore, the willingness to pay a premium for a similar service for which he/she has the disposition to acquire at reduced cost declines.

Conclusion

This study investigated the challenges facing agricultural cooperative system by analysing the factors that influence farmers' willingness to participate. According to the literature review on the role of government and the theory of new institutional economics, government intervention, transaction cost, agency relations and property right have been found to play a crucial role in the organisation, growth and development of cooperatives. By embarking on this study, the extent of deterioration or breakdown in the cooperative order was put to a test. The result shows that farmers' demographic characteristics such as age, gender, experience do not contribute to explaining reasons why they join a cooperative. Farmer education and technical constraints such as information dissemination negatively affect the probability to join a cooperative. It was found in this study that, farmers are reluctant to participate in cooperative because of lack of trust, transparency and the suspect that their interest may not be adequately sustained. This attitude reflected in the findings of the study. On average, the probability that a farmer will join a cooperative was found to be 29.50%. It was further determined in the study that the younger and inexperienced farmers are more likely to join cooperative than older and experienced one. The results highlight a general lack of knowledge about the cooperative system by farmers. Therefore, there is a need for information and training in the livestock sector about the benefits of having cooperatives. Policy directives are needed to either strengthen the existing cooperative system or encourage them to transform into Investor-Oriented Firms (IOF).

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This study is part of the research commissioned by the National Namibian Farmers' Union. Gratitude is hereby expressed for their financial role. Special thanks are owed to South African Confederation of Agricultural Unions (SACAU), the regional farmers' associations' representatives, communal farmers' associations, communal farmers, auctioneers, livestock transporters, abattoirs, red meat exporters and all other value chain actors who assisted in one way the other to make the study a success.

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APPENDIX

Table A1. Descriptive statistics of the variables.

Variable	Mean	Std.Dev	Min	Max
Memcop	0.2941	0.4563	0	1
Farm experience	22.1976	12.3867	1	65
Gender	0.8118	0.3915	0	1
Age	45.8088	12.6088	18	83
Loan	0.1471	0.3547	0	1
Hardap	0.1500	0.3576	0	1
Karas	0.1000	0.3004	0	1
Kunene South & Erongo	0.1471	0.3547	0	1
Omaheke	0.2824	0.4508	0	1
Secondary	0.5676	0.4961	0	1
Tertiary	0.1676	0.3741	0	1

academicJournals

Vol. 11(31), pp. 2871-2881, 4 August, 2016
DOI: 10.5897/AJAR2016.11016
Article Number: 710EA6A59815
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Nitrate leaching in irrigated inorganic agriculture: A case study of Mashare commercial farm in Namibia, Okavango River Basin

Andrea Vushe[1]*, Loffie von Landsberg[2], Alexander Groengroeft[3] and Damas A. Mashauri[4]

[1]Department of Civil and Environmental Engineering, Namibia University of Science & Technology, Namibia.
[2]Mashare Commercial Farm, Namibia.
[3]Institute of Soil Science, University of Hamburg, Germany.
[4]Department of Civil and Environmental Engineering, Namibia University of Science & Technology, Namibia.

Received 19 March, 2016; Accepted 16 June, 2016

Irrigation schemes in old flood plains of the Okavango River were identified as major non-point sources of sediment nutrients and leachates despite lack of supporting evidence from studies that measured nutrient levels in the river's mainstream using grab samples. Hence this study sought to check for evidence of loss and transport of nitrates from an irrigated field into the uncultivated riparian zones of the Okavango River. Soil nitrates were tested for using an Eutech ion 6+ pH/mV meter and a nitrate ion selective electrode, in soil samples taken from an irrigated field, a control site and a depression receiving storm water drained from the irrigated field at the Mashare commercial farm. Based on analysis of farm records' fertilizer application rates and soil nitrates results, it was inferred that maize crops grown in the rainy summer seasons contributed more nitrogen fertilizer losses compared to wheat crops planted in dry winter seasons. The top soil derived from Kalahari sandy soils retained more nitrates compared to the subsoil which had high contents of light-coloured calcrete, which contained low nitrate levels especially when dry. High nitrate levels in horizons 150 cm below the root zone, at a 240 cm depth, and more than twice nitrate levels in the vlei compared to the irrigated field and an uncultivated field proved that there was leaching of nitrates from the irrigated into the uncultivated riparian zones of the Okavango River.

Key words: Calcrete, irrigated field, leachate, maize, nitrates loss, Okavango River, wheat.

INTRODUCTION

Agricultural activities and deforested lands are the major non-point sources of sediment, pesticides, nutrients, and pathogens, which are difficult to measure and control (FAO, 2005 a). Loss and transport of nutrients from agricultural fields into rivers occurs through soil erosion and leaching which can be major factors that limit the nitrogen utilisation efficient of crops.

Loss of nitrate-nitrogen because of leaching (washing) from the bottom of the crop root zone is a loss of money to farmers as it result in reduced yields or a need to apply

*Corresponding author. E-mail: avushe@nust.na. Tel: +264 (85) 6377112.

more nitrogen, and the fertilizer contaminates water resources (FAO, 1985). The lost nutrients are pollution threats to receiving water bodies and may increase water treatment costs.

Periodic measurements of nutrient concentration in river water can be appropriate for assessing impact of non-industrialised settlements and agricultural activities (Williamson et al., 1999; Mathuthu et al., 1997; Zaranyika, 1997). Okacom (2010) and Trewby (2003) suggested that Okavango River basin's commercial farms irrigating crops on highly permeable sandy soils could be polluting the river with nutrients more than any other land use. Vushe et al. (2014) found out that Okavango River water flows from Angola were of excellent quality (according to Namibian Standards), and the nutrient load was attenuated in the Namibia reach despite over hundred percent increase in irrigated area in Namibia in a period of ten years. There were no statistically significant differences in total phosphorus and total nitrogen between 1993 and 2012, and nutrient measurements in 2011 and 2012 showed that the water was passed on to the Okavango delta (in Botswana), with a lower nutrient load. The assays of nutrient levels in the mainstream water failed to prove that nutrients lost from the irrigated fields were entering the Okavango River's mainstream.

Generally, transports of nutrients from farms into mainstreams through groundwater contamination develop gradually for several years (20 to 30 years) before it becomes apparent (Covert, 2014). Annually, the irrigated farms of the Okavango River basin produce two crops (maize and wheat), with a nitrogen application rate of over 400 kg ha^{-1} per year (Trewby, 2003; Vushe et al., 2014). The general target for nitrogen fertilizer application rates for maize is about 250 kg ha^{-1}, which is about 1.5 times more than the average amounts recommended in South Africa (du Plessis, 2003) and USA's Corn Belt (Sawyer, 2007; Alley et al., 2009). Between 1993 and 2012 human activities along the river increased and intensified, especially irrigated agriculture increased from 300 Ha to 2600 Ha (Vushe et al, 2014).

Therefore, for the nutrient load to be apparent in the Okavango's mainstream, the attenuation capacity of the river's ecosystem must be overwhelmed by the nutrient load from the anthropogenic activities. Vushe et al. (2014) found out that the land use changes in the Kavango Region had low impact on the nutrient water quality of the Okavango River, although Okacom (2010) and Trewby (2003), had suggested that irrigation schemes could be the worst polluters.

Therefore, this study sought to check for evidence of loss and transport of nutrients from an accessible irrigated field into the uncultivated riparian zones of the Okavango River. Nitrates were selected as the indicator nutrient for an assay of loss of nutrients from the irrigated fields because the highly soluble nitrate anion is normally repelled by the soil particles leaving it free to be transported by the water in the soil. This form of N is of special environmental concern and it is generally the form found in groundwater (Hermanson et al., 2000).

METHODOLOGY

In two field trips, soil samples were taken from the irrigated field, a vlei receiving storm water drained from the irrigated field, and a control site located between the irrigated field and the annually flooded riparian zone of the river. The soil samples were tested for nitrates. The control site was on an area elevated 2m above the vlei and cleared of vegetation. A soil auger was used to make a hole and for taking soil samples at 30 cm depth intervals down to hard formations, and sampling points were as shown on a map in Figure 1.

Soil sampling in the vlei was limited to a 210 cm depth. In the field, sampling was limited to 110 cm depth because of hard rocky layers. Hence a pit was dug in the field to a depth of 240 cm for testing for nitrates leached far below the root zone. During the first field trips the highest soil nitrate concentrations were obtained in the low lying northern edge of the field, therefore the position of the pit was selected on the low lying area of the field close to the vlei. The soil samples from each 30 cm horizon were mixed and 50 g of soil was weighed and 100 mL of deionised water was added to form a soil slurry. The soil slurry was allowed to settle and the supernatant water was filtered. Nitrate concentration and pH of the soil were measured using a Eutech ion 6+ pH/mV meter, pH electrode and a nitrate ion selective electrode. Soil nitrate measurements were done during the two separate field trips. The first set of field measurements was done a week after the harvest of a winter wheat crop. The second field trip was taken 35 days after a maize crop had been planted and after the onset of the summer rainy season.

Study area

The study area, the Mashare commercial farm on latitude S 19°53' and longitude E 020°11' is located on the southern old flood plains of the Okavango River 40 km east of Rundu Town in the Kavango East Region of Namibia. The region is a broad sandy plateau dominated by sandy soils, with an average slope of less than 0.07%. The area experiences semi-arid climatic conditions with an average rainfall of 577 mm and potential evaporation is more than 2600 mm per year (Vushe et al, 2013). Namibia contributes almost no surface water to the Okavango, although the length of the Okavango is over 400 kilometres, because Namibian tributaries drain predominantly semi-arid and arid areas (El Obeid and Mendelsohn, 2001; Okacom, 2010). The Okavango River basin's headwaters are located in the Angolan highlands, where the stream flow is generated. Namibia's intensive commercial crop production, even in the rainy summer season relies on irrigation water pumped from the perennial Okavango River.

The commercial farm under study uses a combination of two centre pivots each covering 30 hectares, linear systems and drag horse irrigation systems for irrigating mainly maize and wheat crops. Inorganic fertilizers are applied as a compound (NPK) at sowing and nitrogen is also applied in splits every week through fertigation. The total area under irrigation is 150 Ha and the field is located on the old flood plains of the Okavango River. Between the river's levee and the fields is about 100 m swath of savannah woodland. One thirty hectare centre pivot was selected as the field for this study as shown on the map in Figure 1.

The centre pivot under study has a gentle slope of 0.16% in the south to north direction, and hence surface water draining from the field at the northern edge flow via a steeper area with a 2.19%

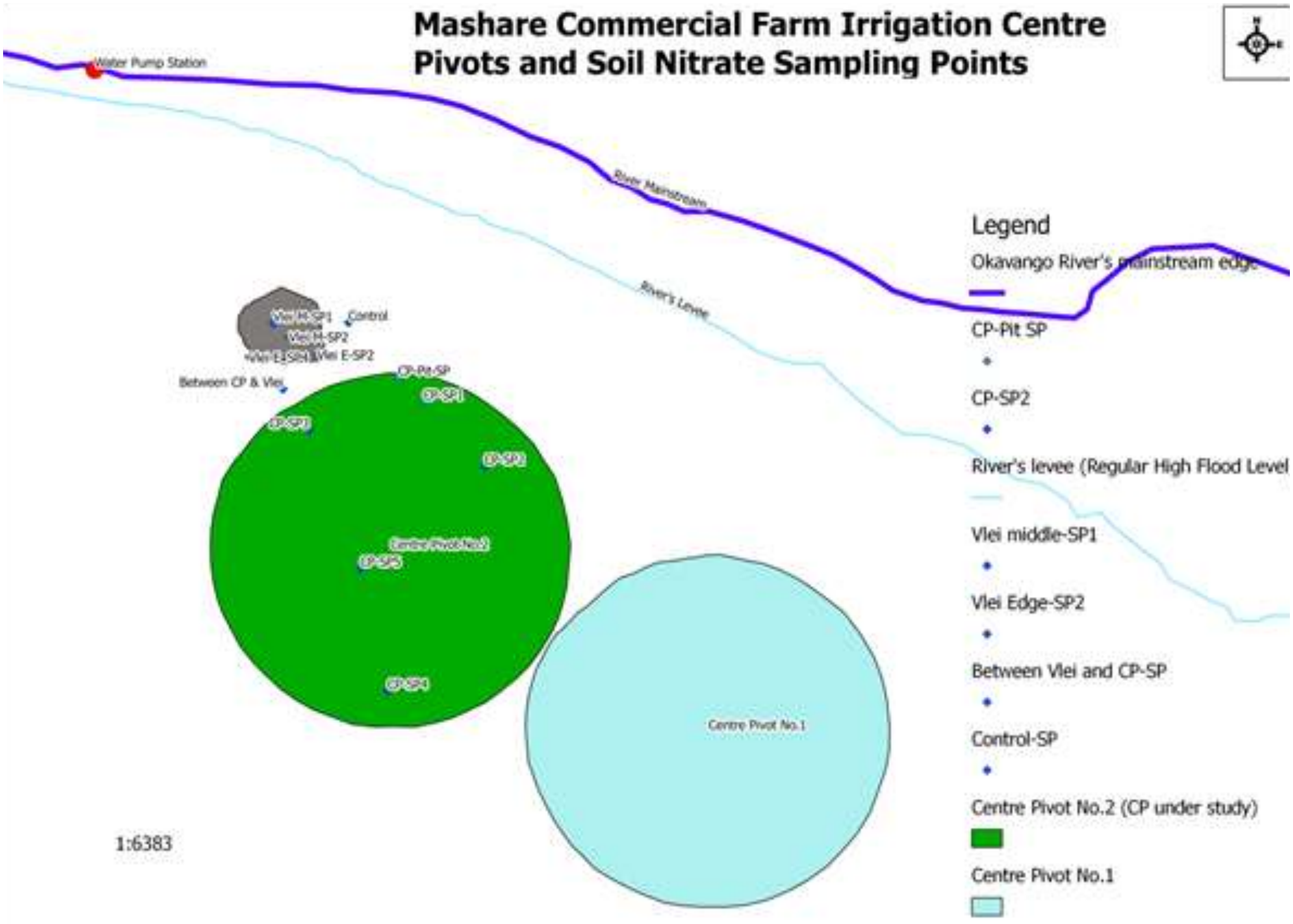

Figure 1. Map of Mashare Irrigation centre pivots and the soil sampling positions.

slope, into a depression or vlei. The vlei is a low lying area of approximately 135 m in diameter, which is located in the old flood plains, between the Okavango River levee and the field under study. Storm water does not flow directly into the Okavango mainstream but ponds in the vlei, and most likely it percolate through ground water flow channels into the river's mainstream. Also, when the Okavango is under extreme flood conditions the vlei is sometimes inundated by the mainstream's flood water. The field's northern edge and the vlei are separated by a 60 m stretch of uncultivated land, which was cleared of vegetation (trees, thorn bushes and grasses) in 2015. The northern edge of the vlei is about 140 m away from the Okavango River perennial high flood edge; the levees. The vlei's edge has sparse perennial grasses and the middle has sparse seasonal grasses. Also, scattered thorny bushes and trees thrive in the black clayey soils of the vlei that have high swell and shrink capacity.

Soils of the study area

The landscape at the study area is composed of three relatively flat land features. Directly south of the river's mainstream is a stretch of frequently flooded plain with dark coloured loamy soils that crack when dry, but the southern boundary of this plain is characterised by sandy levees, and humus rich depressions exist south of the levees. Further to the south a nearly plain area elevated a few meters above the highest river water level follows, which was formed in earlier phases of higher flood levels. These old floodplains are the preferred areas for irrigated crop production due to their soil fertility and safety from frequent flooding, and the Mashare Commercial farm is completely located in this landscape unit. About 150 m from the river's levees the area's gradient generally rises in the southwards direction. Here the land is composed of windblown sandy soils, called Kalahari sands, which have been levelled in part and nowadays forms a slightly undulating landscape covered with Baikiaea-Woodlands or used for cropping activities.

The distribution of soil properties is related to the landscape unit. Soils on the far expanding sandveld of the Kalahari sands are called Arenosols (Driessen and Dudal, 1991; FAO, 2006), which have high infiltration rates, due to coarse granular textures and lack of surface crust development (Ministry of Environment and Tourism, 2000; Andersson, 2006). Medium sands are the dominant size fraction of the Kalahari sands where the total medium sand content is generally about 60% and fine sand about 30%. Clay and silt are less than 10% and often around 6%. The soils are highly permeable and storage of available water is low within normal rooting depths. This is supported by high porosity values of about 42% with a predominance of large and free-draining pores. The sandy textures, cause poor moisture retention and hence rain fed crops can experience prolonged periods of moisture stress. Surface horizons hold slightly higher moisture contents of about 8% compared to subsurface horizons which hold 3 to 6% (Ministry of Environment and Tourism, 2000).

Within the old floodplains where the Mashare Irrigation Farm is located, soils textures were variable whereby soils in the fields are loamier than the Arenosols of the Kalahari sandveld. The mean topsoil texture was 10% clay, 6% silt, 38% fine sand and 45% medium sand. Due to clay illuviation, clay and silt content increases with depth up to 24% on average, therefore most soils in this part of the landscape were classified as Luvisols (Driessen and Dudal, 1991 in FAO, 2006). In the one to two metre depths of the old flood plain, the occurrence of calcrete was responsible for the high pH values and base saturation of the soils. Termites of the Macrotermes genus constructed their mounds, through material transports and construction of holes which improved soil conditions into finer textured soils hence the soils vary from loamy sands to sandy loams around the termite mounds which averaged ten mounds per hectare in some places (Turner et al., 2015). The finer texture improved the water holding capacity significantly compared to the pure sandy Arenosols, hence plant-available water may be about 100 mm per metre depth (Saxton and Rawls, 2006). Although the infiltration capacity of the soils is high because of the loamy texture, the presence of calcrete in subsoil and the compact soil structure may lead to perched water in the subsoil (Grigsby, 1992; National Research Council, 1995), probably causing root aeration problems. Irrigation thus has to be applied carefully not to over-saturate the soil with water and to damage plant growth.

Generally, the Kalahari sandy soils' surface clay contents are lower than subsurface horizons with an average of 6%, increasing irregularly to 14% in lower horizons (Ministry of Environment and Tourism, 2000), which may indicate higher water holding capacities and possibly nutrient retention capacities, in lower horizons (McClellan et al., 2014; University of Hawai'i at Manoa, 2015). Low clay contents combined with low and irregular levels of organic matter are also linked to relatively low nutrient concentrations. These soils are not infertile and they are also not highly productive (Ministry of environment and Tourism, 2000).

Maize is produced under supplementary irrigation in the rainy summer seasons, while wheat is produced in the dry winter seasons under full scale irrigation. For a target of 10 t ha^{-1} maize grain yield, farmers in the Okavango River basin apply about 250 kg per hectare of inorganic nitrogen fertilizers, but in the 2012/13 summer season some farmers applied above 400 kg N ha^{-1} for the maize crop (Vushe et al., 2014), in order to replaced fertilizer lost during numerous long wet spells caused by some torrential rainfall events. On average nitrogen fertilizer application rates are over 40% higher than the recommended rates in South Africa and USA's corn belt (Vushe et al., 2014). There was a high possibility of nitrate leaching in the highly permeable soils of the inorganic commercial farms in the Okavango River basin; hence the main objective of this study was to assess the occurrence of nitrate transport from the irrigated fields. The transport of leached nutrients into the rivers mainstream is expected to occur intermittently but peaks during the river's low flood periods, especially because groundwater flow direction is chiefly from the hinterland towards the river.

There is hardly any recharge of river water into the hinterland's Kalahari aquifer, evidenced by groundwater in the Kalahari aquifer along the banks of the river, which often shows poor quality due to its iron and manganese content, which exceeds the limits for drinking water and high fluoride concentrations in some places (Ministry of Agriculture, Water and Forestry and Ministry of Mines, 2001). Water in the Okavango's mainstream was classified as excellent for drinking (Group A) according to Drinking Water Standards of Namibia (Vushe et al, 2014), but the hinterland's groundwater was classified under poor quality, which indicated that there was low groundwater movement and dilution effects of water from the mainstream into the hinterland aquifer. The Kalahari aquifer recharge by the Okavango River only occured on rare occasions when the Okavango River floods the old flood plains, and this inflow of river water could locally dilute and improve the Kalahari aquifer's groundwater quality (Ministry of Agriculture, Water and Forestry, and Ministry of Mines, 2001). Therefore during the low flow seasons there could be a net flow of leachates from the irrigated farms into the river's ecosystem, hence this study sought for some evidence on presence of transported nitrate nutrients in the river's riparian zone.

RESULTS

Soil profiles

In the irrigated fields and the control site, top soils were mainly Kalahari sand types, but low lying areas and flattened termite mounds in the field had loam soils. A subsoil layer, which was a mixture of calcrete and the Kalahari sands, was observed in the 30 cm to 60 cm depths. In the field, soil sampling with an auger was limited to a depth range of 60 to 110 cm due to the presence of a light-coloured hard calcrete layer.

On the control site and between the field and the vlei, a combination of calcium silicate and hard calcrete rocks also limited soil sampling to a depth range of 60 to 110 cm. The manually dug pit in the irrigated filed had sandy loams in the top 30 cm, a mixture of sandy loam soil and hard light-coloured calcrete rocks in the 30 to 60 cm horizon, and in the 60 to 180 cm horizon there was a hard layer of the light-coloured calcrete rocks, which overlaid a softer reddish-brown calcrete layer. The softer reddish-brown calcrete layer was harder to dig as depth increased and hence the manual digging was limited to a 240 cm depth.

The vlei had a different soil profile to the irrigated field, since it is located at the lowest point, and it is the receiving or illuviation zone of the local soil catena that include the centre pivot irrigated field under study. The vlei has a soil depth of more than 200 cm, which is predominantly a black clay soil (vertisol) which cracks extensively when dry. The top horizons in the 120 cm depth were predominantly black clay soils while the lower horizons had mixtures of clay and light-coloured calcrete, and the amount of calcrete increased with increase in depth. At a depth of 210 cm there was a hard horizon consisting of a mixture of hard calcrete and calcium silicate stones.

Soil pH

Generally, the pH increased with increase in depth in the irrigated field, control site, the vlei and between the vlei and the irrigated field. In the irrigated field, the pH of top soil ranged from 5.81 to 6.77, while the 30 to 60 cm horizon had a pH ranging from 6.68 to 7.73. The control site's pH was 7.01 in the top soil and 7.46 in the 30 to 60cm horizon, but the vlei had the highest pH which ranged from 7.98 to 9.55.

Fertilizer application rates at Mashare commercial farm

For the 2015 wheat crop, Mashare commercial farm applied 194.6 kg N ha^{-1} of inorganic nitrogen fertilizers, harvested 5.5 t ha^{-1} and the protein content of the wheat was above 12%. The N fertilizer was applied as a compound fertilizer (2:3:2 NPK with Zn) at a rate of 21 kg N ha^{-1} at planting, followed by fertigation with 30.6 kgNha^{-1} as urea and 10 kgNha^{-1} as calcium nitrate in the first week. Fertigation with urea was also done in the third and sixth weeks, and at the flag leaf stage at rates of 30.6 kg N ha^{-1}. Fertigation with ammonium sulphate was done in the second and fourth weeks at rates of 10.5 kg N ha^{-1}. In the fifth week fertigation with a compound fertilizer (3:0:1 NPK) was done at a rate of 20.2 kgNha^{-1}.

For maize which is grown in the rainy summer season, the target application rate is 250 kg N ha^{-1}, but 12% more N fertilizer was applied in weekly fertigation splits due to long wet spells in the 2014/15 summer season, and the average yield was 10.5 t ha^{-1}. The nitrogen fertilizer application schedule was 30 kg N ha^{-1} at planting as a basal compound fertilizer (2:3:2 NPK & S + Zn) followed by fertigation with 25.76 kg N ha^{-1} of urea one week after planting and 20.2 kg ha^{-1} of urea in the second week. The weekly urea fertigation schedules of 25.76 kg N ha^{-1} and a 20.2 kg N ha^{-1} application in the following week were repeated five times until the 12th week. Generally, 80% of crop residues are incorporated into the soil for nutrients recycling and soil conditioning.

Soil Nitrates in the control site, irrigated field, between the field and Vlei, the Vlei's Edge and in the Middle of the Vlei

Soil nitrate tests were done on every 30 cm depth down to hard layers which limited the auger's penetration and soil sampling. The depths of the holes were limited between 60 cm and 120 cm except for two holes in the middle of the vlei that had a hard layer at a 210 cm depth. The results of soil nitrate measured per each 30 cm depth at each sampling point in the centre pivot (identified as CP-SP1 to 4), in the middle of the vlei (Vlei M-SP1 to 2), control, manually dug pit (CP-Pit-SP, between the vlei and the centre pivot (between CP and Vlei) and at the vlei's edge (vlei E-SP1 to 4) are shown in Table 1.

Soil Nitrates in centre pivot irrigated field after wheat harvest, before the rains

Five sampling holes were made with an auger and the depths of the holes were limited between 60 cm and 120 cm because of the light-coloured hard calcrete layer. The results of soil nitrate measured per each depth of soil on all five holes were similar to graphs obtained at the two positions shown in Figure 2 and Figure 3.

Soil nitrates tested after some rain events in the centre pivot irrigated field with a maize crop

Eleven days after the onset of the rainy season a pit was dug manually just at the northern edge of the field which had a maize crop, soil nitrates were measured on soil samples obtained on each 30 cm horizon, and results are shown in Figure 4. The top soil (30cm depth) had more nitrates (0.141 g per g soil) compared to the top soil nitrate levels of 0.007 g per g soil which were obtained after the wheat harvest as shown in Figure 3.

Soil nitrates in the Vlei

Soil nitrates measured after harvesting wheat, before the onset of the rainy season showed that the nitrate level in the vlei's top soil was less than 0.002 g per g soil maximum at the 100 cm depth of 0.168 g per g soil and there was a reduction with increase in depth after the 90 to 120 cm horizon as shown in Figure 5. The black clay soil was dry and cracked in the top soil, but soil moisture increased with depth after the 30 cm depth.

Figure 6 shows that after the first summer rains with a total of 82.5 mm in 6 rainfall events in 27 days, soil nitrates levels in the vlei had increased in all horizons to a maximum of 0.311 g per g soil in the 60 to 90 cm horizon compared to nitrate levels measured just after the wheat harvest, before the rains' onset. The top soil had 0.0 g per g soil and the maximum nitrate level was 0.168 g per g soil in the 60 to 90 cm horizon.

DISCUSSION

The nitrogen fertilizer application rates of 194.6 kg ha^{-1} for wheat were similar to rates recommended in Australia, where 176 kg N ha^{-1} could be applied if the target yield is 5 t ha^{-1} of wheat grain with 10% protein, and 210 kg ha^{-1} of N fertilizer was recommended for a target yield of 5 t ha^{-1} of wheat grain with 12% protein (Department of Agriculture and Fisheries, 2012).

In South Africa, the recommended fertilizer application rates for maize was 175 kg N ha-^{1} for a target yield of 8 t

Table 1. Soil nitrate levels test results in first and second field campaigns.

Sampling sites	Depth (cm)	pH	Soil nitrate (g/g soil)
Soil nitrate tests: First campaign			
CP-SP1	30	6.77	0.055
	60	6.97	0.001
	90	6.98	0.001
	120	6.98	0.001
CP-SP2	30	6.77	0.104
	60	7.73	0.020
CP-SP3	30	5.81	0.007
	60	7.17	0.001
	90	7.43	0.001
	120	7.68	0.001
CP-SP4	30	6.48	0.024
	60	6.68	0.002
CP-SP5	30	6.354	0.002
	60	7.065	0.002
Vlei M-SP1	30	8.33	0.004
	60	8.43	0.144
	90	8.47	0.168
	120	8.67	0.164
	150	8.42	0.114
	200	7.98	0.028
Control	30	7.01	0.007
	60	7.46	0.002
Soil nitrate tests: Second campaign			
CP-Pit-SP	30	6.71	0.141
	60	6.95	0.090
	90	8.21	0.118
	120	7.36	0.002
	150	8.09	0.002
	180	6.71	0.013
	210	8.29	0.045
	240	6.94	0.099
Between CP and vlei	30	6.85	0.128
	60	6.69	0.052
	90	8.41	0.002
	110	6.86	0.002
Vlei edge-1	30	6.1	0.030
	60	6.8	0.014
	90	6	0.000
	120	5.85	0.066
	135	6.73	0.066
Vlei E-SP2	30	5.96	0.003
	60	5.91	0.002
	90	6.73	0.004
	120	7.05	0.002
Vlei E-SP3	30	6.57	0.054
	60	6.68	0.001
	90	6.55	0.001
	110	5.75	0.003
Vlei E-SP4	30	5.9	0.223
	60	7.27	0.070
	90	6.95	0.063
	120	6.52	0.020
	150	6.59	0.057
	170	6.77	0.020
Vlei M-SP2	30	8.58	0.098
	60	9.11	0.150
	90	9.55	0.311
	120	9.5	0.221
	150	8.33	0.084
	180	8.59	0.233
	210	8.52	0.163

ha^{-1} (FAO, 2005b; van der Linde and Pitse, 2006), which were similar to rates recommended in USA's Corn Belt (Sawyer, 2007; Alley et al., 2009). A maize yield of more than 10.2 t ha^{-1} can be harvested if 250 kg N ha^{-1} is applied on a field with a maize plant population of 60000 plants ha^{-1} (FAO, 2006).

For the 2014/15 summer maize crop, the Mashare farmer applied 280 kg ha^{-1} of N fertilizer and obtained a yield of 10.5 t ha^{-1} which indicated more N losses. The increase in N fertilizer application rates from 175 to 286 kg ha^{-1} led to an increase in yield from 8 to 10.5 t ha^{-1}, which indicated a reduction in fertilizer use efficiency from 45.7 to 36.7 kg maize per kg N, and hence there were higher losses of the N fertilizer at the application rate of 280 kg ha^{-1}. This was similar to findings by Zotarelli et al. (2008) that an increase in N fertilizer application rates from 163 to 246 kg/ha, did not increase yield of a zucchini squash crop but decreased nitrogen use efficiency and increased the amount of leached nitrates by 73%.

The Mashare farmer applied more N fertilizer (through a weekly fertigation schedule) in order to compensate for N losses to leaching and gaseous emissions during long wet spells caused by the erratic summer rainfall. Leaching could be the main cause of higher N fertilizer application rates, since the farmer applied more inorganic

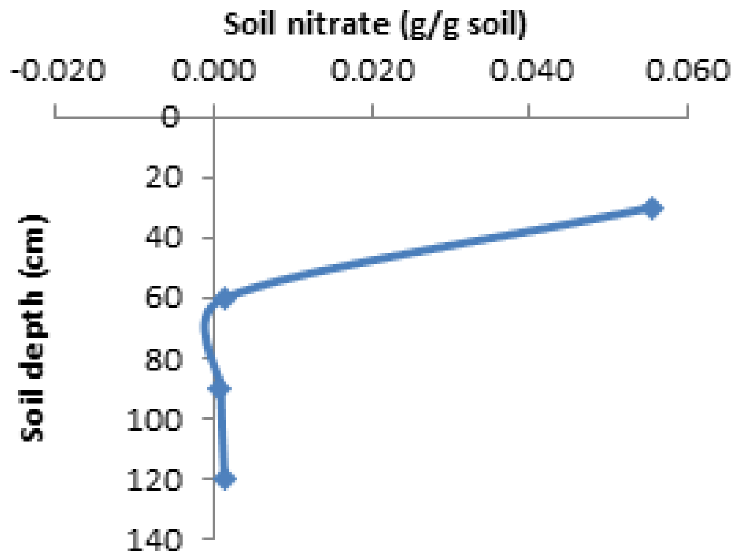

Figure 2. Soil nitrates measured in the centre pivot irrigated field after wheat harvest, sampled using an auger down to 120cm, on position CP-SP1.

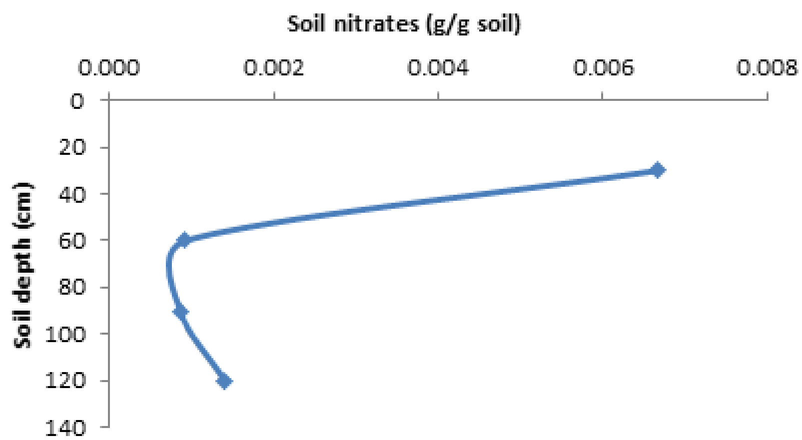

Figure 3. Soil nitrates measured in the centre pivot irrigated field after wheat harvest, sampled using an auger down to 120cm, on position CP-SP3.

fertilizers on a soil which had high hydraulic conductivity and a low water retention capacity. Application of fertilizers (especially organic fertilizers) that improve water and nitrate retention of top soil, and release the nitrates slowly just to meet crop nitrate demand even under saturated moisture conditions might reduce leaching of nitrates during the long wet spells. The farm manager rubbed soil samples between the finger and thumb for soil moisture measurement and irrigation scheduling. The method could be useful but it was less accurate compared to other irrigation scheduling methods that could be used to minimize the excessive fertigation.

An analysis of root zone soil nitrate concentration and matching fertigation schedules with a maize crop nitrate demand curve might help in reducing the N fertilizer over application and nitrates leaching during the long wet spells. Further studies may be required, in order to evaluate if more accurate supplementary irrigation scheduling methods complimented with agronomic practices that increase nitrate retention capacity of the root zone can reduce fertilizer application, and hence reduce leaching, without causing maize yield reductions.

According to Bowman et al. (1998), environmental conditions and management practices may affect N nutrition and nitrate leaching. Wheat is grown in the cool and dry winter months while maize is grown in the rainy

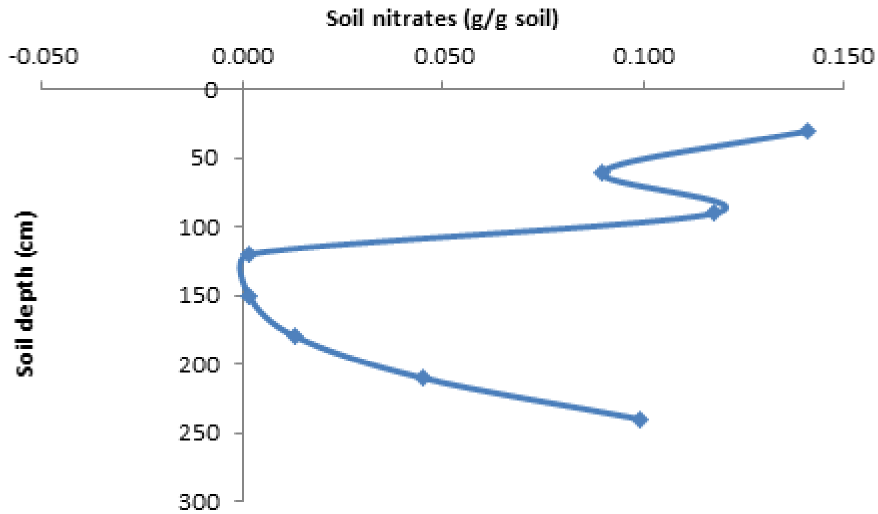

Figure 4. Soil nitrates measured after onset of rainy season, in pit dug down to 240 cm, on position CP-Pit-SP on the northern edge of centre pivot irrigated field.

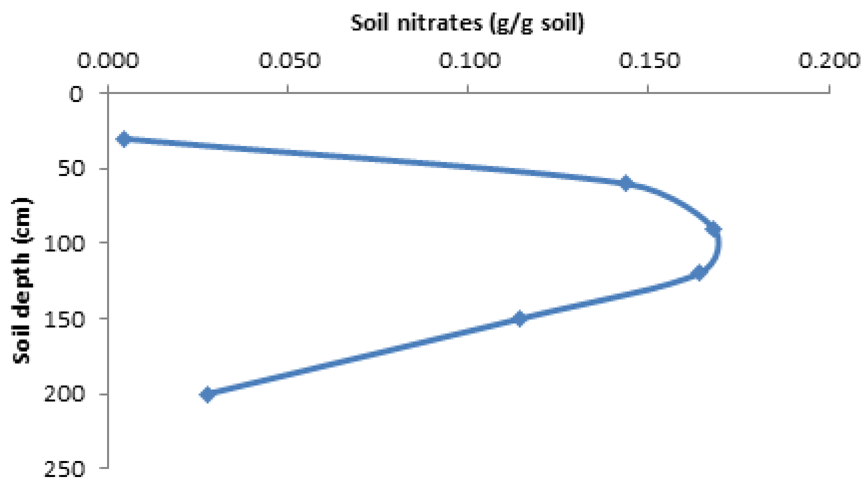

Figure 5. Soil nitrates measured in the vlei before just after the wheat harvest, sampled with an auger down to 200 cm.

summer season, and the agronomic management practices are different, hence for reducing nitrate leaching, different soil fertility and moisture management strategies are required for the two crops. The earlier mentioned strategies for minimizing nitrate losses to leaching are:

Improving the soil properties for higher soil nitrate retention and leaching reduction, and Use of more accurate fertigation schedules; may be complimented with methods that enhance recovery of nitrates and

curtail transport of nitrates from the old flood plains into the river's current flood plains. For example, identifying subsurface leachate draining zones between the field's edge and the river's levee, and planting deep rooted crops or tress to take up the nitrates in the drainage flow lines may mitigate nutrient pollution of the Okavango River's ecosystem.

Table 1 showed that after the wheat harvest; nitrates in the top soil were in the range 0.001 to 0.104 g per g soil, and the subsoils nitrate levels ranged 0.001 to 0.02 g per g soil. The lowest nitrate levels were obtained near the middle of the centre pivot irrigated field. The highest soil nitrate concentration was obtained in the low lying northern edge of the field probably due to transportation and deposition of nitrates from upslope to lower lying parts of the field by storm water runoff. The top soil had

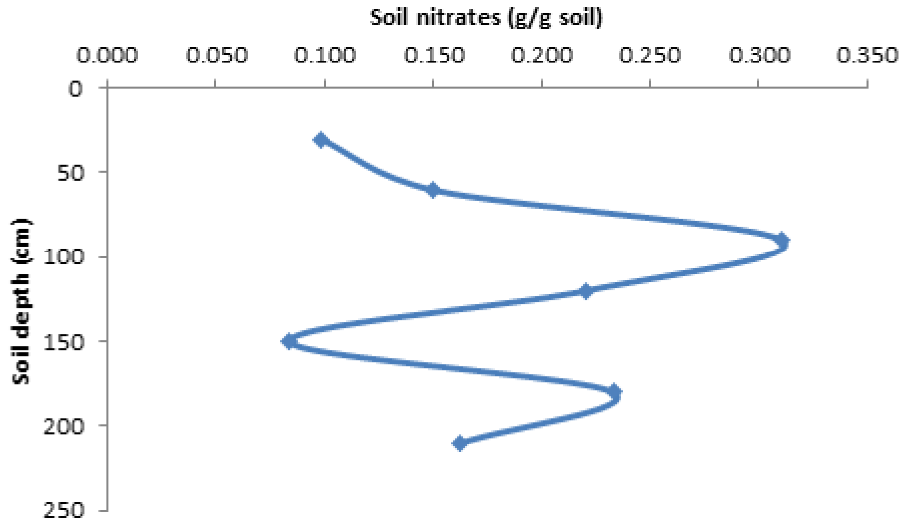

Figure 6. Soil nitrates measured in vlei after the first rains sampled with an auger down to 210 cm.

higher nitrate levels probably due to a higher nitrate retention capacity because of a higher water holding capacity which is usually enhanced by a higher content of organic matter of top soils of the Kalahari sands (Ministry of environment and Tourism, 2000). Also, organic matter provides a source of nitrogen, increases water-holding capacity, and improves nutrient holding and release (McClellan et al., 2014; University of Hawai'i at Manoa, 2015).

The elevated nitrate levels in the irrigated field, the vlei's edge and in the middle of the vlei (ranged 0.004 to 0.311 g per g soil) compared to the control (ranged 0.002 to 0.007 g per g soil) showed that the vlei area's nitrate level was receiving nitrates from the agricultural activities either as overland flow or through groundwater leachate flows. This was indicated by the high nitrates levels in both the top soil and the subsoil of the sampling points at the edge of the vlei, and between the vlei and the field. The control site had lower nitrate levels which were contributed by non-anthropogenic natural sources.

When the irrigated field had a maize crop, the nitrates levels of 0.141 g per g soil were highest in the top soil (0 to 30 cm depth) of the field (as shown in Figure 4), because N fertilizers were applied weekly through fertigation. The relatively high nitrate levels (0.118 g per g soil) in the 60 to 90 cm horizon might be due to nitrates that were transported by percolating water from upper horizons (subsoil), because 82.5 mm of rainfall was received after the maize crop's planting date (11/11/2015), and in particular two consecutive days got heavy rainfall amounts of 30 mm on 16/11/2015 and 23 mm on 17/11/2015. The two consecutive rainfall events caused a long term wetting of the partial impermeable calcrete, which only allow downward water movement when subjected to continuous wet conditions for over three hours (Grigsby, 1992; National Research Council, 1995).

Hence leaching of nitrates could have occurred, and probably aggravated by antecedent moisture from preceding irrigation events. The tests for soil nitrates were done 11 days after the last heavy rainfall of 22 mm on 04/12/2015, and the soil nitrate levels were 0.141 g per g soil in the top soil (0 to 30 cm depth). Zotarelli et al. (2008) measured nitrate levels in 0-30cm depth of a sandy soil (with 97% sand-sized particles) 51 days after planting a zucchini squash crop, 163 and 246 kg/ha N fertilizer had been applied, and the soil nitrate levels were 113 and 274 mg/L in the soil solution. This can be compared to the soil nitrate levels of 1410 mg/L in soil solution (0.141 nitrates per g soil shown in Figure 4). The higher nitrate levels showed that the Mashare farmer applied some nitrogen fertilizer after the heavy rains in order to compensate for possible losses of nitrogen nutrients.

Figure 4 showed that nitrates were almost absent (0.002 g nitrates per g soil) in the light-coloured calcrete layer which dominated the 120 to 180 cm horizon, although the layer overlaid the 180 cm to 240 cm horizon, which had higher nitrate levels (0.013 to 0.099 g nitrates per g soil). This indicated a low nitrate retention capacity of hard light-coloured calcrete layer and the resistance of the hard calcrete layer to capillary movement of the leachate. Therefore the nitrates under 180 cm depth was identified as the leached nitrates and hence lost from the field to the environment because the maize and wheat crops (with less than 100 cm effective root depths) were unable to utilize the nitrates leached below the 180 cm depth. The increased nitrate concentration under the 180 cm horizon corresponded to a change in geological formation from hard light-coloured calcrete to a softer reddish-brown calcrete formation, and the later could have higher water retention capacity due to a higher porosity. The soft reddish-brown calcrete zone could be one of the main geological formations through which the nitrate leachate is transported from the irrigated field into the vlei. Further studies could give details on the effectiveness of the soft reddish-brown calcrete horizon in transportation of leachates from the irrigated fields.

As shown in Figure 5, the dry and cracked top soil (0 to 30cm depth) had low nitrate levels of 0.004 g per g soil which indicated that nitrates could have been lost, probably to the atmosphere through denitrification (Tindall et al., 1995). Generally moist black clay soil has low permeability, but under arid conditions it dries and cracks, and the cracks can conduct water rapidly as preferred pathways downward water flow (Yong and Warkentin, 1975; Mitchell and van Genuchten, 1993). Also, Tindall et al. (1995) stated that clay may only retard leaching of nitrate and leaching loss is significant in both sand and clay. Therefore the vlei's black clay could allow percolation of the nitrate leachate to lower horizons. Figure 5 showed an increased levels of moisture content and nitrate levels in the 30 to 150 cm depths of the vlei, which had a range of 0.114 to 0.168 g nitrate per g soil, while the control site had 0.002 to 0.007 g nitrate per g soil, which showed that the nitrates transported from the field by the leachate and overland flow were being deposited in the vlei.

Generally, denitrification is relatively low in moist clay soils compared to sandy soils, because under unsaturated conditions, the clay has little to no tendency to denitrify due to high moisture content of the clay (Tindall et al., 1995). This explained why the moist horizons of the black clay soil had higher levels of nitrates compared to the dry top soil. The light coloured clayey soils at the 200 cm depth had high amounts of calcrete and calcium silicate stones and lower levels of nitrates despite a higher moisture content compared to upper horizons. The lower nitrate levels at the 200 cm depth showed that the calcrete and calcium silicate stones had probably lost some nitrates through leaching into deeper horizons. As shown in Figure 5 and Figure 6, the nitrate levels in the 120 to 150 cm horizon, before the rains and after the rains remained almost constant (decreased by 0.03 g per g soil), probably because the soil in the 120 to 150cm horizon had a low nitrate retention capacity that got saturated at a level less than 0.12 g nitrate per g soil. Therefore the horizon released excess nitrates to lower horizons. Therefore, if the vlei is hydro geologically connected to the Okavango's main stream the leached nitrates eventually enter the mainstream through groundwater flow channels.

Conclusion

For a target yield of 10.5 t ha^{-1}, more than 286 kg ha^{-1} of N fertilizer was applied to maize crops grown in the rainy summer seasons compared to 170 N kg ha^{-1} application rates recommended internationally for a target yield of 8 t ha^{-1}. Hence maize production might have contributed more leached nitrates compared to the wheat crop produced in the dry winter season, and a yield of 5.5 t ha^{-1} was harvested when the fertilizer application rate was 194.6 kg N ha^{-1}, which was within international recommendations.

Evidence of nitrate leaching was shown by the high nitrate levels in soil horizons at 240cm depths, which were 150 cm deeper than the root zone. The hard light-coloured calcrete had low nitrate levels compared to the underlying soft reddish-brown calcrete horizon located below the 180 cm depth, which might be the most likely horizon and pathway for transportation of nitrate leachate. Evidence of nitrate transport from the irrigated field into the uncultivated riparian zones of the Okavango River were proved by nitrate levels in the vlei which were more than twice the soil nitrate levels of irrigated field and the control site.

The top soil (0 to 30 cm horizon) had higher nitrate levels compared to the subsoil which had higher contents of light-coloured calcrete, and the calcrete contained no nitrates when dry. The nitrate concentration in the soil decreased with increase in depth in the irrigated field and uncultivated fields in all soil horizons containing high calcrete content, showing that the calcrete had low nitrate retention capacity.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are very grateful to the BMZ (Germany Government) for financial support under The Future Okavango Research Project, Management of Mashare Commercial Farm for giving us assistance, agronomic data and permission to do field tests. The data and information and support from managers of several other irrigated commercial farms in the Okavango River basin, Namibia University of Science and Technology and Namibian government departments is also appreciated.

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academicJournals

Vol. 11(31), pp. 2882-2892, 4 August, 2016
DOI: 10.5897/AJAR2016.11025
Article Number: E969FD559817
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Study of the variability, correlation and importance of chemical and nutritional characteristics in cactus pear (*Opuntia* and *Nopalea*)

Francisco Abel Lemos Alves[1]*, Albericio Pereira de Andrade[2], Riselane de Lucena Alcântara Bruno[3] and Djalma Cordeiro dos Santos[1]

[1]Agronomic Institute of Pernambuco, Av. General San Martin, 1371, Bongi, 50761-000, Recife-PE, Brazil.
[2]Federal University of Paraíba, Centre of Agricultural Sciences, Campus II, 58397-000, Areia-PB, Brazil.

Received 21 March, 2016; Accepted 20 May, 2016

Cactus pear is a widely cultivated plant in the Brazilian semiarid region that contributes significantly to the feeding of livestock, especially in times of drought. Because it has a great phenotypic variability among the varieties cultivated in Brazil, there is need to characterize the diversity of chemical and nutritional characteristics. The objectives of this study were to characterize the diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 20 chemical and nutritional characteristics, and to determine the correlation and importance of these characteristics in the variability among genotypes, using multivariate analysis techniques. The study was conducted at the IPA (disambiguation) experimental station in Arcoverde-PE, using randomly designed blocks with three replications. The materials IPA-100003, IPA-200016, IPA-200008, IPA-100004, IPA-200021, IPA-200205 and IPA-200149 were evaluated for 20 quantitative characteristics of the plants. The collected data were analyzed by analysis of variance by F test and means grouped by the Scott-Knott test ($p < 0.05$). The broad-sense heritability and correlation among characteristics were estimated. The diversity was estimated by multivariate methods. Analyses of variance and diversity revealed significant differences among genotypes, with the possible formation of three or four genetically distinct groups. The heritability values ranged from 78.04 to 99.99%. The content of flavonoids and potassium were the characteristics that contributed most to the divergence among the materials. These characteristics are significantly correlated with the nitrogen-free extract and phenolic compounds. The confirmation of variability among the cactus pear varieties studied serves as potential materials in breeding programs. Multivariate analysis techniques are effective in the study of diversity of species of the genera *Opuntia* and *Nopalea*.

Key words: Brazilian semiarid region, characterization of forage, food analysis, genetic distance, grouping, multivariate analysis.

INTRODUCTION

Cactus pear (*Opuntia* spp. and *Nopalea* spp.) is native in tropical and subtropical America, but currently is in a wide range of soil and climatic conditions in all continents (America, Africa, Asia, Europe and Oceania), both in cultivated and wild forms. These plants are used in these countries for various purposes: production of fruits and vegetables for human consumption; fodder for animal feed; soil conservation; biomass for energy (biogas and ethanol); production of cochineal for carmine production; and numerous by-products, such as drinks, vegetarian cheese, medicines and cosmetics (Dubeux-Junior et al., 2013).

In Brazil, there are currently about 600,000 ha cultivated with species *Opuntia ficus indica* (cv. Giant and Round) and *Nopalea cochenillifera* (cv. Small palm). Despite recent government efforts to diversify the use of this culture, most planted areas is still devoted to forage production. For being cactaceous, with numerous anatomical, morphological, physiological and chemical characteristics of adaptation to the ecological conditions of arid and semiarid environments, it has become one of the main forage plants used in ruminant feed, whether goats, sheep and cattle in the Brazilian semiarid. This region is characterized by having a high index of annual evaporation, higher than 2,000 mm, and annual average rainfall of less than 750 mm, concentrated in a single period of 3 to 5 months. In addition, many areas in the region are salinized (> 4, 0 $dS.m^{-1}$) (Araújo et al., 2005; Dubeux-Junior et al., 2013).

Research has revealed that cactus pear has a high content of various chemical compounds considered natural herbal remedies that can add value to their products. The cladodes, fruit, seeds, flowers and roots are used in the treatment of gastritis, fatigue, liver damage, digestion, general detoxifying, hyperglycemia, hyperlipidemia, acidosis, arteriosclerosis, wound healing, gastric ulcer, anti-inflammatory, neuroprotective, antimicrobial, antioxidant, etc. In those parts, hydrocolloid fibers, pigments, minerals, vitamins and bioactive substances are found with nutraceutical action (Nazareno, 2013; El-Mostafa et al., 2014).

By having a wide genetic diversity in genera *Opuntia* and *Nopalea* with about 300 species (Mondragón-Jacobo and Pérez-González, 2001), there is the need to characterize the genetic diversity of the varieties grown in Brazil. In the studies of the genetic diversity of plant species, researchers are interested in grouping similar genotypes, so that the greatest differences occur among the groups formed. In this respect, multivariate techniques, such as discriminant, principal component, coordinate and grouping analyses may be applied. The adoption of one of the techniques mentioned varies according to the desired pattern result, precision, ease of analysis and available information, whether a morphological, physiological, ecological, chemical or molecular characteristic (Ferreira et al., 2003).

The objectives of this study were to characterize the diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 20 chemical and nutritional characteristics, and to determine the correlation and importance of these characteristics in the variability between genotypes, using multivariate analysis techniques.

MATERIAL AND METHODS

Location of the experiment

The study was conducted at the experimental station of Arcoverde, of the Agronomic Institute of Pernambuco (IPA), located in Arcoverde-PE (8°25'S 37°05'W), altitude of 680.70 m, average annual temperature (22.90±1.68°C), average annual relative humidity (69.60±5.30%), average annual wind speed (3.92±0.48 m s^{-1}), average cumulative evaporation (1700.40 mm), average annual cumulative rainfall of 798.1 mm, microregion of sertão of Moxotó (Inmet, 2015).

Plant material and conducting the experiment

The materials used are listed in Table 1. The cladodes of the clones were planted on the 22 and 23 of April 2010, spaced 1.0 x 0.5 m; using one cladode per hole. The experimental design was a randomized block design with three replications. Each block consisted of three rows with eight plants of each variety. The experimental plot was composed by the middle row, with six useful plants, 3.0 m^2 of useful area. The soil was fertilized 30 days after planting, with 20 $t.ha^{-1}$ of manure spread between the lines. Periodically, cultural practices were carried out in the form of weeding with hoe in all the cultivated area. The collection of materials were held at 8:00 a.m. on February 19, 2013 (dry season). After collection, the material was cleaned, cut into small pieces (2 to 3 cm in length), and dried in a forced-air oven at 55°C, where it remained for 72 h until constant weight, in which the air-dried mass was obtained. The dried material was crushed in a Willey® type mill and packed in sealed plastic containers for the chemical and nutritional determinations.

Determination of chemical and nutritional characteristics

These were determined: the contents of total phenolic compounds (PC), total flavonoid (FLAV), total anthocyanins (ANT), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulfur (S), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE).

The PC were determined according to the methodology described by Gulcin et al. (2004) using spectrophotometric method, and the results expressed in mg of GAE (gallic acid equivalent) per gram of dry mass (mg GAE g^{-1} DM). The FLAV were determined according to Pereira et al. (2009) using spectrophotometric method and the results expressed in mg of QE (quercetin equivalent) per gram of dry mass (mg QE g^{-1} DM). The ANT, according to Lemos (2008) using spectrophotometric method, the results were expressed in µg of QE (quercetin equivalent) per 100 gram dry matter (µg QE. 100 g^{-1} DM).

The N was determined by the Kjeldahl method; P by blue

*Corresponding author. E-mail: abel.alves@ipa.br. Tel: +55 081 31847200; fax: +55 081 31847200.

Table 1. Cactus pear varieties, genera *Opuntia* and *Nopalea*, used in the study and grown in the state of Pernambuco, Brazil.

Number	Varieties	Species	Common name
1	IPA-100003	*Opuntia fícus indica*	IPA-20
2	IPA-200016	*Opuntia stricta*	Elephant Ear Mexican
3	IPA-200008	*Opuntia atropes*	F-08
4	IPA-100004	*Nopalea cochenillifera*	Small palm
5	IPA-200021	*Nopalea cochenillifera*	F-21
6	IPA-200205	*Nopalea cochenillifera*	IPA-Sertânia
7	IPA-200149	*Opuntia larreri*	-

colorimetry molybdenum; K and Na by flame photometry of emission; S by turbidimetric; Ca, Mg, Fe, Cu, Zn and Mn by spectrophotometry atomic absorption of accordance with Malavolta et al. (1997) and the results were expressed in $g.kg^{-1}$ or $mg.kg^{-1}$. DM, MM, CP, EE, CF and NFE were determined according to the study of Messias et al. (2013) using physical and chemicals methods, and the results were expressed in %.

Statistical analysis

The data were initially evaluated by analysis of variance (ANOVA), and the means were compared by the Scott and Knott test (1974), at the level of 5% probability. The broad-sense heritability was calculated by the estimator: $h^2 = \sigma^2_g / \sigma^2_p \times 100$; where: σ^2_g = genetic variance and σ^2_p = phenotypic variance. The genetic variance was calculated by the estimator σ^2_g = MSTreat – MSRes / J; where: MSTreat = Mean square of the treatment; MSRes = Mean square of the residue and J = number of repetitions (Rêgo et al., 2011).

Diversity among varieties was estimated using a measure of dissimilarity expressed by the Mahalanobis distance (D^2) according to Cruz et al. (2012). The hierarchical grouping method according to Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Tocher optimization method (Rao, 1952) and the method of Principal Component Analysis (Cruz et al., 2012) were performed.

The relative importance of characters in relation to diversity was studied according to the methodology described by Singh (1981) and the principal component analysis (Cruz et al., 2012). The correlations among the characteristics were obtained as described by Rêgo et al. (2011), and the probability of 1 and 5% were tested by t-test. Data analyzes were performed with the help of the statistical program GENES®- Computer Application in Genetics and Statistics (Cruz, 2001) and Assistat® 7.7 (Silva and Azevedo, 2006).

RESULTS AND DISCUSSION

The analysis of variance by F test ($p \leq 0.01$) showed significant differences in the content of phenolic compounds (PC), total flavonoid (FLAV), anthocyanins (ANT), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), iron (Fe), copper (Cu), zinc (Zn), total protein (TP), ether extract (EE), nitrogen-free extract (NFE), and $p \leq 0, 05$ for the nitrogen content (N), which confirms a variability among the varieties of cactus pear to most chemical and nutritional characteristics. There were no significant differences in the content of magnesium (Mg), sodium (Na), manganese (Mn), dry matter (DM), mineral matter (MM) and crude fiber (CF) in the plants (Table 2).

Guevara-Figueroa et al. (2010), studying the centesimal composition, content of cladode phenolic compounds of 10 cultivated and wild genotypes of *Opuntia* spp., reported differences among them in relation to PC, FLAV, CP, EE, CF, NFE and MM. Furthermore, Chahdoura et al. (2015) in a study about the nutritional composition of cladodes of *Opuntia microdasys* and *Opuntia macrorhiza* reported differences for EE, MM, NFE, CF, Cu, Ca, Mg, Na and K between them. These authors found no differences in DM, CP, Fe, Mn and Zn between these species.

Bensadón et al. (2010), studying the nutritional value of cladodes of *Opuntia ficus indica*, varieties Milpa Alta and Atlixco, found no difference between them in DM, CP, EE, MM, CF and PC. Furthermore, Batista et al. (2003), studying the chemical composition of cladodes of two varieties of the genus *Opuntia* (Giant palm and IPA-20) and one variety of genus *Nopalea* (Small palm) also reported no difference among them in relation to DM, CP, EE, MM, NFE, P, Mg, Fe, Cu and Zn. However, the researchers reported significant differences among these genotypes for Ca and Mn contents.

The genotypes showed an average variation in the content of PC (1.23 to 2.84 mg GAE g^{-1} DM), FLAV (1.52 to 3.02 mg QE g^{-1} DM), ANT (0.05 to 0.34 µg QE. 100 g^{-1} DM), N (6.41 to 13.73 $g.kg^{-1}$ DM), P (0.63 to 2.08 $g.kg^{-1}$ DM), K (1.83 to 7.74 $g.kg^{-1}$ DM), Ca (7.30 to 17.22 $g.kg^{-1}$ DM), Mg (4.63 to 7.09 $g.kg^{-1}$ DM), Na (0.11 to 0.14 $g.kg^{-1}$ DM), S (0.44 to 1.30 $g.kg^{-1}$ DM), Fe (14.68 to 69.27 $mg.kg^{-1}$ DM), Cu (2.07 to 93.02 $mg.kg^{-1}$ DM), Zn (23.33 to 63.79 $mg.kg^{-1}$ DM), Mn (246.12 to 598.45 $mg.kg^{-1}$ DM), DM (10.09 to 11.12%), MM (4.12 to 8.09% in the DM), CP (3.94 to 14.90% in the DM), EE (1.34 to 2.73% in the DM), CF (7.37 to 12.59% in the DM) and NFE (56.07 to 71.87% in the DM) (Table 3). Most of the values found in this study is in line with those reported in the literature (Batista et al., 2003; Bensadón et al., 2010; Dubeux-Junior et al., 2010; Guevara-Figueroa et al., 2010; Silva et al., 2012; Chahdoura et al., 2015).

The chemical components of plants vary both in

Table 2. Analysis of variance and estimates of the environmental variation coefficient (EV), ratio of genetic (GV) and environmental variation (EV) coefficients, broad-sense heritability (h^2), chemical and nutritional characteristics of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Mean squares											
VF	DF	PC	FLAV	ANT	N	P	K	Ca	Mg	Na	S
Block	2	0.00	0.00	0.00	7.51	0.04	0.09	13.23	2.82	0.00	0.00
Varieties	6	0.95**	0.85**	0.03**	20.22*	0.75**	4.97**	39.16**	1.87ns	0.00ns	0.25**
Residues	12	0.00	0.00	0.00	4.44	0.05	0.83	1.08	1.51	0.00	0.05
EV (%)	-	1.93	0.05	4.43	20.19	21.38	24.86	8.15	21.03	20.63	21.45
GV/EV	-	15.03	514.56	19.77	1.09	2.12	1.29	3.43	0.28	0.10	1.22
h^2 (%)	-	99.85	99.99	99.91	78.04	93.12	83.29	97.25	19.31	2.96	81.77
Mean squares											
VF	DF	Fe	Cu	Zn	Mn	DM	MM	CP	EE	CF	NFE
Block	2	251.18	60.54	32.88	792.28	0.15	3.48	0.06	0.01	17.50	35.58
Varieties	6	1151.08**	4240.17**	547.01**	41180.40ns	0.60ns	4.25ns	43.35**	0.67**	11.98ns	68.46**
Residues	12	187.74	269.76	69.17	18222.31	0.36	1.46	0.67	0.04	8.19	7.54
EV (%)	-	35.07	31.19	23.77	33.15	0.67	19.73	12.09	11.03	29.03	4.24
GV/EV	-	1.31	2.22	1.52	0.65	0.46	0.80	4.59	2.17	0.39	1.64
h^2 (%)	-	83.69	93.64	87.35	55.75	39.29	65.73	98.44	93.37	31.66	88.99

PC total phenolic compounds, FLAV total flavonoids, ANT total anthocyanins, N nitrogen, P phosphorus, K potassium, Ca calcium, Mg magnesium, Na sodium, (S) sulfur, (Fe) iron, (Cu) copper, (Zn) zinc, (Mn) manganese, (DM) dry matter, (MM) mineral matter, (CP) crude protein, (EE) ether extract, (CF) crude fiber, (NFE) nitrogen-free extract, (ns) not significant, * significant at 5%, ** significant at 1% by F test.

composition and in content, and vary between species and within species. Factors that contribute to this difference in the genus *Opuntia* are genetic factors, environmental growth conditions, soil, cultivation, collection period, stress, age of the plants, order of the analyzed cladode, analyzed tissues, drying temperature of cladodes, extraction methods and differences in methodologies used in determinations (Santos-Zea et al., 2011; Bari et al., 2012).

Significant differences among the varieties, for some chemical components, were expected, knowing that they are plant materials that have diverging phenotypical characteristics, even they being seeded and grown in the same area and exposed to the same environmental conditions. This situation is a favorable indication for the study of genetic diversity.

The ratio between the genetic variation (GV) and environmental variation (EV) coefficients was above one for the characteristics PC, FLAV, ANT, N, P, K, Ca, S, Fe, Cu, Zn, CP, EE, and NFE with heritability values (h^2) between 78.04 and 99.99%, indicating high genetic control of these characteristics. For the characteristics Mg, Na, Mn, DM, MM and CF, the GV/EV was less than one, indicating possibly the influence of the environment on these characteristics (Table 2).

Given the existence of genetic variability among genotypes, we proceeded to the study of genetic divergence among the materials. For the characteristics in which the ANOVA was significant, differences were identified ($p \leq 0.05$) by the Scott-Knott test, and the formation of two (N, P, K, Fe and Zn), three (S, Cu, CP, EE, NFE), four (ANT and Ca), six (PC) and seven (FLAV) groups of means (Table 3). The Tocher grouping method gathered the seven varieties into three groups. The group I was represented by genotypes 3, 5, 4 and 7; group II by 2 and 6; and group III by genotype 1 (Table 4).

According to the dendrogram obtained by UPGMA hierarchical grouping method, the varieties of cactus pear were gathered into three groups, considering the cut off 45%, respectively, of the relative genetic distance (Cruz et al., 2012). This distribution is similar to that obtained by the Tocher method (Table 4 and Figure 1). The principal component analysis demonstrated that the use of the first three variables was sufficient to account for almost 77% of the total variation obtained in the seven genotypes (Table 5).

Thus, a reasonable description of the diversity of genotypes can be made by these components in two-dimensional or three-dimensional plane. According to Cruz et al. (2012), it is necessary that the first principal components should be close to 80% of the total value to explain the variability manifested among individuals, taking the interpretation of the phenomenon with considerable simplification of the characteristics in two-dimensional or three-dimensional plane. By analyzing the chart of score dispersions of principal component analysis, we observed the formation of four groups. The genotypes were divided into group I (2, 4 and 6), group II (5 and 7), group III (3) and group IV (1) (Figure 2).

The grouping of genotypes by the principal component

Table 3. Means of chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Characteristics	Varieties						
	1	2	3	4	5	6	7
PC	2.84[a]	1.84[e]	1.28[f]	1.93[d]	2.16[c]	1.23[f]	2.25[b]
FLAV	3.02[a]	1.57[f]	2.26[d]	2.51[b]	2.33[c]	1.52[g]	1.93[e]
ANT	0.16[b]	0.05[d]	0.05[d]	0.08[c]	0.07[c]	0.06[d]	0.34[a]
N	12.15[a]	12.93[a]	9.20[b]	9.21[b]	9.36[b]	6.41[b]	13.73 [a]
P	1.29[b]	0.74[b]	2.08[a]	0.86[b]	0.78[b]	0.63[b]	1.03 [b]
K	4.74[a]	4.35[a]	2.35[b]	2.88[b]	5.13[a]	1.83[b]	4.34 [a]
Ca	15.08[b]	13.46[b]	17.22[a]	10.00[c]	10.26[c]	15.75[a]	7.30 [d]
Mg	4.63[a]	6.15[a]	5.70[a]	5.58[a]	7.09[a]	5.36[a]	6.38 [a]
Na	0.11[a]	0.11[a]	0.14[a]	0.11[a]	0.11[a]	0.11[a]	0.11 [a]
S	0.89[b]	0.86[b]	1.30[a]	1.12[a]	0.44[c]	1.12[a]	1.18 [a]
Fe	58.49[a]	69.27[a]	44.49[a]	28.20[b]	23.08[b]	35.22[b]	14.68 [b]
Cu	2.07[c]	2.14[c]	86.96[a]	49.74[b]	59.26[b]	93.02[a]	75.28 [a]
Zn	63.79[a]	34.73[b]	31.06[b]	27.97[b]	23.33[b]	27.22[b]	36.75 [b]
Mn	598.45[a]	418.93[a]	246.12[a]	450.37[a]	294.64[a]	469.75[a]	371.77[a]
DM	10.09[a]	11.12[a]	10.60[a]	11.01[a]	10.30[a]	11.10[a]	10.14[a]
MM	6.20[a]	6.48[a]	8.09[a]	5.45[a]	6.18[a]	4.12[a]	6.26[a]
CP	14.90[a]	7.18[b]	4.17[c]	5.39[c]	4.86[c]	3.94[c]	7.08[b]
EE	2.32[b]	1.34[c]	1.70[c]	1.76[c]	2.73[a]	1.59[c]	1.95[c]
CF	10.40[a]	8.69[a]	12.59[a]	10.13[a]	11.96[a]	7.37[a]	7.82[a]
NFE	56.07[c]	65.18[b]	62.83[b]	66.24[b]	63.86[b]	71.87[a]	66.73[b]

PC total phenolic compounds (mg GAE g^{-1} DM), FLAV total flavonoids (mg QE g^{-1} DM), ANT total anthocyanins (µg QE. 100 g^{-1} DM), N nitrogen ($g.kg^{-1}$ DM) P phosphorus ($g.kg^{-1}$ DM), potassium K ($g.kg^{-1}$ DM), Ca calcium ($g.kg^{-1}$ DM), Mg magnesium ($g.kg^{-1}$ DM), Na sodium ($g.kg^{-1}$ DM); S sulfur ($g.kg^{-1}$ DM), Fe iron ($mg.kg^{-1}$ DM), Cu copper ($mg.kg^{-1}$ DM), Zn zinc ($mg.kg^{-1}$ DM), Mn manganese ($mg.kg^{-1}$ DM), DM dry matter (%), MM mineral matter (% DM), CP crude protein (% DM), ether extract EE (% DM), CF crude fiber (% DM), NFE nitrogen-free extract (% DM); * Means followed by the same letter on the line do not differ by the Scott-Knott grouping test ($p < 0.05$).

Table 4. Grouping of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, based on the chemical and nutritional characteristics, the Mahalanobis distance and Tocher optimization method.

Group	Varieties
I	3, 5, 4, 7
II	2, 6
III	1

method differed a little to that obtained by the UPGMA hierarchical method and Tocher method. It classified genotype 3 in a separate group, and genotype 4 shows similarity to 2 and 6 (Figure 1 and 2; Table 4). The grouping analysis (cluster analysis) identifies groups of similar individuals after estimation of a dissimilarity matrix. There are several grouping methods, example of Tocher, UPGMA and principal component that differ by the type of results, and the different ways to define the closeness between individuals or groups formed. In all cases, it is not known, a priori, the number of groups to be established and different methods give different results (Cruz et al., 2012).

The grouping methods are based mainly on hierarchical methods of optimization and ordination. In the hierarchical methods, there is the method of the average distance among groups (UPGMA), in which the groups are identified in the form of dendrograms, arranged on multiple levels and does not take into account the optimal number of groups. In optimization, there is the Tocher algorithm, which aims to achieve a partition of individuals that optimize (maximize or minimize) some predefined measure. It is based on the formation of groups in which the distances within the groups are smaller than the distances among groups, obtaining the optimal number of groups. In ordination, we highlight the principal component analysis technique that aims to reduce the dimensionality of the variables so that the new combination of resulting uncorrelated linear variables explain the structure of variance and covariance of the original set of variables, resulting in the grouping of subjects based on dispersions in relation to the Cartesian axes (Cruz et al., 2012).

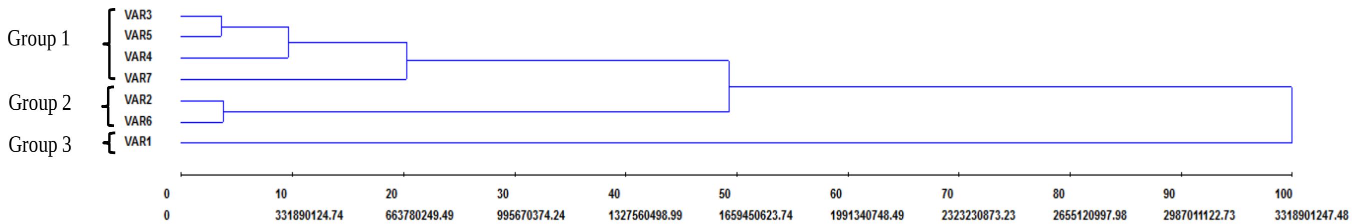

Figure 1. Representative dendrogram of the grouping by UPGMA of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, based on chemical and nutritional characteristics.

Table 5. Estimates of the eigenvalues associated with the principal components and relative importance (eigenvectors) for 20 chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Components	Root (eigenvalue)	Rood (%)	Accumulated (%)	Relative importance (eigenvectors)									
				PC	FLAV	ANT	N	P	K	Ca	Mg	Na	S
1	7.07	35.34	35.34	0.35	0.26	0.16	0.25	0.04	0.29	-0.06	-0.08	-0.12	-0.14
2	4.43	22.13	57.47	-0.12	0.17	-0.16	-0.06	0.43	-0.15	0.33	-0.18	0.42	0.17
3	3.82	19.09	76.56	0.04	0.07	0.11	0.04	0.11	0.18	-0.25	0.40	0.11	-0.15
4	2.33	11.64	88.20	0.02	-0.02	0.51	0.18	0.19	-0.16	-0.15	-0.15	0.08	0.49
5	1.82	9.08	97.28	-0.05	-0.36	0.05	0.48	0.01	0.20	-0.07	0.26	0.06	0.03
6	0.54	2.72	99.99	-0.12	-0.45	0.12	-0.08	0.02	0.13	0.50	0.08	0.04	-0.27
7	0.00	0.00	99.99	0.03	0.07	-0.13	0.02	-0.06	-0.02	-0.01	-0.10	-0.06	0.01
-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	0.00	0.00	99.99	-0.17	-0.03	-0.29	0.02	-0.09	-0.19	-0.17	0.02	0.02	0.19
20	0.00	0.00	100.00	-0.02	-0.06	0.02	0.21	0.05	-0.06	-0.01	-0.11	0.04	0.23

Components	Root (eigenvalue)	Root (%)	Accumulated (%)	Relative importance (eigenvectors)									
				Fe	Cu	Zn	Mn	DM	MM	CP	EE	CF	NFE
1	7.07	35.34	35.34	0.10	-0.27	0.31	0.18	0.27	0.09	0.34	0.20	0.07	-0.32
2	4.43	22.13	57.47	0.19	0.02	0.10	-0.12	0.04	0.34	0.02	-0.05	-0.32	-0.23
3	3.82	19.09	76.56	-0.32	0.19	-0.21	-0.39	0.24	0.23	-0.18	0.30	0.26	-0.03
4	2.33	11.64	88.20	-0.26	0.26	0.14	0.03	0.24	0.02	0.05	-0.14	-0.26	0.09
5	1.82	9.08	97.28	0.31	-0.26	-0.27	-0.19	-0.13	0.34	-0.02	-0.37	-0.13	0.01
6	0.54	2.72	99.99	0.15	0.26	0.18	-0.07	0.36	-0.06	0.09	0.23	-0.24	0.05
7	0.00	0.00	99.99	0.14	-0.20	0.02	0.13	0.61	-0.02	-0.67	-0.17	0.01	0.06
-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	0.00	0.00	99.99	0.11	-0.20	-0.28	-0.04	0.44	0.05	0.52	0.14	0.03	0.35
20	0.00	0.00	99.99	0.18	-0.16	0.06	0.07	-0.26	0.10	-0.30	0.75	-0.11	0.19

PC total phenolic compounds (mg GAE g^{-1} DM), FLAV total flavonoids (mg QE g^{-1} DM), ANT total anthocyanins (µg QE. 100 g^{-1} DM), N nitrogen ($g.kg^{-1}$ DM) P phosphorus ($g.kg^{-1}$ DM), potassium K ($g.kg^{-1}$ DM), Ca calcium ($g.kg^{-1}$ DM), Mg magnesium ($g.kg^{-1}$ DM), Na sodium ($g.kg^{-1}$ DM); S sulfur ($g.kg^{-1}$ DM), Fe iron ($mg.kg^{-1}$ MS), Cu copper ($mg.kg^{-1}$ MS), Zn zinc ($mg.kg^{-1}$ DM), Mn manganese ($mg.kg^{-1}$ DM), DM dry matter (%), MM mineral matter (% DM), CP crude protein (% DM), EE ether extract (% DM), CF crude fiber (% DM), NFE nitrogen-free extract (% DM).

The use of multivariate techniques in the detection of diversity requires a certain degree of structure in the data. Therefore, it is important that different grouping criteria should be used, and that the consensus structure of most of them be considered as correct in order to assure that the result is not an artifact of the technique used (Arriel et al., 2006; Viana, 2013). The characteristics that contributed most to the total variance of the first component were PC and CP; in the second component, P and Na; in the third component, Mg and Mn (Table 5). The most important characteristics are those whose weighting coefficients (eigenvectors) are of greater magnitude, in absolute value, in the first principal components (Cruz et al., 2012). These, then, would be the most responsive characteristics in the selection process among cactus pear populations. The other

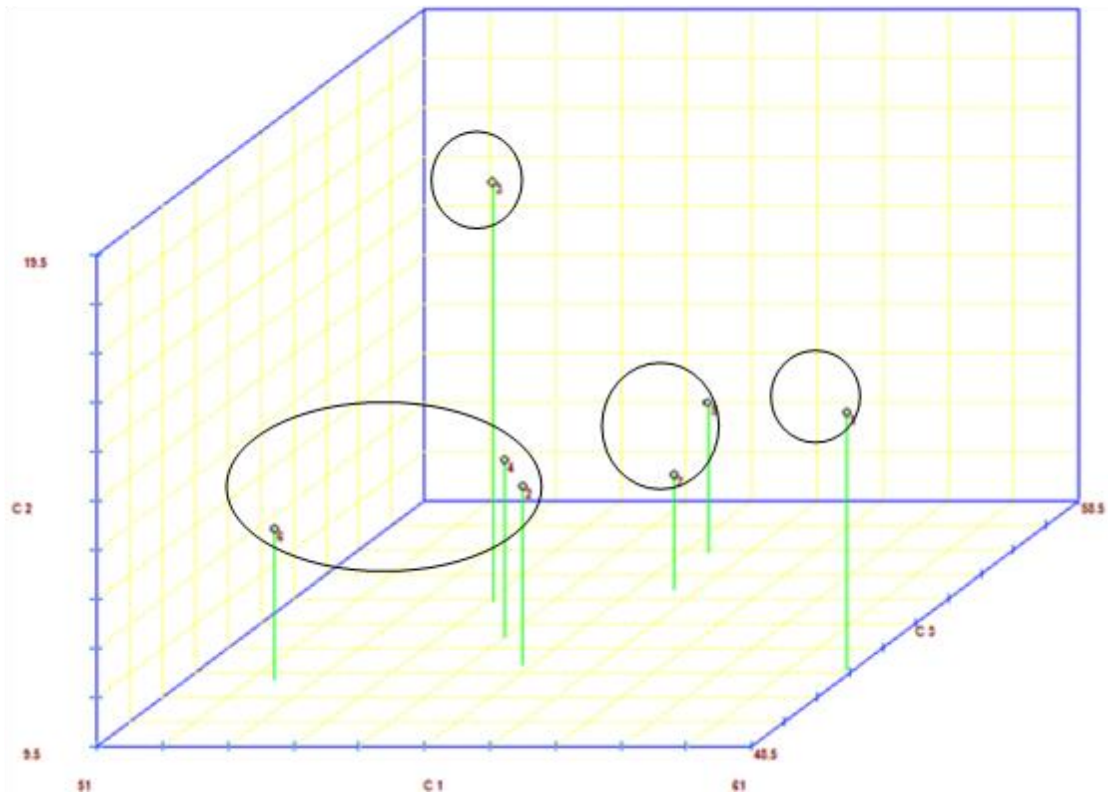

Figure 2. Graphic dispersion of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, in relation to the first, second and third principal component (C1, C2, C3), based on twenty chemical and nutritional characteristics.

characteristics contributed little to the divergence among the genotypes and could be neglected (Rêgo et al., 2011).

The formation of three groups of cactus pear by UPGMA and Tocher methods, and four groups by the principal components method provide relevant information to the conservation of genetic material as a source for breeding programs. According to Silva et al. (2011), crossings between genotypes from different groups provide superior lineages for the improvement of characteristics of interest. Obtaining lineages from commercial varieties is a viable alternative because they are already-improved and tested genotypes in various growing environments. Considering hybrids, it is also possible to have a high proportion of different fixed loci, facilitating the selection and recombination of favorable alleles (Amorim and Souza, 2005).

Thus, as a suggestion for the breeding program with genera *Opuntia* and *Nopalea*, the breeder must consider not only the distance between groups as a criterion to guide the crossings, but also the genotype individual performance for each characteristic of agronomic and zootechnical interest (Ferreira et al., 2003). Besides, there is the possibility and ease of obtaining viable crossings between individuals of different genera or species. In many cases, it is not possible to obtain viable crossings between different species of plants by natural means, which is possible only by non-conventional methods or by biotechnology techniques (Paixão, 2012).

In the analysis of relative contribution of characteristics for the diversity among the seven varieties of cactus pear by Singh methodology (1981), the FLAV contributed with 46.30% and K with 14.07% for the variability among genotypes. These two characteristics contributed with 60.37% of the total variability among the materials. The characteristics that have contributed least to the divergence were Cu, EE, Mn and Mg (Table 6). The lower contribution variables are little informative in the characterization of variability, and can be discarded in diversity studies (Rêgo et al., 2011).

Most chemical and nutritional characteristics were not significantly correlated with each other, except for Na, that had a positive correlation ($p \leq 0.01$) with P (0.88) and Zn with CP (0.97) (Table 7). Sodium is not an essential nutrient for most plants. In fact, the greatest interest in the study of this ion is because its concentration in soils of arid and semiarid regions is high, hindering or even preventing the growth and development of plants. However, this ion plays an important role in osmotic adjustment of plants exposed to salt or water stress in these regions. In addition, it acts as an essential nutrient

Table 6. Relative contribution of 20 chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in semiarid region of Pernambuco through Singh methodology (1981).

Caracteristics	Relative contribution (%)
PC	7.33
FLAV	46.30
ANT	0.80
N	1.78
P	5.03
K	14.07
Ca	1.33
Mg	0.34
Na	0.00
S	8.06
Fe	0.71
Cu	0.08
Zn	2.42
Mn	0.19
DM	1.10
MM	0.63
CP	3.13
EE	0.12
CF	1.95
NFE	4.63

PC total phenolic compounds (mg GAE g^{-1} DM), FLAV total flavonoids (mg QE g^{-1} DM), ANT total anthocyanins (µg QE. 100 g^{-1} DM), N nitrogen ($g.kg^{-1}$ DM) P phosphorus ($g.kg^{-1}$ DM), K potassium ($g.kg^{-1}$ DM), Ca calcium ($g.kg^{-1}$ DM), Mg magnesium ($g.kg^{-1}$ DM), Na sodium ($g.kg^{-1}$ DM); S sulfur ($g.kg^{-1}$ DM), Fe iron ($mg.kg^{-1}$ MS), Cu copper ($mg.kg^{-1}$ MS), Zn zinc ($mg.kg^{-1}$ DM), Mn manganese ($mg.kg^{-1}$ DM), DM dry matter (%), MM mineral matter (% DM), CP crude protein (% DM), EE ether extract (% DM), CF crude fiber (% DM), NFE nitrogen-free extract (% DM).

for growth of some C4 and CAM plants, example of cactus pear (Epstein and Bloom, 2006).

Some C4 and CAM plants need this nutrient to make photosynthesis; however, it is not known whether this essential function has a biochemical or biophysical role. What is known is that this process focuses on the initial metabolism of four carbons that takes place in the mesophyll cells, or in the transport of this molecule between the mesophyll cells and vascular bundle sheath. Studies indicate that sodium facilitates the absorption of pyruvate by the chloroplasts of the mesophyll, perhaps activating the pyruvate carrier; it could still maintain the functional integrity of chloroplasts of the mesophyll (Epstein and Bloom, 2006). In addition, Na^+ is involved in the regeneration of phosphoenolpyruvate, and replaces K^+ in some functions in C4 and CAM plants (Taiz and Zeiger, 2013).

Phosphorus participates in reactions of photosynthesis and respiration as part in the molecules of NADPH, ATP, and various intermediates generated by these processes. The positive correlation between P and Na can be explained by the fact that cactus pear is a plant with CAM photosynthetic metabolism and need Na^+ to perform photosynthesis. Zinc is part of many proteins; in many enzymes, that metal ion is required in the active site (carbonic anhydrase, superoxide dismutase, alcohol dehydrogenase, glutamate dehydrogenase); in others, it is an integral component, not participating in the active site (Epstein and Bloom, 2006; Taiz and Zeiger, 2013). Thus, the positive correlation between Zn and CP. Positive correlations ($p \leq 0.05$) were also found in PC and K (0.82) or CP (0.83); and between P and MM (0.80) (Table 7).

Phenolic compounds belong to a class of secondary metabolites that includes a wide variety of structures, both simple and complex, and have at least one aromatic ring in which at least one hydrogen is replaced by a hydroxyl group. Among these substances, there are structures as varied as the phenolic acids, coumarin derivatives, water-soluble pigments of flowers, fruits and leaves, lignins, tannins, etc. In addition, the phenolic compounds are part of the structure of proteins, alkaloids and terpenoids (Carvalho et al., 2010). Various functions are assigned to these compounds, from protection to biotic and abiotic stresses, pollinator attractants and seed dispersers, mechanical support, ultraviolet radiation protection, etc. (Taiz and Zeiger, 2013). Thus, there was a positive correlation between PC and CP for being part of the structure of various proteins.

Potassium, despite being the most abundant cationic mineral constituent of plants, and constituting up to 10% of the dry weight of a plant, is not an integral constituent of any metabolite that can be isolated from plant material. It is present in the cytosol and cell vacuoles as a free ion (K^+) in high concentrations. The main functions of this ion are the osmotic adjustment in plants exposed to salt or drought stress, activation of enzymes, stabilization of the functional configuration of macromolecules, participation in transport through the ion membrane, anion neutralization, maintenance of the osmotic potential and transport of organic and inorganic nutrients (Epstein and Bloom, 2006). Therefore, positive correlation between K and PC was present, since many of these substances are part of proteins or other macromolecules that needs to be chemically stabilized.

Ashes, or mineral matter, provide an indication of the wealth of the sample in mineral elements. The major elements found are the cations: calcium, potassium, sodium, magnesium, iron, copper, cobalt and aluminum; and the anions: sulfate, chloride, silicate and phosphate, which are absorbed from the environment, either by the roots or shoot of the plant (Messias et al., 2013). Therefore, was a positive correlation between the P and MM in the cactus pear samples. Negative correlations ($p \leq 0.05$) were found between FLAV with NFE (-0.81), and

Table 7. Correlations between the chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Caracteristics	PC	FLAV	ANT	N	P	K	Ca	Mg	Na	S	Fe	Cu	Zn	Mn	DM	MM	CP	EE	CF	NFE
PC	1	0.65ns	0.53ns	0.65ns	-0.11ns	0.82*	-0.42ns	-0.09ns	-0.53ns	-0.45ns	0.04ns	-0.65ns	0.70ns	0.49ns	0.70ns	0.05ns	0.83*	0.61ns	0.05ns	-0.69ns
FLAV	-	1	0.11ns	0.13ns	0.40ns	0.33ns	-0.00ns	-0.36ns	0.06ns	-0.16ns	0.01ns	-0.34ns	0.56ns	0.28ns	0.59ns	0.28ns	0.60ns	0.62ns	0.61ns	-0.81*
ANT	-	-	1	0.63ns	0.02ns	0.37ns	-0.59ns	0.05ns	-0.28ns	0.22ns	-0.44ns	0.05ns	0.38ns	0.14ns	0.69ns	0.03ns	0.37ns	0.22ns	-0.38ns	-0.10ns
N	-	-	-	1	0.06ns	0.70ns	-0.42ns	0.12ns	-0.23ns	-0.07ns	0.22ns	-0.59ns	-0.53ns	0.14ns	0.48ns	0.42ns	0.58ns	0.03ns	-0.14ns	-0.46ns
P	-	-	-	-	1	-0.20ns	0.46ns	-0.26ns	0.88**	0.46ns	0.16ns	0.17ns	0.26ns	-0.35ns	0.38ns	0.80*	0.09ns	-0.00ns	0.62ns	-0.50ns
K	-	-	-	-	-	1	-0.48ns	0.39ns	-0.48ns	-0.73ns	0.06ns	-0.62ns	0.38ns	0.09ns	0.59ns	0.23ns	0.55ns	0.62ns	0.14ns	-0.56ns
Ca	-	-	-	-	-	-	1	-0.58ns	0.55ns	0.22ns	0.66ns	-0.01ns	0.22ns	0.10ns	-0.26ns	0.16ns	0.07ns	-0.31ns	0.23ns	-0.19ns
Mg	-	-	-	-	-	-	-	1	-0.09ns	-0.46ns	-0.46ns	0.23ns	-0.66ns	-0.73ns	0.03ns	0.18ns	-0.54ns	0.26ns	0.13ns	0.31ns
Na	-	-	-	-	-	-	-	-	1	0.49ns	0.12ns	0.41ns	-0.14ns	-0.59ns	-0.00ns	0.71ns	-0.32ns	-0.22ns	0.59ns	-0.14ns
S	-	-	-	-	-	-	-	-	-	1	-0.06ns	0.43ns	0.02ns	-0.01ns	-0.16ns	0.09ns	-0.17ns	-0.65ns	-0.25ns	-0.27ns
Fe	-	-	-	-	-	-	-	-	-	-	1	-0.70ns	0.49ns	0.37ns	-0.26ns	0.24ns	0.45ns	-0.38ns	0.05ns	-0.43ns
Cu	-	-	-	-	-	-	-	-	-	-	-	1	0.62ns	-0.56ns	-0.07ns	-0.11ns	-0.74ns	-0.05ns	-0.01ns	0.62ns
Zn	-	-	-	-	-	-	-		-	-	-	-	1	0.69ns	0.55ns	0.14ns	0.97**	0.18ns	-0.03ns	-0.76*
Mn	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.01ns	-0.55ns	0.72ns	-0.04ns	-0.46ns	-0.24ns
DM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.37ns	0.56ns	0.72ns	0.28ns	-0.63ns
MM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.08ns	0.06ns	0.67ns	-0.56ns
CP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.30ns	-0.02ns	-0.78*
EE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.47ns	-0.48ns
CF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.58ns
NFE	-	-	-	-	-	-	-	-		-	-	-	-	-	--	-	-	-	-	1

* and ** significant at 5 and 1% probability, respectively, by the t test; (ns) not significant.

between NFE with Zn (-0.76) or CP (-0.78) (Table 7).

In animal feed, nitrogen-free extract represents the nonstructural carbohydrates, soluble in acids and bases, usually consisting of starch, sugar and pectin. It indicates the energy value of a food, and is calculated by the difference of the remaining fractions of organic or dry matter (CF, EE, CP and MM) (Detmann et al., 2012; Messias et al., 2013). Therefore, there was a negative correlation between NFE and CP.

Several functions are attributed to flavonoids in plants. The most important include: protection against the incidence of ultraviolet and visible rays; protection against attacks of insects, fungi, viruses and bacteria; attraction of animals and insects for pollination and seed dispersal purposes; antioxidants; hormonal action control; allelopathic agents; enzyme inhibitors; protection against abiotic stresses, etc. (Zuanazzi and Montanha, 2010; Bartwal et al, 2013). Therefore, the flavonoids are the most abundant class of secondary metabolites, present in species of genera *Opuntia* and *Nopalea*.

The flavonoid content is influenced by environmental factors. The salt stress, water stress, temperature and light to which the plants of cactus pear are exposed, increase the content of these metabolites in the plant (Ramakrishna and Ravishankar, 2011; Rodziewicz et al., 2014). However, these stresses reduce the growth and development of cactus pear by negatively influencing the photosynthesis and, thereby, the production of carbohydrates (Bartwal et al., 2013).

As the primary metabolism (carbohydrate) is closely related to secondary metabolism (phenolic compounds), alterations in the first can profoundly affect the second. In addition, many secondary metabolites are formed by sequences of reactions analogous to those of primary metabolism. Thus, under stress conditions, the carbon that would be used in the production of carbohydrate is diverted to the production of secondary metabolites (Ramakrishna and Ravishankar, 2011; Santos, 2010). Then, the negative correlation between NFE and FLAV was found.

In addition, to be protected from abiotic stresses(drought, salt, temperature and high radiation), plants produce several antioxidant enzymes, such as the superoxide dismutase, which has zinc as one of its cofactors. Therefore, the negative correlation between Zn and NFE is found, once the carbon that would be used in the production of carbohydrate is diverted to the production of these enzymes (Epstein and Bloom, 2006; Taiz and Zeiger, 2013).

According to Cruz et al. (2012), the existence of significant correlations indicates the feasibility of indirect selection in order to obtain gains in the characteristic of greatest importance. Among the genotypes that stood out with the highest, overall means for the chemical and nutritional characteristics were 1, 3 and 5 (Table 3). Thus, crossings involving these genotypes could generate superior progenies in characteristics of agronomic, zootechnical or physiological interest.

These results suggest future work aimed to explore the variability found among the cactus pear genotypes studied, and the possibility of using other methods such as protein molecular markers, chemical markers (secondary metabolites), physiological and biochemical characteristics, and chromosomal variation for the determination of genetic variability, providing a complementary analysis to studies by chemical and nutritional characteristics.

Conclusions

The seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, present divergence. Multivariate methods used for divergence gather these genotypes into three or four groups. The characteristics that contribute most to the diversity among the varieties are the flavonoid and potassium contents. These characteristics are correlated with nitrogen-free extract and total phenolic compounds. Multivariate analysis techniques are effective in the study of diversity of species of the genera *Opuntia* and *Nopalea*.

Conflict of Interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 11(31), pp. 2893-2902, 4 August, 2016
DOI: 10.5897/AJAR2016.11144
Article Number: 7E7FBB459819
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of seed component ratios and cutting regime on the performances of annual ryegrass and burr medic mixtures

Giovanni Antonio Re*, Claudio Porqueddu, Federico Sanna, Leonardo Sulas, Antonio Franca, Giovanna Piluzza and Simonetta Bullitta

Consiglio Nazionale delle Ricerche, Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo, Traversa La Crucca 3, località Baldinca, 07100 Sassari, Italy.

Received 21 April, 2016; Accepted 8 July, 2016

A research was carried out in Sardinia (Italy) to identify the best combination and management in binary mixtures of *Lolium rigidum* Gaudin Nurra (L) and *Medicago polymorpha* L. Anglona (P) new released varieties of both species. Two pure stands ($L_{100}P_0$ and L_0P_{100}) and three mixtures ($L_{75}P_{25}$, $L_{50}P_{50}$ and $L_{25}P_{75}$) were compared under two cutting regimes; a commercial mixture was also included in the experiment as test. Forage yield and quality, biological efficiency, interspecific interference and competitive ability of both species were assessed. Total dry matter yield ranged from 2.2 to 5.6 t ha^{-1} (two-cuttings) and from 2.3 to 4.9 t ha^{-1} in commercial mixture and $L_{25}P_{75}$ (three-cuttings). The association grass-legume showed positive effects on the control of unsown species. Crude protein yield, neutral detergent fibre, acid detergent fibre and acid detergent lignin concentration significantly varied between mixtures. The highest protein yield was obtained in the $L_{25}P_{75}$ mixture, reaching 1308 kg ha^{-1} in two-cuttings, as well as the best combination for quality and yield that maximised the synergic interaction effects between species.

Key words: Annual self-reseeding species, forage quality, grass legume competition, mixtures.

INTRODUCTION

Agricultural sustainability can be improve by using multispecies plant mixtures, which can exploit complementary and interspecific interactions within more intensively managed grassland system (Finn et al., 2013). Usually grass-legume mixtures are established to improve pasture and field conditions and are preferred over pure-grass forage stands throughout the world because they increase the total yields of herbage and protein and offer balanced nutrition (Albayrak and Ekiz, 2005). Maintenance of the balance between grasses and legumes in the mixed stand is of great importance as grasses are more efficient than legumes for nutrient uptake (Kyriazopoulos et al., 2012).

Mixtures offer several potential advantages over stands

*Corresponding author. E-mail: gianni.re@ispaam.cnr.it.

of pure grass or pure legume, including, soil erosion reduction, weed control and prolonged stand longevity (Casler, 1988). The benefits of forage legumes are well documented, in addition to their role in nitrogen fixation (Peoples et al., 2009), legumes have a high nutritive value (Wilkins and Jones, 2000) and for several species, there are other beneficial effects on nutrition associated with the presence of condensed tannins and other plant secondary metabolites (Piluzza et al*.,* 2013). Grass-legume mixtures can yield more nitrogen (N) than legumes in pure stands, due to mutual stimulation of nitrogen uptake from both symbiotic and non-symbiotic sources (Nyfeler et al*.,* 2009). Mutual grass-legume interactions also stimulate the efficient transformation of N into biomass, compared to either monocultures. The effects of this functional diversity can substantially contribute to improve the productivity and the efficiency of the resource use in agricultural grassland systems. Nyfeler et al*.* (2011) found that the maximum benefits are reached in mixtures with 40 to 60% of legumes.

Mediterranean basin, due to its rich native flora, represents the current and future world source of germplasm of the most annual forage and pasture legumes and grass, which are important components of production systems in Mediterranean-type climate areas (Bennett and Cocks, 1999; Sulas, 2005). Burr medic (*Medicago polymorpha* L.) is among the more widespread annual self-generating legume in Mediterranean pastures (Loi et al., 1995; Brundu et al., 2004) and represents a valuable resource for grazing sheep and multiple uses (Rochon et al., 2004). Annual ryegrass (*Lolium rigidum* Gaudin) is an important annual self-reseeding grass native to the Mediterranean region, characterized by high winter growth rates, good forage, seed production and high forage palatability, and is well adapted to drought and grazing (Franca et al., 1998; Sanna et al*.,* 2014).

Since early 90's, a selection program aimed at identify elite germplasm of both burr medic and annual ryegrass has been started in Sardinia (Italy) by CNR-ISPAAM. Moreover, the potential of this species for quality and productive improvement of marginal pastures in Mediterranean areas (Sulas and Sitzia, 2004; Sanna et al*.,* 2014), restoring mine or sand quarries (Porqueddu et al., 2013), revegetation of firebreaks and as cover crops in orchards or vineyards (Mercenaro et al., 2014) was ascertained. The aforementioned selection program has resulted in the release of two news Italian varieties, *M. polymorpha* "Anglona" (P) and *L. rigidum* "Nurra" that are registered in the Italian Forage Variety List (Official Journal, 2016).

Several authors have investigated the effects of different seeding ratios in mixtures, based on annual legumes and cereals or perennial legumes and grasses species, for providing out-of-season forage to cover forage seasonal deficits or to reach satisfactory production levels for many categories of livestock (Lithourgidis et al*.,* 2006; Kyriazopoulos et al*.,* 2012; Kocer and Albayrak, 2012; Uzun and Asik, 2012; Cinar and Hatipoglu, 2015). Nevertheless, very few papers focused on annual self-reseeding grass-legume mixtures. Even if abundant literature is available for each species alone as pure sward, no detailed information is available, to our knowledge, regarding burr medic and annual ryegrass grown in mixtures.

Therefore, the main objective of the present work was to identify best seed ratios combination and management of the new released varieties of burr medic and annual ryegrass in mixtures. For such purposes, different seed ratios arranged in binary mixtures of the two annual self-reseeding species were evaluated for (i) forage yield, quality and competition outcomes and (ii) effects of sward management.

MATERIALS AND METHODS

Location, experimental design and crop management

The experiment was carried out during two consecutive years (2002 to 2004) in North-West Sardinia (Italy) (40°46'28'' N, 8°29'17'' E, 80 m a.s.l.), under rainfed regime. The climate is typical of the central Mediterranean basin with long-term average annual rainfall of 540 mm and mean annual temperature of 16.2°C. The soil, classified as Eutric Leptosols and Vertic Cambisols (FAO, 2006), is clay-loam calcareous, with pH 7.5, low N and P_2O_5 content and adequate K_2O content. The accessions used in the experiment were *L. rigidum* "Nurra" (L) and *M. polymorpha* "Anglona" (P). Five plots of 20 m^2 each (5 m × 4 m) were hand sown in autumn 2002 in a split-plot randomized block design with four replicates. The plots included two pure stands ($L_{100}P_0$ and L_0P_{100}) and three mixtures ($L_{75}P_{25}$, $L_{50}P_{50}$ and $L_{25}P_{75}$) where 100 represented the standard dense sowing rate of each component in pure stand (25 and 20 kg ha^{-1} for L and P, respectively). A commercial mixture (CM), constituted by Australian varieties of annual legumes (*Medicago truncatula "*Paraggio", *Medicago rugosa "*Sapo" and *Trifolium brachycalycinum* "Clare"), well suited to soil at the experimental site, was also used as control. After a common cut performed in late winter, each plot was splitted in order to compare different cutting regimes, according to the burr medic phenological stages: two cuts, T1 = Early Flowering (EF) and Pod Maturing (PM) vs three cuts, T2 = Early Flowering (EF), Full Flowering (FF) and Pod Maturing (PM). Before sowing, all plots were fertilized with 36 kg ha^{-1} of N and 92 kg ha^{-1} of P_2O_5. No irrigation or weeding were applied. Dry matter yield (DMY) and botanic composition were determined on two sample areas of 0.5 m^2 per plot. Dry matter content was determined oven drying each phytomass at 80°C until a constant weight is obtained.

Relative Yield Total (RYT) of a mixture measures its biology efficiency quantifying the effects of competition on growth, reproduction or survival of plants (Weigelt and Jolliffe, 2003), comparing the forage production in pure stands respect to the forage production in mixtures. According to Lithourgidis et al*.* (2006) and Kyriazopoulos et al*.* (2012), RYT was calculated as:

$$RYT = \frac{RYp + RYl}{PpYp + PlYl}$$

Where RYp and RYl are the relative yields of P and L, respectively; Yp and Yl are yields of P and L in monocultures and (Pp and Pl) the relative proportions of P and L*,* in the mixtures. A RYT= 1 indicates that species in the mixtures are competing for resources, with facilitation for RYT > 1 and antagonism for RYT < 1 (Williams and McCarthy, 2001). *RYp* and *RYl* were computed as:

$$RYp = \frac{Ypl}{PpYp}, \qquad RYl = \frac{Ylp}{PlYl}$$

where Ypl represents yield of P in the presence of L and Ylp is yield of L in the presence of P. If RY=1 indicates that the species have an equal intraspecific and interspecific competition. When RY>1, the tested species better competes against the other species. When RY<1 means that for the tested species, the interspecific competition is higher than the intraspecific one.

Forage oven dried subsamples were ground to 1 mm screen to be analysed for quality. Total N was determined using Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral, acid detergent fibres and lignin (NDF, ADF and ADL), were determined according to Van Soest (1994) procedure. Total Digestible Nutrients (TDN), Digestible Dry Matter (DDM), Dry Matter Intake (DMI), Relative Feed Value (RFV) and Net Energy for lactation (NE_l) were estimated according to the following equations adapted from Lithourgidis et al. (2006) and Sadeghpour et al. (2014):

TDN = (-1.291 × ADF) + 101.35,
DMI = 120 / %NDF dry matter basis,
DDM = 88.9 - (0.779 × %ADF) dry matter basis,
RFV = %DDM × %DMI × 0.775,
NE_l = (1.044 - (0.0119 × %ADF)) × 2.205.

Statistical analysis

Forage yield, quality parameters and competitive ability data, were analysed using Statgraphics Centurion XVI version (StatPoint Technologies Inc., 2009). Homogeneity test of variance and arcsin transformation of percentages relative to data were performed. Angular values were subjected to analysis of variance (ANOVA) to test for differences between mixtures and between cutting regimes. Fisher's test and Tukey's HSD test were used for post hoc tests of significant differences between means as indicated. The significance level was fixed at 0.05 for all statistical analysis.

RESULTS AND DISCUSSION

In the first year, total rainfall and temperature did not substantially differ from climatic data for the same location. In the 2nd year, rainfall exceeded climatic value and a total rainfall of 440 mm was recorded in autumn-winter (with a peak of 200 mm in October) advantaging unsown species.

Forage yield

The concurrent presence of the two species positively affected dry matter yield in mixtures, except in T2 at PM. Statistically, significant differences among mixtures and between cutting regimes were found for DMY, without significant interaction in each year (Table 1). At the first year, total DMY of pure stands and mixtures ranged from about 2.2 to 5.6 t ha^{-1} in T1 cutting regime and from 2.3 to 4.9 t ha^{-1} in T2 cutting regime, respectively. In T1, $L_{25}P_{75}$ produced almost twice than the burr medic in pure stand and the CM, and about 35% more than $L_{100}P_0$. All mixtures ($L_{50}P_{50}$, $L_{75}P_{25}$ and $L_{25}P_{75}$) gave higher productions than pure sward at both cutting regimes (T1 and T2). Thalooth et al. (2015) found similar trend, in mixtures of *Trifolium alexandrinum* L. and *Lolium multiflorum* L.

The best performance in term of DMY of annual ryegrass-burr medic mixtures compared to pure stands found in our experiment is in accordance to the findings of Hauggaard-Nielsen et al. (2006), who reported that mixture components might use ecological resources more efficiently than sole crops. Annicchiarico and Tomasoni (2010) observed that the advantage in terms of DMY of the *L. multiflorum* L. × *Trifolium repens* L. association over the mean response of its components in pure stands, arises mainly from transfer of biologically fixed N from clover to grass. For *M. polymorpha* in the same site and period of our experiment, Sulas and Sitzia (2004) reported a value of 1.9 kg of fixed N per 100 kg of above ground DM. This figure can be higher in L+P mixture because grasses compete strongly for soil N and, consequently, legumes are forced to rely on N fixation as N source (Loiseau et al., 2001). According to Nyfeler et al. (2009, 2011), the positive interactions between N-fixing legumes and non-N-fixing plant species can contribute to a significantly larger extent to the beneficial mixing effects on forage yield, than pure stands, or than interaction between other functional groups. The same authors reported that in mixtures of grasses and legumes (with legume proportion from 50 to 70%) fertilized with 50 kg ha^{-1} year^{-1} of N, produced DMY comparable to that of a grass monoculture fertilized with 450 kg ha^{-1} year^{-1} of N.

Regardless of the cutting regimes, the binary mixtures $L_{25}P_{75}$ and $L_{50}P_{50}$ showed a synergistic interaction (Table 1) over yielding their monocultures, as found also by Cardinale et al. (2007) and Finn et al. (2013).

In the second year, overall DMY of sown species was markedly lower (minus 70%) than the first year. However, $L_{25}P_{75}$ produced again higher DM than the other mixtures in both cutting regimes. The main factor responsible for DM decrease was a reduced seedling density in the mixture species at the beginning of second growing season.

Unsown species

Compared to pure stands and CM, the grass-legume associations have shown a positive effect on unsown species control. In the first year, at early flowering (EF), unsown species represented about 40% in L0P100 and CM, whereas unsown species were about 30% of the total DM in three other different mixtures and L100P0 stand (Figure 1a). In addition, similar values were recorded at full flowering (FF) under T2 cutting regime (Figure 1b); only in L100P0, unsown species presence

Table 1. Dry matter yield (t ha^{-1}) of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Main effect (Mixtures)		Cutting regimes			
		2002-2003		2003-2004	
		T1 (EF+PM)	T2 (EF+FF+PM)	T1 (EF+PM)	T2 (EF+PM)
L_0P_{100}	-	3.10^{cd}	2.53^{b}	0.85^{cd}	0.46^{c}
	P	1.83	1.90	0.36	0.23
$L_{25}P_{75}$	L	3.76	2.95	1.45	1.47
	Total	5.59^{a}	4.85^{a}	1.81^{a}	1.70^{a}
	P	1.04	1,28	0.24	0.23
$L_{50}P_{50}$	L	4.15	3,09	0.98	1.11
	Total	5.19^{a}	4.37^{a}	1.22^{bc}	1.34^{ab}
	P	1.47	0.95	0.13	0.15
$L_{75}P_{25}$	L	3.14	3.34	0.83	0.66
	Total	4.61^{ab}	4.29^{a}	0.96^{bcd}	0.81^{bc}
$L_{100}P_0$		3.64^{bc}	2.29^{b}	1.42^{ab}	0.47^{c}
CM		2.24^{d}	2.33^{b}	0.57^{d}	0.43^{c}
*Cutting regimes		0.94		1.24	

*HSD of Tukey 95% for pair comparison between total T1 and T2 values within mixture. EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at p ≤ 0.05 (Fisher's test).

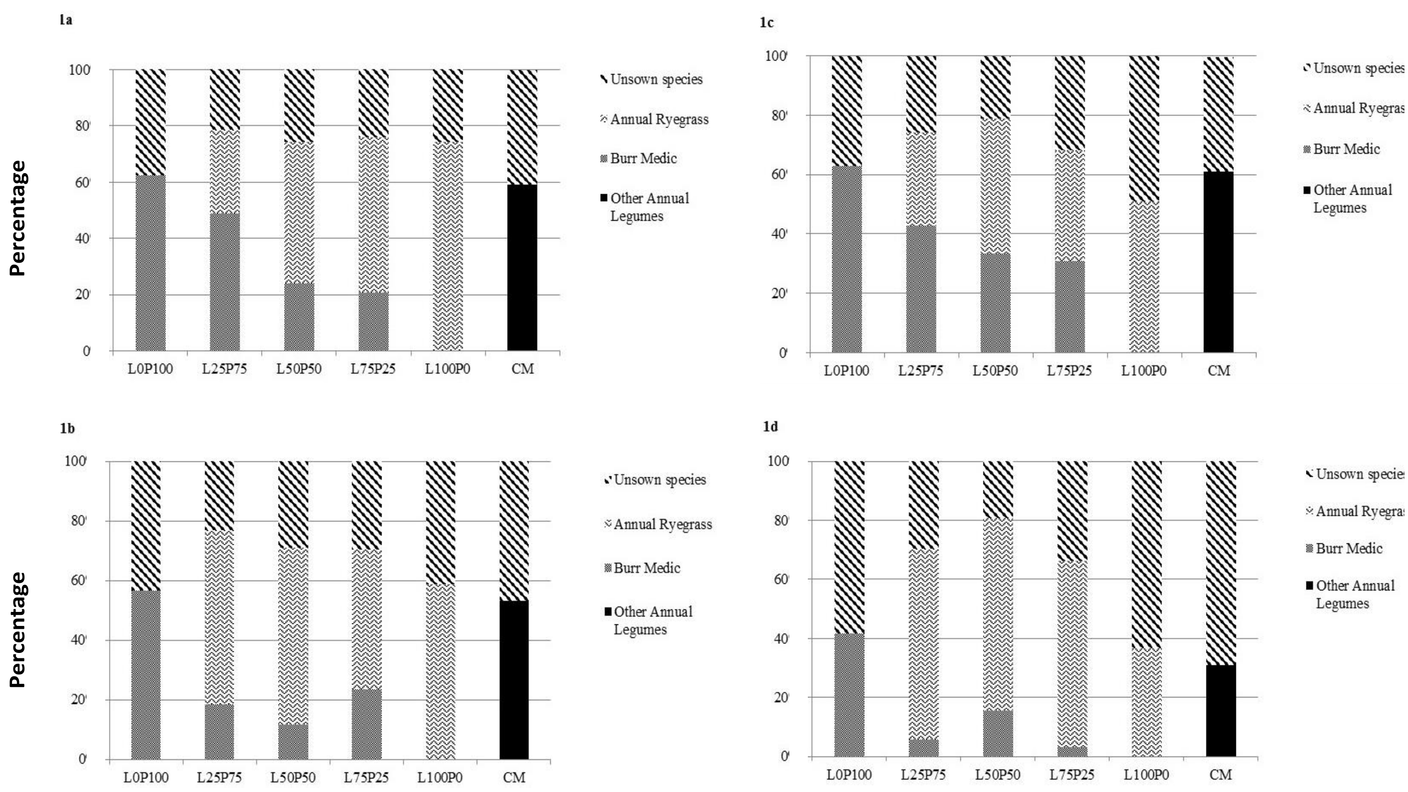

Figure 1. Floristic compositions for each phenological stage of harvesting (2002-2003), (1a) Early Flowering in T1 and T2 (1st cut), (1b) Pod Maturing in T1 (2nd cut), (1c) Full Flowering in T2 (2nd cut) and (1d) Pod Maturing in T2 (3rd cut).

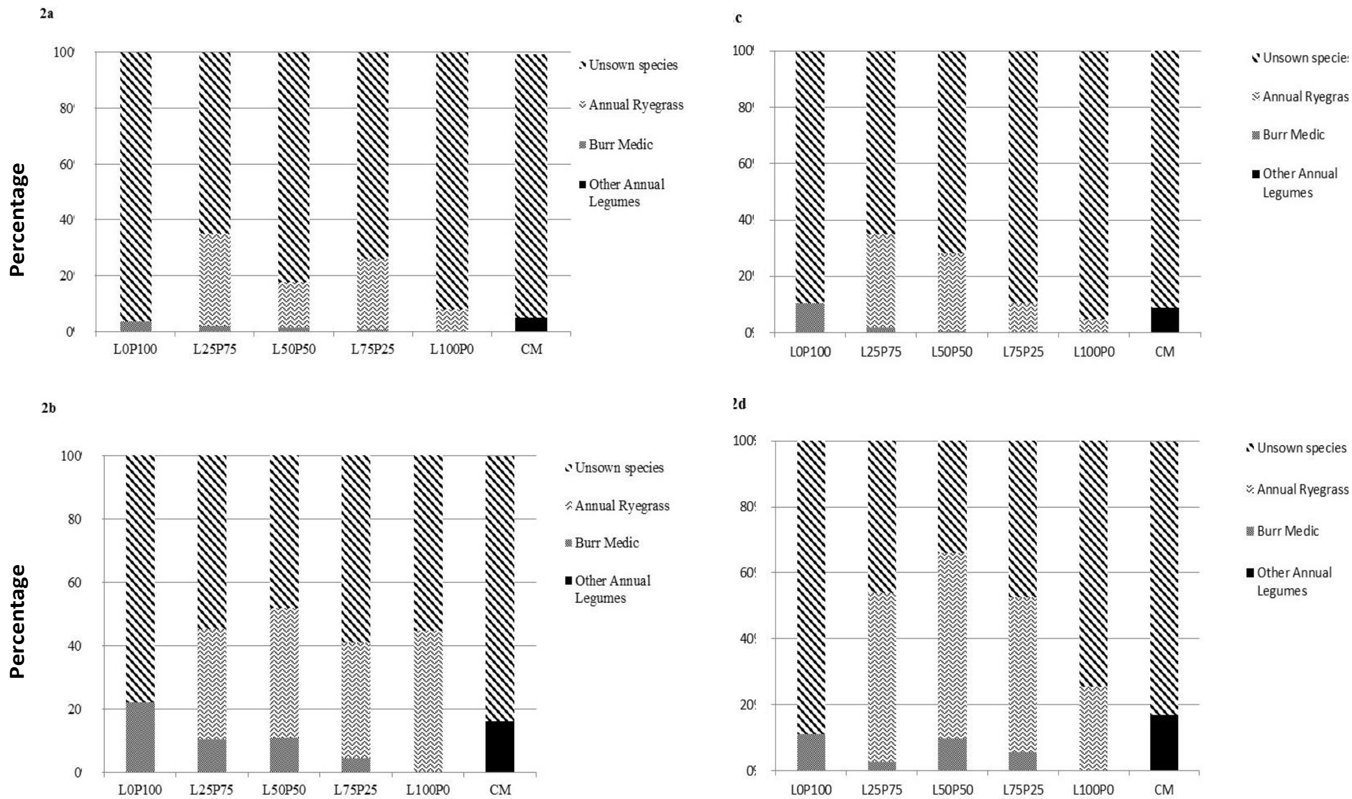

Figure 2. Floristic compositions for each phenological stage of harvesting (2003-2004), (2a) Vegetative stage in T1 (3^{th} cut), (2b) Early Flowering in T1 (4^{th} cut), (2c) Vegetative stage in T2 (4^{th} cut) and (2d) Early Flowering in T2 (5^{th} cut).

was higher than 50%. At pod maturing (PM), pure stands and CM showed higher percentage of unsown species compared to mixtures (Figure 1c and d). On average, the percentage of unsown species was 60% higher in T2 compared to T1 cutting regime. In the second year (Figure 2), when seedlings density of the mixture species was markedly affected by the low number of germinating seeds at the end of summer (data not shown), unsown species were much more competitive representing about 80% of DMY at winter season for both treatments (Figure 2a and c). Only $L_{25}P_{75}$ showed, in both cutting regimes, a more competitive ability mainly due to annual ryegrass. In spring 2004 at EF, the contribution of mixture components increased to 60% in $L_{50}P_{50}$ (Figure 2b and d).

Competitive ability

In accordance to Kyriazopoulos et al. (2012), average RYT value (index indicating whether facilitation, suppression and interferences occurs between mixture components), in both cutting regimes did not show statistical significant differences (data not reported) even if in $L_{25}P_{75}$ was higher than 1 indicating that species in the mixture were competing for resources with facilitation.

Forage quality

As a general trend, CP concentration (Figure 3a) was negatively affected by the grass proportion in mixtures and, as it was expected, by phenological stages (from 30% for L_0P_{100} at EF to about 8 to 9% for $L_{100}P_0$ at PM). Moreover, the concentration of CP was lower in CM than in P in pure stand. Crude protein yield, NDF, ADF and ADL contents significantly varied between mixtures and cutting regimes. At both cutting regimes, the highest total protein yield was obtained in $L_{25}P_{75}$: 1308 and 1221 kg ha^{-1} in T1 and T2, respectively (Table 2). In the first cut $L_{25}P_{75}$ produced about 100 kg ha^{-1} more than the burr medic pure stand, about twice the production of the other mixtures and four times compared to the pure grass stand. Total CP of burr medic pure stand was almost double than that of grass in pure stand in both T1 and T2. In accordance to Albayrak et al*.* (2005) and Thalooth et al. (2015), mixtures with high proportion of legumes such as our $L_{25}P_{75,}$ produced more dry matter and crude protein. Uzun and Asik (2012) in *Pisum sativum* L. + *Avena sativa* L. mixtures found that the highest CP was obtained from 50% pea+50% oat. Other studies reported that grass-legume mixtures had higher CP contents than grasses alone (Sanderson, 2010; Kim and Albrecht, 2011; Kocer and Albayrak, 2012) and the average of the

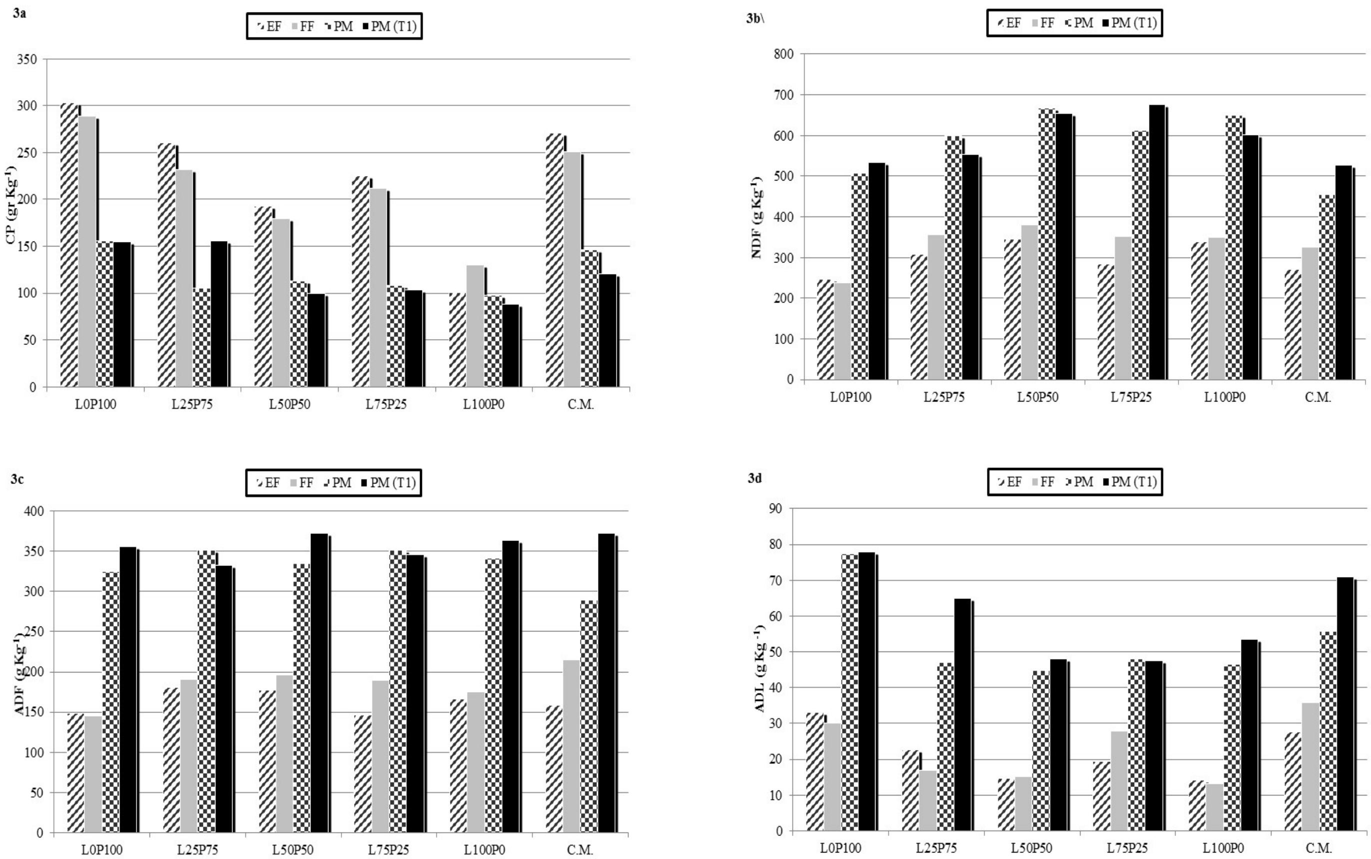

Figure 3. Forage chemical composition as g kg^{-1} of (3a) crude protein (CP), (3b) neutral detergent fibre (NDF), (3c) acid detergent fibre (ADF) and (3d) acid detergent lignin (ADL) concentration in the pure stands, grass-legume mixtures and Commercial Mixture at different phenological stage. EF: Early flowering; FF: full flowering; PM: pod maturing; and cutting regimes. Values with different letters are significantly differences at $p \leq 0.05$ (HSD Tukey test).

Table 2. Crude protein yield (CP, kg ha^{-1}) of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Cutting regimes	**CP (kg ha^{-1})**					
	T1=T2	**T1**	**Total**	**T2**		**Total**
Main effect (Mixtures)	**EF**	**PM**	**(EF+PM)**	**FF**	**PM**	**(EF+FF+PM)**
L_0P_{100}	358^{b}	639^{b}	997^{b}	550^{c}	223^{nc}	1131^{ab}
$L_{25}P_{75}$	448^{a}	860^{a}	1308^{a}	481^{c}	292^{nc}	1221^{a}
$L_{50}P_{50}$	270^{c}	583^{bc}	853^{bc}	332^{bc}	275^{nc}	877^{c}
$L_{75}P_{25}$	256^{c}	551^{bcd}	807^{cd}	444^{bc}	241^{nc}	941^{bc}
$L_{100}P_0$	105^{d}	428^{cd}	533^{e}	167^{a}	276^{nc}	548^{d}
CM	296^{bc}	366^{d}	662^{de}	499^{c}	257^{nc}	1052^{abc}
*Cutting regimes			194			

*HSD of Tukey 95% for pair comparison between total T1 and T2 values within mixture. EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

monocultures. Nevertheless, this result is in contrast to Lithourgidis et al. (2006), who reported that the higher grass ratio in the mixture produced higher protein per unit area, but in presence of low DMY per ha.

Caballero et al. (1995) in agreement with NRC standards (NRC, 1985) reported that a mixed hay with 130 g CP Kg^{-1} would meet requirements for most sheep. Dry matter production and CP concentration, as shown in Table 1 and Figure 3, highlight the high nutritive value of our binary mixtures, especially in early cuts for both

Table 3. (a) Neutral detergent fibre (NDF), (b) acid detergent fibre (ADF) and (c) acid detergent lignin (ADL) production (Kg ha^{-1}) of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

a			**NDF (kg ha^{-1})**			
Cutting regimes	**T1=T2**	**T1**	**Total**	**T2**		**Total**
Main effect (Mixtures)	**EF**	**PM**	**(EF+PM)**	**FF**	**PM**	**(EF+FF+PM)**
L_0P_{100}	293^{b}	2206^{bc}	2499^{cd}	444^{c}	727^{b}	1464^{b}
$L_{25}P_{75}$	528^{a}	3047^{ab}	3575^{ab}	741^{a}	1523^{a}	2791^{a}
$L_{50}P_{50}$	484^{a}	3827^{a}	4311^{a}	655^{ab}	1624^{a}	2763^{a}
$L_{75}P_{25}$	322^{b}	3614^{a}	3936^{ab}	691^{a}	1930^{a}	2943^{a}
$L_{100}P_0$	355^{b}	2908^{ab}	3263^{bc}	447^{c}	1824^{a}	2626^{a}
CM	296^{b}	1603^{c}	1899^{d}	647^{ab}	768^{b}	1711^{b}
*Cutting regimes				771		

b			**ADF (kg ha^{-1})**			
Cutting regimes	**T1=T2**	**T1**	**Total**	**T2**		**Total**
Main effect (Mixtures)	**EF**	**PM**	**(EF+PM)**	**FF**	**PM**	**(EF+FF+PM)**
L_0P_{100}	176^{c}	1471^{bc}	1647^{bc}	277^{bc}	464^{b}	917^{b}
$L_{25}P_{75}$	311^{a}	1833^{ab}	2144^{a}	397^{ab}	974^{a}	1682^{a}
$L_{50}P_{50}$	238^{b}	2172^{a}	2410^{a}	337^{abc}	814^{a}	1389^{a}
$L_{75}P_{25}$	166^{c}	1842^{ab}	2008^{ab}	373^{ab}	1108^{a}	1647^{a}
$L_{100}P_0$	174^{c}	1762^{ab}	1936^{ab}	428^{a}	965^{a}	1567^{a}
CM	174^{c}	1133^{c}	1307^{c}	223^{c}	489^{b}	886^{b}
*Cutting regimes				437		

c			**ADL (kg ha^{-1})**			
Cutting regimes	**T1=T2**	**T1**	**Total**	**T2**		**Total**
Main effect (Mixtures)	**EF**	**PM**	**(EF+PM)**	**FF**	**PM**	**(EF+FF+PM)**
L_0P_{100}	39^{a}	322^{ab}	361^{b}	58^{b}	111$^{n.s.}$	208^{ab}
$L_{25}P_{75}$	39^{a}	357^{a}	379^{b}	35^{c}	131$^{n.s.}$	205^{ab}
$L_{50}P_{50}$	20^{c}	280^{abc}	300^{ab}	25^{bc}	109$^{n.s.}$	154^{b}
$L_{75}P_{25}$	22^{c}	254^{bc}	293^{ab}	55^{b}	152$^{n.s.}$	228^{a}
$L_{100}P_0$	15^{c}	260^{bc}	275^{a}	17^{c}	132$^{n.s.}$	164^{b}
CM	30^{b}	216^{c}	246^{a}	71^{a}	94$^{n.s.}$	195^{ab}
*Cutting regimes				69		

*HSD of Tukey 95% for pair comparison between total T1 and T2 values within mixture. EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

treatments and mainly for L25P75.

The NDF concentration increased with the grass percentage in the mixtures and at the later phenological stages (Figure 3b). At EF, NDF ranged from 23% in L0P100 to 33% in L100P0; while at PM, it ranged from 52% in L0P100 to 65% in L75P25. Total NDF yield (Table 3a) ranged from 1900 (CM) to about 4300 kg ha-1 (L50P50) in T1, and from 1464 (L0P100) to about 3000 kg ha-1 (L75P25) in T2. Mixtures showed significantly higher NDF content than burr medic pure stand and CM. Cuts at EF and FF showed lower NDF concentration (from about 20% in L_0P_{100} to 35% in $L_{50}P_{50}$) compared to cuts at the later stage PM. Total ADF yield (Table 3b) ranged from 886 kg ha^{-1} (CM) in T2 to 2410 kg ha^{-1} ($L_{50}P_{50}$) in T1. L_0P_{100} and CM showed significantly lower ADF than grass-legume mixtures in T1. In T2, ADF content of L_0P_{100}, was significantly lower than the remaining ones.

Total lignin content (ADL) of $L_{100}P_0$, $L_{75}P_{25}$ and CM was significantly lower than $L_{25}P_{75}$ and L_0P_{100} in T1, while in T2 the differences were less evident. At PM, the lignin content was more than double compared to the previous cuts both in T1 and T2. Significant differences were found between treatments: the lignin content in T1 was always higher than in T2, excluding $L_{75}P_{25}$ and CM. No interactions were found among different mixtures and treatments (Table 3c). For other mixtures containing burr medic, lower NDF and ADF concentrations than grasses

Table 4. Total digestible nutrients (TDN), dry matter intake (DMI) and digestible dry matter (DDM) content (%) at the first year of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Cutting regimes	TDN (%)				DMI (%)				DDM (%)			
	T1=T2	T1	T2		T1=T2	T1	T2		T1=T2	T1	T2	
Main effect (Mixtures)	**EF**	**PM**	**FF**	**PM**	**EF**	**PM**	**FF**	**PM**	**EF**	**PM**	**FF**	**PM**
L_0P_{100}	82.1^{b}	55.5^{c}	82.6^{a}	59.4^{b}	4.9^{a}	2.3$_{a}$	5.1^{a}	2.5^{b}	77.3^{b}	61.2^{c}	77.6^{a}	63.6^{b}
$L_{25}P_{75}$	78.0^{f}	58.4^{a}	76.7^{c}	56.0^{b}	3.9^{d}	2.2^{b}	3.4^{c}	2.2^{c}	74.8^{f}	63.0^{a}	74.3^{c}	61.5^{b}
$L_{50}P_{50}$	78.5^{e}	53.3^{e}	76.0^{d}	58.1^{b}	3.5^{f}	1.9^{d}	3.2^{d}	1.9^{d}	75.1^{e}	59.9^{e}	73.6^{d}	62.8^{b}
$L_{75}P_{25}$	82.5^{a}	56.7^{b}	76.8^{c}	56.0^{b}	4.3^{c}	1.9^{d}	3.5^{c}	2.1^{c}	77.5^{a}	62.0^{b}	74.1^{c}	61.5^{b}
$L_{100}P_0$	79.9^{d}	54.4^{d}	78.8^{b}	57.3^{b}	3.6^{e}	2.1^{c}	3.5^{c}	1.9^{d}	75.9^{d}	60.6^{d}	75.3^{b}	62.3^{b}
CM	80.8^{c}	53.3^{e}	73.5^{e}	64.0^{a}	4.5^{b}	2.4^{a}	3.7^{b}	2.8^{a}	76.5^{c}	59.9^{e}	72.1^{e}	66.4^{a}

EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at p ≤ 0.05 (Fisher's test).

Table 5. Relative feed value (RFV) and net energy for lactation (NE_l) at the first year of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Cutting regimes	RFV (%)				NE_l (Mcal Kg^{-1})			
	T1=T2	T1	T2		T1=T2	T1	T2	
Main effect (Mixtures)	**EF**	**PM**	**FF**	**PM**	**EF**	**PM**	**FF**	**PM**
L_0P_{100}	294^{a}	114^{a}	308^{a}	106^{bc}	1.912^{a}	1.369^{c}	1.920^{a}	1.451^{b}
$L_{25}P_{75}$	229^{e}	109^{a}	196^{c}	116^{b}	1.826^{e}	1.429^{a}	1.801^{c}	1.381^{b}
$L_{50}P_{50}$	204^{f}	91^{c}	183^{d}	93^{d}	1.837^{d}	1.326^{e}	1.788^{d}	1.423^{b}
$L_{75}P_{25}$	256^{c}	91^{c}	199^{c}	100^{cd}	1.918^{a}	1.394^{b}	1.804^{c}	1.380^{b}
$L_{100}P_0$	210^{e}	98^{b}	203^{cd}	95^{cd}	1.865^{c}	1.347^{d}	1.844^{b}	1.406^{b}
CM	265^{b}	112^{a}	209^{b}	143^{a}	1.885^{b}	1.326^{e}	1.737^{e}	1.543^{a}

EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at p ≤ 0.05 (Fisher's test).

monoculture were reported (Caballero et al., 1995; Albayrak and Tùrk, 2013), because the proportion of cell wall constituents is larger in grasses than in legumes and the former have quicker lignin accumulation (Buxton et al., 1991). As it was expected, concentration of NDF and ADF, increase throughout the vegetative period in our study. ADL was lower in both grass pure stand and in the mixture compared to legumes in pure stand and CM. Lithourgidis et al. (2006) found a lower lignin content in grass than in common vetch monoculture. As general trend, TDN, DMI, DDM, RFV and NE_l values showed high variation among phenological phases but did not show significant differences between cutting regimes. TDN, as it was expected, was higher on average at EF (80.3%) and FF (77.4%) compared to PM (55.3% at T1 and 58.5% at T2, respectively) (Table 4). As well as TDN, DMI and DDM were higher at EF and FF compared to PM. In both cutting regimes for DMI, L_0P_{100} and CM showed higher values. First cuts at EF and FF showed 18% higher DDM compared to PM stage. TDN shows at EF and FF a higher value in burr medic pure stand compared to the annual ryegrass pure stand and decreased at PM both in T1 and T2. On the contrary, Lithourgidis et al. (2006) found a higher TDN in triticale and oat monocultures than monoculture of common vetch, and it was inversely related to ADF. As ADF increases, there is a decline in TDN, which means that animals cannot completely utilize forage nutrients. DMI and DDM were higher both in grass and legume monoculture. RFV was affected by component ratios (Table 5); P in pure stand (294 at EF and 308 at FF) showed higher RFV than the other mixtures and $L_{100}P_0$. The NE_l was higher at EF and FF than at PM. NE_l showed a similar trend to those recorded for RFV. Although is not a direct measure of the nutritional content of forage (Van Soest, 1996), many authors affirmed that it is important for estimating the value of forage. Uzun (2010) provided a RFV rank, categorizing RFV respectively from rejected (< 75%) to prime (> 151%). In our binary mixtures, RFV was about 200% at EF and FF and it was one third higher than that of *Medicago sativa* L. + *Lolium perenne* L. mixtures under irrigated conditions (Cinar and Hatipoglu, 2015). Moreover, it ranged from 91 to 116% in late stages (PM) for both T1 and T2 cutting regimes. Hence, P+L mixtures can be categorized as prime forage at early and full flowering stages and fairgood quality in late stages (PM). According to Markoviæ et al*.* (2011), the energy concentration expressed for dairy cows in MJ NE_l kg^{-1} DM is very important and the highest possible level of energy concentration is a prerequisite to feed highly performing cows successfully. Abaş et al*.* (2005) reported for *M. sativa* L. hay a NE_l value of 5.20 MJ kg^{-1} DM, comparable to our values at the same phenological stage at PM.

Conclusion

The new released varieties, "Anglona" burr medic and "Nurra" annual ryegrass, proved to be productive and well suited to grow in mixture. Forage yield and quality and species interaction were strongly influenced by the different seed ratios. On the contrary, cutting regimes did not substantially affect the mixture performances. Experimental evidence indicates significant yield benefits from simple binary mixtures, which yielded more than their monocultures. The best combination for quality and yield was performed by $L_{25}P_{75}$ mixture, which maximised the synergic interaction effects between annual ryegrass and burr medic and improved their complementary use of resources, in both cutting regimes. Therefore, such a combination is to be suggested to farmers for an effective and efficient exploitation of the two species in mixtures.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of Fondazione Banco di Sardegna and Salvatore Nieddu, Daniele Dettori, Anton Pietro Stangoni, Piero Saba and Maddalena Sassu for their excellent technical assistance.

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Vol. 11(31), pp. 2903-2911, 4 August, 2016
DOI: 10.5897/AJAR2016.10837
Article Number: 7BB528959821
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Drying of hulled naturally processed coffee with high moisture content and its impacts on quality

Valdiney Cambuy Siqueira[1], Flavio Meira Borem[2], Eder Pedroza Isquierdo[3], Guilherme Euripedes Alves[4], Diego Egidio Ribeiro[5]*, Afonso Celso Ferreira Pinto[6], and Jose Henrique da Silva Taveira[7]

[1]Faculty of Agricultural Sciences, Federal University of Grande Dourados – S/N, Cx. P. 533, 79.804-970, Dourados, MS, Brazil.
[2]Department of Agricultural Engineering, Federal University of Lavras – S/N, Cx. P. 3037, 37.200-000, Lavras, MG, Brazil.
[3]University of the State of Mato Grosso – Av. São João, S/N, 78.200-000, Cáceres, MT, Brazil.
[4]Department of Agricultural Engineering, Federal University of Lavras – S/N, Cx. P. 3037, 37.200-000, Lavras, MG, Brazil.
[5]Department of Agricultural Engineering, Federal University of Lavras – S/N, Cx. P. 3037, 37.200-000, Lavras, MG, Brazil.
[6]Department of Agricultural Engineering, Federal University of Lavras – S/N, Cx. P. 3037, 37.200-000, Lavras, MG, Brazil.
[7]Department of Agricultural Engineering, University of the State of Goias – Av. Protestato Joaquim Bueno, 945, 75920-000, Santa Helena de Goias, GO, Brazil.

Received 24 January, 2016; Accepted 14 July, 2016

This study aimed to propose a new method to reduce the time needed for coffee processing and drying and to verify this method's possible impact on both the sensorial and physiological qualities of coffee. The method, which entails hulling coffee with different moisture contents by removing the entire pericarp before completing the drying, reduced the operating time by more than 50% as compared to the normal drying of natural process coffees. The temperatures used for drying the hulled coffee did not compromise its quality; coffee hulled with 36 ± 2% (w.b.) resulted in higher sensory analysis scores; and coffee hulled with higher moisture content yielded a better physical appearance.

Key words: Mechanical drying, *Coffea arabica* L., processing, physiological and sensory quality.

INTRODUCTION

In recent decades, coffee production has gone through several transformations such as the introduction of varieties that are more productive, have greater resistance to pests and diseases, and have greater tolerance to drought as well as the implementation of modern production systems with a high standard of crop protection. In addition, there have been important technological advances such as the introduction of irrigation

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

systems, crop management and mechanical harvesting. Thus, in Brazil, coffee farming is becoming a growing business, which occupies extensive agricultural areas of high productivity (Carvalho et al., 2010; Faria and Siqueira, 2005; Lanna and Reis, 2012; Silva et al., 2008, 2013).

However, advances in the field have not been accompanied by comparable technological innovations in the post-harvest to allow for the assimilation of the increased yields and efficiencies. In fact, increased harvest yields combined with the introduction of mechanical harvesting have turned the post-harvest stage into the main bottleneck of the coffee production chain, with drying being the main limitation in the flow between harvest and storage (Borém et al., 2008b).

When compared with other commodities such as corn, soy, and wheat, coffee is the only agricultural grain with drying times that exceed 40 h. In the case of naturally processed coffees, drying times may even exceed 200 h in order to ensure a moisture content that is suitable for storage (Donzeles et al., 2007; Lacerda Filho and Silva, 2006; Resende et al., 2007, 2011a; Simões et al., 2008).

The longer drying times are due both to the high initial moisture content of the coffee upon harvesting and slower drying given the presence of the thick, fleshy and moist pericarp. Besides exposing the coffee to various risks that may alter its quality, these long drying times result in higher energy and labor consumption and thus a higher processing cost (Berbert et al., 2001; Borém et al., 2008b; Finzer et al., 1997; Ghosh and Venkatachalapathy, 2014; Palacin et al., 2009; Resende et al., 2007).

The coffee drying rate can be increased by reducing relative humidity, increasing dry bulb temperature, or increasing the flow rate of the drying air (Alves, 2013; Isquierdo et al., 2011; Ribeiro et al., 2003). However, there are some limitations that impede the implementation of these techniques on a large scale.

Equipment used to reduce relative humidity is costly and, as of yet, has not been designed for use with commercial coffee dryers. The use of high air flow has a greater effect when the drying temperature is low and in the early stages of drying, when the product has a high moisture content. High drying air temperatures can cause thermal and physical damage to the product and compromise its quality (Borém et al., 2006; Isquierdo et al., 2011, 2013; Ribeiro et al., 2003).

Basically, coffee can be processed by two methods: dry and wet. The dry method consists of drying the whole fruit with all anatomical components intact. This results in a dried fruit also known as dried coffee pods or natural coffee. In wet processing, the fruit is pulped, that is, the epicarp and part of the mesocarp are removed, leaving just the seed, endocarp and potentially some of the remaining mucilage that adheres to the endocarp. This coffee is commonly known as parchment coffee (Borém et al., 2008b; Esquivel and Jiménez, 2012; Selmar et al., 2006).

The greatest reduction in drying time is reported from wet processing which can be up to 6.8 times faster than dry process coffee, depending on the temperature and air flow used (Alves, 2013). Nonetheless, in Brazil most farmers still process their coffee using the dry process.

Drying efficiency is not solely related to time, costs, equipment, use of renewable and nonrenewable resources, etc., but it is also tied to product quality since quality is a key factor at the time of sale. Several studies show that drying has a direct influence on the final coffee quality (Borém et al., 2008a; Coradi et al., 2008; Isquierdo, 2011; Isquierdo et al., 2013, 2012; Kleinwächter and Selmar, 2010; Reinato et al., 2011b, 2012; Rosa et al., 2005). It is therefore necessary to develop new technologies for processing and drying natural coffees in order to meet increasing post-harvest demands, improve drying efficiencies and do so without compromising the quality of the final product. This study analyzed the technical feasibility of drying natural coffees with full removal of the pericarp at higher moisture contents. The reduction of the total drying time and possible impacts on the physiological and sensory quality of coffee was analyzed.

MATERIALS AND METHODS

Characterization of the experiment

Coffee fruits (*Coffea arabica* L. cv. Bourbon Amarelo) were harvested in commercial crops at 1,246 m asl (S22° 06' 7.6'' and W45° 12' 15.5'') in the municipality of Carmo de Minas, Minas Gerais, Brazil. The harvest was performed manually, by collecting only fruit at maximum maturity. After the harvest, fruits were subjected to hydraulic separation to remove those with lower density, such as overripe, underdeveloped and insect-damaged fruit. After this step, a new manual selection removed any remaining immature and overripe fruits that were harvested but not removed in the hydraulic separation process.

The material was then separated into two groups. The first group, consisting of fruits with initial moisture content of 70.6% (w.b.), was dried at 40°C until reaching a final moisture content of 11 ± 0.5% (w.b.). The second group was dried at 40°C for periods of 36, 48, 60 and 72 h, corresponding respectively to moisture contents of 36±2; 29±2; 22±2 and 17±2% (w.b.). Fruits were then hulled at these respective periods and after hulling subject to continuous drying at 35 ± 1°C and 40 ± 1°C, until reaching a final moisture content of 11 ± 0.5% (w.b.) as described in Figure 1. Drying was carried out by forced convection in fixed bed dryers, composed of six square perforated trays, each with 0.35 m sides and a depth of 0.4 m, located over a plenum in order to ensure a uniform airflow. Throughout the drying process of both the natural and hulled coffees, airflow was monitored using a vane anemometer set and kept at 24 $m^3 min^{-1} m^{-2}$. Temperature adjustment and control were performed by means of an electronic controller and constant monitoring, with the aid of mercury thermometers inside the coffee mass.

For initial drying, 20 L of fruits were placed in each tray, corresponding to approximately 13 kg. The thickness of the drying fruit layer at the beginning of this process was 16 ± 1 cm. For drying the second group, a portion of fruit was hulled by a sample huller machine CARMOMAQ® DC1. Afterwards, the hulled coffee (beans)

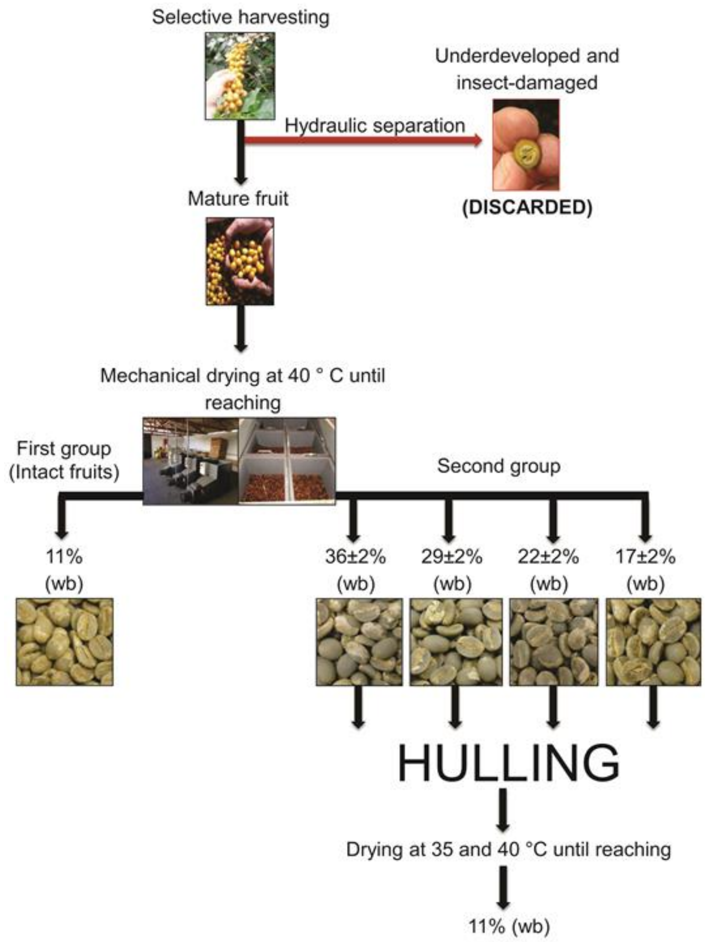

Figure 1. Flowchart of coffee processing and drying.

was subjected to continuous drying according to the treatments. After hulling, the height of the coffee layer was approximately 4 cm.

Determination of moisture content and preparation of the samples

The moisture content of the natural coffee was determined using an oven at 105 ± 3°C for 24 h in three replicates (Brasil, 2009). The moisture content of the hulled coffee was determined by the oven method at 105 ± 1°C for 16 h, according to international standard ISO 6673 (ISO, 2003). The results were expressed as a wet basis percentage (w.b.).

After drying, coffee beans were classified according to size and shape. For analysis, only undamaged plano-convex shaped beans that were retained by sieves with 16 to 18/64 inch diameter holes were used. This was done to standardize the samples and minimize interference unrelated to treatments.

Characterization of coffee quality

In order to evaluate the physiological quality of the bean and the sensory quality of the coffee beverage, physiological tests, color evaluations and sensory analysis was performed on each prepared sample.

Electrical conductivity and potassium leaching

Electrical conductivity of the coffee beans was determined using two replicates of 50 beans per sample. Each replicate was weighed to the nearest 0.001 g and then immersed in 75 mL distilled water, inside plastic cups with a 200 mL capacity. These cups were placed in a BOD incubator with forced air ventilation and a temperature of 25°C for five hours. After incubation, the electrical conductivity of the imbibition water was determined using a benchtop conductivity meter – BEL brand, model W12D – (Krzyzanowski et al., 1991).

The leaching of potassium ions was performed on raw beans using the imbibition water obtained in the prior electrical conductivity test. Levels of leached potassium were determined using a flame photometer – Digimed brand, model NK-2002 (Prete and Abrahão, 1995).

Lercafé test

A Lercafé test was conducted in three replicates of 25 beans each sample. Coffee beans were submerged in 100 mL of a 5% sodium hypochlorite solution for one hour (Reis et al., 2010). A plastic screen from a germination box was used to ensure that all beans were submerged and in contact with the solution. Subsequently, the germination boxes were capped and kept in a BOD incubator under a constant temperature of 25°C for 6 h. The beans were then

Table 1. Drying time (hours) of coffee beans submitted to the new method of processing.

Temperature (°C)	Moisture contents at hulling (% w.b.)			
	36±2	29±2	22±2	17±2
40±1	47.5	57.25	67.25	76.5
35±1	58	67	72.5	81

washed to remove excess solution, immersed in distilled water for 40 min and then arranged on a counter for counting, characterization and visual evaluation. This evaluation was performed by comparing the endosperm color to the total surface area of the beans.

Color evaluation

The color of the raw coffee beans was determined using a Minolta colorimeter CR 300 to directly measure "L", "a", "b" dimensions using the Hunter lab color scale (Nobre, 2005). The samples were placed in Petri dishes and, for each replicate, five readings were taken at the four cardinal points of the plate and at the center point.

Sensory analysis

Sensory analysis was performed by certified specialty coffee judges, using the methodology proposed by the Specialty Coffee American Association – SCAA – (Lingle, 2011). The SCAA sensory analysis protocol was used for coffee brewing and roasting. The coffee was roasted to a level corresponding to 58 points for whole beans and 63 points for ground beans, with a tolerance of ± 1 point. 100 g of beans were roasted from each sample.

For the sensory evaluation, five cups of each sample were tasted, with one session of sensory analysis for each replicate and three replicates for each treatment. The evaluated sensory attributes were grouped into "subjective" and "objective" categories. "Subjective" attributes were fragrance/aroma, flavor, acidity, body, balance, aftertaste and overall impression. They were scored according to their quality on a scale of 6 to 10 points in intervals of 0.25 points. The "objective" category included uniformity, sweetness and clean cup (absence of defects). The objective attributes were scored on a scale from 0 to 10 points, with 2 points awarded for each cup that presented satisfactory levels of each attribute. For the purposes of this study, the final score, obtained from the sum of the scores for each attribute in both categories were only considered.

Experimental design and statistical analysis

The experiment was a completely randomized design with a 4 x 2 + 1 factorial arrangement with four moisture content hulling levels (36 ± 2; 29 ± 2; 22 ± 2 and 17 ± 2% w.b.), two drying temperatures of the hulled coffee (35 ± 1 and 40 ± 1°C) and a control treatment (complete drying of natural coffee at 40 ± 1°C, without hulling), in three replicates. Data was subjected to analysis of variance and the mean values were compared by Scott-Knott test at a 5% significance level. The hulled coffees were compared with the control coffee using Dunnett's test.

RESULTS AND DISCUSSION

The drying time of coffee fruits prior hulling were 36, 48, 60 and 72 h, which correspond to the moisture content 36±2, 29±2, 22±2 and 17±2% w.b. in the beans, respectively. The drying time of the hulled coffee beans was calculated by the difference between the total drying time (Table 1) and the drying time before hulling. Considering the initial moisture content of the coffee beans at 36±2, 29±2, 22±2 and 17±2% w.b. at the moment of hulling, the drying time of the hulled coffee beans was 11.5, 9.25, 7.25, and 4.25 h for the air temperature set at 40±1°C. For the air temperature set at 35±1°C, the drying time of the hulled coffee beans was 22, 19, 12.5, and 9 h. Conversely, the total drying time of the natural coffee (control) with initial moisture content at 70.6% (w.b.) was 108 h.

The total time for drying the hulled coffee beans was 56% lower [(108-47.5)/108 = 56%] than for drying the natural coffees (whole fruits), when the coffee fruits were hulled at 36±2% (w.b.) and submitted to air temperature to 40±1°C. Such behavior is due to the shorter time (36 h) until the hulling moment plus the 11.5 h for drying the hulled coffee beans at this temperature.

The higher drying rates are for the hulled coffees dried at 40 ± 1°C as compared to the ones dried at 35 ± 1°C (Figure 2), regardless of the moisture content at the moment of hulling. It is clear that the lower temperature of drying is the higher in the time needed for drying. The higher decrease in moisture content when air drying temperature was 40 ± 1°C can be explained by the larger difference in the partial vapor pressure of air and inside de coffee beans. These results in higher drying rate (Table 2), which makes the water molecules removed easily and quickly. By hulling the coffee in this manner, its morphological and/or physical characteristics become more similar to other agricultural products such as soy, beans, wheat, etc. This opens up the possibility of using other types of dryers used for drying these products and not restricting coffee drying solely to traditional coffee dryers, such as fixed bed, rotary and cross flow dryers. Table 3 presents a summary of the analysis of variance for all variables. The drying temperature by itself did not change any of the variables studied. However, the moisture content at the time of hulling resulted in both different sensory perceptions of the coffee beverage, as well as in the color evaluation (coordinates "a" and "b" and brightness). The interaction between drying temperature and the moisture content of the hulled coffee is significant only for color evaluations, as well as the effect of this interaction, together with the control

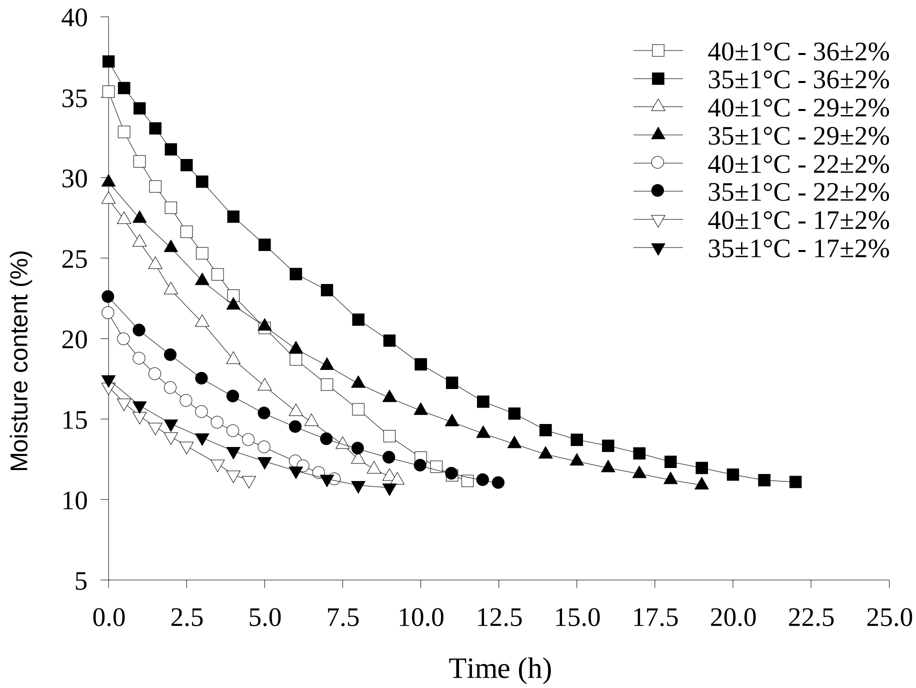

Figure 2. Moisture content of hulled coffee beans during drying with air temperature set at 40 ± 1°C and 35 ± 1°C (drying curves).

Table 2. Average drying rate (kg kg $^{-1}$ h^{-1}) of coffee beans early hulled at high moisture content and submitted to air drying temperature set at 40±1 and 35±1°C.

Temperature (°C)	Moisture content (% w.b.)			
	36±2	29±2	22±2	17±2
40±1	0.035	0.030	0.021	0.018
35±1	0.021	0.016	0.013	0.010

Table 3. Summary of analysis of variance for electrical conductivity, potassium leaching, Lercafé, sensory score (sensory), coordinate "a", coordinate "b" and brightness of coffee beans after different methods for hulling and drying.

Source of variation	EC	KL	Lercafé	Sensory	Coord"a"	Coord"b"	B's
	F_{calc}	F_{calc}	F_{calc}	F_{calc}	F_{calc}	F_{calc}	F_{calc}
Temperature	0.013^{ns}	0.025^{ns}	1.066^{ns}	1.933^{ns}	1.623^{ns}	3.034^{ns}	3.522^{ns}
Moisture content upon hulling	0.354^{ns}	1.710^{ns}	0.643^{ns}	5.694*	98.99*	25.611*	87.692*
Factorial (temperature x moisture content)	0.248^{ns}	1.058^{ns}	1.806^{ns}	2.190^{ns}	4.289*	3.957*	3.364*
Factorial x control	4.249^{ns}	2.376^{ns}	1.415^{ns}	0.024^{ns}	75.241*	9.224*	149.361*
CV (%)	19.2	19.7	15.8	0.8	9.9	4.9	6.4

*Significant at 5%, by F-test. ^{ns} Non-significant. Control: complete drying of natural coffee at 40°C, without hulling.

(complete drying of the natural coffee).

Table 4 shows the average values of the physiological tests, color evaluation and sensory analysis performed on coffee hulled with high moisture content and subjected to drying at different temperatures, as compared to natural coffee (control). With respect to physiological testing and

Table 4. Electrical conductivity, potassium leaching, Lercafé, sensory score (Sensory), coordinate "a", coordinate "b" and brightness of coffee beans subjected to drying after hulling as compared to drying of control.

Treatments	EC ($\mu S.cm^{-1}.g^{-1}$)	KL (ppm)	Lercafé (%)	Sensory	Coord"a"	Coord"b"	B's
35±1/36±2	12.039	23.103	75.407	85.611	2.396*	24.679*	19.049*
35±1/29±2	11.390	23.368	71.414	84.555	1.444*	19.461	27.553*
35±1/22±2	11.861	20.214	66.370	85.833	1.091	19.460	29.016*
35±1/17±2	12.944	22.147	60.559	84.611	0.834	18.674	40.472
40±1/36±2	13.242	26.926	66.734	86.111	1.684*	22.702*	22.355*
40±1/29±2	11.216	18.462	55.852	84.472	2.007*	23.908*	20.589*
40±1/22±2	12.362	18.886	62.068	84.333	1.085	20.782	30.600
40±1/17±2	11.860	23.403	71.703	84.111	0.783	20.002	33.304
Control	15.140	26.253	58.748	84.889	0.673	19.320	43.046

Control: complete drying of natural coffee at 40°C, without hulling.

Table 5. Coordinates "a" and "b" and brightness of coffees hulled with high moisture content and subjected to drying at different temperatures.

Variables analyzed	Temperature (°C)	Moisture content (% w.b.)			
		36±2	29±2	22±2	17±2
Coordinate "a"	35±1	2.39 aA	1.44 aB	1.09 aB	0.83 aB
	40±1	1.68 bA	2.01 aA	1.08 aB	0.78 aB
Coordinate "b"	35±1	24.68 aA	19.46 bB	19.99 aB	18.67 aB
	40±1	22.70 aA	23.91 aA	20.78 aB	20.00 aB
Brightness	35±1	19.05 aC	27.55 aB	29.01 aB	40.47 aA
	40±1	22.35 aB	20.59 bB	30.60 aA	33.30 bA

Means followed by different lowercase letters in the same column and uppercase letters in the same row are significantly different (P< 0.05) by Scott-Knott test.

sensory analysis, none of the treatments were significantly different from the control.

In the color evaluation, some of the hulled coffees presented different color tones than the control. Coffees dried at 40 ± 1°C and hulled at moisture contents of 36 ± 2 and 29 ± 2% (w.b.) had different "a" and "b" coordinates and brightness levels as compared to the control coffee. For the drying temperature of 35 ± 1°C similar results were found as well as a difference in the brightness observed in the coffee hulled with a moisture content of 22 ± 2% (w.b.). However, unlike the coffee dried at 40 ± 1°C, the coffee hulled at 29 ± 2% (w.b.) did not present changes in bluish and yellowish colors (coordinate "b") as compared to the control. Higher values of the coordinates "a" and "b" represent higher saturation of the colors, red and yellow, respectively, and lower values of these coordinates indicate greater saturation of green and blue, respectively.

Differences in brightness were found between coffees hulled with high moisture content and coffee processed by the standard dry method with 11 ± 0.5% (w.b.). The higher the moisture content upon hulling, the greater the difference in tonality. It is interesting to also note that coffees hulled with high moisture content presented, at the end of the drying process, a darker tone that is common to wet processed coffees. Coffee beans processed in the wet way have higher activity of polyphenol oxidase (Saath et al., 2014) and these coffee beans are darker than the coffee beans processed using the natural method (Abreu et al., 2015). It suggests that the high activity of polyphenol oxidase is a possible cause of the darker color of the coffee beans hulled with high moisture content.

Results of the interaction between moisture content and drying temperature for the color analysis are shown in Table 5. It was not possible to observe a clear behavior for the same moisture contents in function of the two drying temperatures used. The higher the drying temperature, the lower the intensity of the color green for coffees processed by dry and wet methods (Corrêa et al., 2002). However, for the temperature range used in this experiment, this behavior was only observed when the

Table 6. Average values of electrical conductivity, potassium leaching, Lercafé, sensory score (Sensory), coordinate "a", coordinate "b" and brightness for the coffee hulled and dried at temperatures of 35 ± 1 and 40 ± 1°C.

Temperature	EC (µS cm^{-1} g^{-1})	KL (ppm)	Lercafé (%)	Sensory	Coord"a"	Coord"b"	B's
35±1°C	12.06	22.21	68.44	85.15	1.44	20.7	29.02
40±1°C	12.17	21.92	64.09	84.75	1.39	21.85	26.71

Table 7. Electrical conductivity, potassium leaching, Lercafé and sensory score (Sensory) for the coffee hulled and dried at temperatures of 35 ± 1 and 40 ± 1°C.

Moisture content (% w.b.)	EC (µS cm^{-1} g^{-1})	KL (ppm)	Lercafé (%)	Sensory
36±2	12.64	25.01	71.07	85.86a
29±2	11.30	20.91	63.63	84.51b
22±2	12.11	19.55	64.22	85.08b
17±2	12.40	22.77	66.13	84.36b

Means followed by different lowercase letters in the same column are significantly different (P< 0.05) by Scott-Knott test.

coffee was processed with a moisture content of 36 ± 2% (w.b.).

In general, values of the coordinates "a" and "b" decrease and brightness values increase when the hulling was carried out with drier coffee. This implies a blue-green color with light shading, an aspect that is usually related to good quality coffee (Afonso Júnior and Corrêa, 2003; Selmar et al., 2007). Increased brightness is more evident in the temperature of 35 ± 1°C, perhaps favored by the longer drying time after hulling. The quality of coffee showed no significant differences for the two temperatures used in drying the coffee hulled with high moisture contents (Table 3). The average values of the analyses performed to characterize the physiological quality of the bean and sensory quality of the beverage are shown in Table 6.

For artificial drying with forced ventilation, the maximum temperature that can be used without compromising quality is 40 ± 1°C (Isquierdo, 2011). The same was observed in the present study, in which the temperature of 40 ± 1°C applied in drying coffee hulled with high moisture content had no negative effects on the final product quality. This information is relevant, since the drying times are significantly reduced when the drying is performed at 40 ± 1°C when compared with the temperature of 35 ± 1°C.

Table 7 lists the average values of the analyses used to determine the quality of coffee subjected to two drying temperatures after hulling with different moisture contents. The moisture content of the coffee upon hulling did not influence the values of electrical conductivity, potassium leaching and Lercafé. These analyses indicate the physical integrity of the bean and indicate its physiological potential. Thus, for these ranges of moisture content upon hulling, there was no great physical or thermal damages to the beans during the drying process.

It was expected that the higher moisture content would cause greater damage since the bean would be more sensitive to hulling and, in fact, mechanical damage to the beans was observed during this operation. Moreover, hulling promotes an increased drying rate, since by removing the pericarp (skin, mucilage and parchment), a barrier to the water removal is also removed. This fact, coupled with high moisture content of the bean, favors a significant increase in the drying rate (Burmester and Eggers, 2010; Guida and Vilela, 1996; Isquierdo et al., 2013; Resende et al., 2009; Sfredo et al., 2005), which may cause internal cracks or microscopic fissures in the bean (Kirleis and Stroshine, 1990; Yang et al., 2003b; 2003a). Nevertheless, this hypothesis was not confirmed in this study as no immediate damage was found. Contrary to the main hypothesis, coffee gulled with the highest moisture content (36±2% w.b.) showed more pleasant sensory characteristics, resulting in a higher final score, as compared to the other treatments (Table 7). In fact, tasters found the sensory attributes of this coffee were similar to those of pulped coffee, describing it as having a more pleasant acidity with better aftertaste and balance.

The highest score in the sensory analysis was for the coffee hulled with a moisture content of 36±2% (w.b.), which was also the treatment with the lowest total drying time (47.5 and 58 h for temperatures of 40 and 35°C, respectively). Therefore, early removal of parts of the fruit reduces the total drying time without changing the sensory quality of the beverage as compared to the control treatment (Table 4).

Therefore, this drying technology causes no immediate physiological and sensory damage while providing a reduction in coffee drying time of over 50% as compared to the traditional drying method for natural coffees.

Conclusions

The new technology proposed in this paper, which comprises the hulling of coffee fruits at high moisture content, decreased the total time of drying in more than 50% as compared to the traditional method currently used by farmers. In addition, this new method does not cause instant damages to the coffee beans, and does not decrease physiological and sensorial quality.

Another advantage of the early hulling of coffee fruits at high moisture content is that the dried coffee beans have better aspect, with typical color and luminosity of a high quality product.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the FAPEMIG, the CAPES, the CNPq, the INCT-Café and the Federal University of Lavras for the support of this study.

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Vol. 11(31), pp. 2912-2919, 4 August, 2016
DOI: 10.5897/AJAR2016.11284
Article Number: 0D0DA1D59825
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Morphological analysis of fruits, seeds, and seedling germination *Acacia farnesiana* (L.) Willd

Paulo Alexandre Fernandes Rodrigues de Melo[1], Kelina Bernardo Silva[2], Edna Ursulino Alves[3], Robson Luis Silva de Medeiros[1*], Antônio Pereira dos Anjos Neto[4], Kedma Maria Silva Pinto[5], Wallace de Sousa Leite[1] and Valderez Pontes Matos[6]

[1]São Paulo State University "Júlio Mesquita Filho", Plant Production Department. Jaboticabal–SP, CEP: 14883-125, Brazil.
[2]State University of Paraíba, Catole do Rocha-PB, CEP: 58884-000, Brazil.
[3]Department of Environmental Sciences and Fitotecnia, Paraiba Federal University, Areia-PB, CEP: 58397-000, Brazil.
[4]Paraiba Federal University, Cidade Universitária, Areia-PB, CEP: 58397-000, Brazil.
[5]Federal Rural University of Pernambuco, Av. Bom Pastor, s/n - Boa Vista, Garanhuns-PE, CEP: 55292-270, Brazil.
[6]Department of Agronomic, Federal Rural University of Pernambuco, Manuel de Medeiros, s/n, Dois Irmãos, Recife-PE, CEP 52171-900, Brazil.

Received 2 June, 2016; Accepted 14 July, 2016

The understanding of the morphology of fruits, seeds and seedlings is very important on the identification and the preservation of species. The work describes and illustrates morphological characteristics of fruits, seeds, germination, and seedlings of *Acasia farnesiana*. The fruits were collected manually from parent trees selected at random, in the municipality of Sousa State of Paraíba. After collection, they were packed in polyethylene bags and taken to the Seed Analysis Laboratory, Universidade Federal da Paraiba, Areia-PB. Subsequently, evaluations were made of the morphological and morphometric characteristics of fruit, seed germination stages, and seedling. The fruit is of type nucule vegetable, plain, dry, polysemy, glabrous, linear-format wavy and late dehiscence. The seeds are stenospermics, format obovoid, apex rounded, and base slightly rounded. The germination is epigeal - phanerocotyledonal, starting on the third day and may be terminated on the seventh day after sowing. It was possible to describe and illustrate in detail the morphology of fruit, seeds, germination stages, and seedling of the species, considering the characteristics of easy recognition, standardization, rapid achievement, and high probability of establishment in the field.

Key words: *Vachellia farnesiana*, biometry, native species, forest seeds.

INTRODUCTION

Acacia farnesiana L. Willd. is trees or shrub, which belongs to Fabacea family and Acacia genus. It has large distribution in tropical and sub-tropical areas all over the world due to its high capacity of adaptation to diverse weather conditions, as well as geographic and altitude conditions, making the adaptation of this species easy in different environments (Erkovan et al., 2013). This aspect has facilitated its introduction in different regions. In addition, several species of this genus are used as ornamental lumberyard, in tanneries due to the presence of tannins, for extraction of gums. Its flower essences are used in perfumery, fixing dunes, and formation of hedges

(Ramli et al., 2011; Kingsley et al., 2014).

For wild plants, one of the biggest problems found by experts is the lack of information related to species identification, once not always identified botanical material is available (Amaro et al., 2006).

In Leguminosae family, subfamily Mimosoideae, vegetative and floral characters, in which the systematic study of Angiosperm is fundamentally based, are not always sufficient for characterization of some taxonomy groups, reason why the fruits and seeds have been used as a decisive character. Without these, recognizing certain genus become difficult (Camacho et al., 2012). According to the author, morphological descriptions of such structures are usually quite large at subfamily level or they are found in brief generic diagnoses. The morphological identification of seedlings also allows to characterize families, genera, and even species, having been applied in studies of forest inventories in regions of temperate and tropical climates (Anez et al., 2005).

In the environment, there is morphological diversity of fruit that in the elapse of their evolution have passed through a series of adaptation and have acquired different forms (Cosmo et al., 2009; Paoli and Bianconi, 2008). Thus, the description of the fruit within an ecological context is a new way to understand the biology of reproduction (Anez et al., 2005). Biometrics of fruits and seeds, as well as the knowledge of morphology and seedling development is critical to support studies of germination and seedling production for plant restoration (Leonhardt et al., 2008).

About the development of seedlings, Cosmo et al. (2009) reported that this stadium deserves proper attention, because it can be used to elucidate morphological and anatomical aspects, other than promoting species' recognition of certain regions in ecological studies. In this context, Silva (2012) stated that information about the development stage can be used with great reliability for such purposes, helping the immediate identification of the species.

Despite the importance of morphological studies of seedlings, there are a few studies of that in Brazil, especially when it comes to native forest species, like this study was conducted aiming to characterize morphologically fruits, seeds, germination, and seedlings of *A. farnesiana*.

MATERIALS AND METHODS

Mature fruits of *A. farnesiana* were harvested by hand from matrix plants located in Sousa State of Paraíba and were put in plastic bags and carried to the Laboratory of Seed Analyses, Department of Fitotecnia and Environmental Science, in the Center of Agricultural Science of the Federal University of Paraiba, in Areia–PB, Brazil. There, fruits were handly opened to remove the seeds.

Morphological characterization of fruits and seeds

For biometric determinations, hundred fruits and seeds were selected randomly for individual measurement of length, width, and thickness, using a digital caliper with a precision of 0.001 mm. The length was measured from the base to the apex, excluding the stalk and the width and thickness measurements were measured in the midline of the fruits and seeds (CHEIB, 2009); after measurement of fruits and seeds, they were individually weighed on an analytical balance accurate to 0.001 g. The quantitative characteristic data were submitted to descriptive analysis, calculated with Excel application, arithmetic mean, standard deviation, coefficient of variation, range of variation, and relative frequency of fruit and seeds.

The considered aspects to describe fruits were weight, type, color, size, texture and consistency of the pericarp and seed number per fruit; in seeds, the external morphological characteristics were weight, type, color, size, texture, and consistency of integuments, form, edge, position of the hilum, micropyle, and raphe, while the intern characteristics observed were embryo (cotyledon, hypocotyl-radicle axis, plumule) and the presence of endosperm.

To make the intern morphologic study of seeds easier, they were manually scarified with sandpaper number 80 in the side opposite the hilum region and then immersed in water at room temperature for a period of 24 h.

In addition to the analyzes described earlier, the weight of a thousand seeds was determined, using eight replicates of 100 seeds each, following the methodology described in the Rules for Seed Analysis (Brasil, 2009a).

Morphological characterization of germination and seedling

In the description and illustration of the external morphology of germination and seedling, 100 seeds were sown in a polyethylene tray (40 × 30 × 8 cm) containing vermiculite as substrate, moistened with distilled water in 60% retention capacity, and maintained in a greenhouse with replacement of water daily. Based on preliminary tests it was observed that the seeds of this species have tegumentary numbness and to accelerate the germination process, seeds were manually scarified with sandpaper number 80 in the opposite side of the micropyle. The steps of the germination process were evaluated daily from sowing to seedling stage, considering as seedling when the protophilus of the first order were fully expanded.

The methods and terms used to describe morphologic aspects of fruits, seeds, germination, and seedlings of *A. farnesiana* are according to Barroso et al. (1999), Brasil (2009b) and Silva et al. (2012), and were drawn by hand.

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

Table 1. Physical characteristics of the fruits of *Acacia farnesiana*.

Variable	Average	Standard deviation	CV (%)	Variation range
Length (mm)	54.47	7.36	13.52	38.61-75.88
Width (mm)	12.52	1.07	8.57	9.82-14.98
Thickness (mm)	11.89	1.34	11.28	8.49-14.70
Weight (g)	3.06	0.75	24.52	1.018-5.493
Number of seeds per fruit	17.89	3.77	21.05	10-27

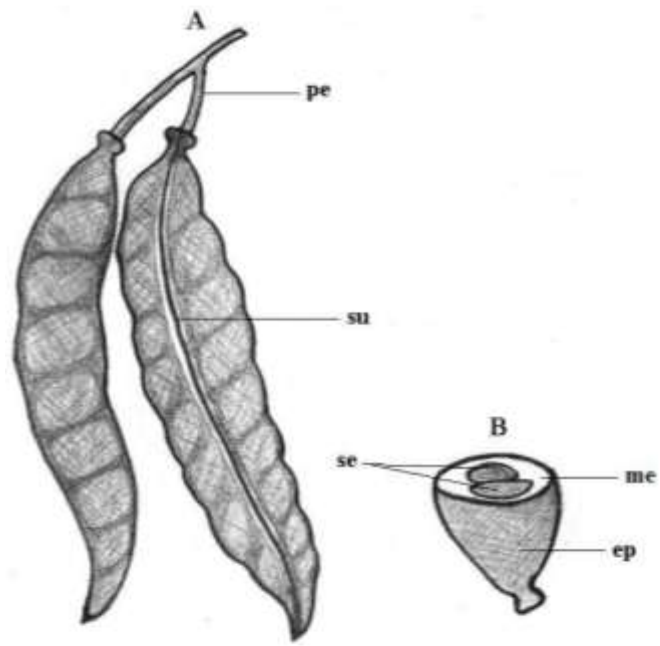

Figure 1. Aspects of the external morphology of the fruit of *Acacia farnesiana*. A - fruit; B - cross section of fruit (EG - peduncle; SU - suture if seed - EP - epicarp; myself - mesocarp) (fonte: Kelina Bernardo Silva).

RESULTS AND DISCUSSION

Morphologic characterization of fruits

The fruit of *A. farnesiana* is legume nucoide, simple, dry, polysemy (presenting 10 to 27 seeds), glabrous, straight-wavy shape, and late dehiscence (Table 1). This species of fruit has a pleasant odor, depressions on the sides and on one suture line in the ventral and dorsal portion from the stem to the apex. The base is acuminate, stipellate with persistent stems and ligneous consistency (Figure 1A). The epicarp is dry, opaque, with slightly striated surface, glabrous and dark brown; the mesocarp is dry, with light brown color and consistency of cork-fibrous, which is divided by false septa where the seeds are (Figure 1B); it is possible see to one yellowish sticky substance between the epicarp and mesocarp.

The legume fruit has origin from the supper ovarian, which is unicarpellate, dehiscent at the junction point of the edges of the carpel and the dorsal region of the midrib, forming two valves. Those are characteristic only of the Leguminosae family, who is open along two sutures (often of equal length, nonligneous) causing the separation of the valves which keep them together in the base (Barroso et al., 1999).

Studying fruits of *Erythrina velutina* Willd., Silva et al. (2008) observed similar results regarding fruit type and coloration. Likewise, Abud et al. (2009) assessed the fruit morphology of *Mucuna aterrima* (Piper and Tracy) and got the same information related to the fruit type, epicarp, and seed numbers per fruit.

Similar results were found by Nogueira et al. (2010) studying the fruit morphology of pau-violeta (*Dalbergia cearensis* Ducke) looking for the irregular form of size and length, which ranged from 30.61 to 47.57 mm. In addition, the width was between 7.83 and 10.88 mm, and the thickness around 2.94 and 4.95 mm.

Caatinga species in conditions of water stress caused by drought have functional changes, such as anatomical and morphological changes, including deep root growth, decrease in leaf size, stem growth and leaf loss (Trovão et al., 2007). Therefore, it is believed that changes in size of fruits and seeds are not only related to genetic resources, but also to the conditions determined by the environment (Figure 2).

Morphological characterization of seeds

The seeds of *A. farnesiana* are stenospermics of obovoid shape, apex rounded, and slightly rounded base (Figure 3). The obovoid shape was described by Barroso et al. (1999) referring to seeds of other species of *Acacia*. The testa is smooth, dark brown color, hard consistency when dried, and leathery when hydrated. This agrees with Sousa (2010) when he reported that the testa of the leguminous seeds is usually brown or black.

The seed has on both sides apical-basal pleurograma, which represents about 85% of its length; the hilum is small, circular format, located at the base of the seed (Figure 3A); the micropyle has spot-on format and is located just below the hilum, being barely noticeable to the naked eye and the raphe is absent. The storage tissue is cotyledonary, whitish color, firm consistency,

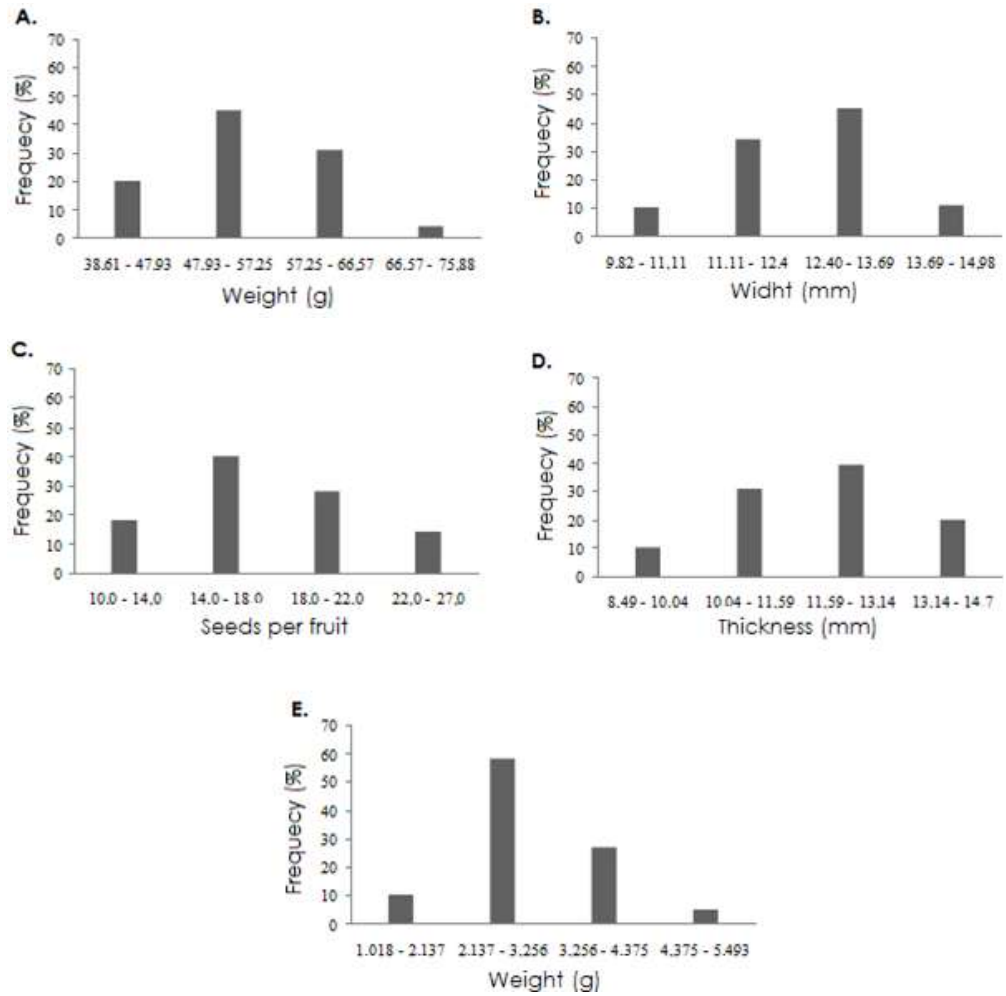

Figure 2. Distribution of relative frequencies of length, width, thickness, weight, and number of seeds per fruit of *Acacia farnesiana*.

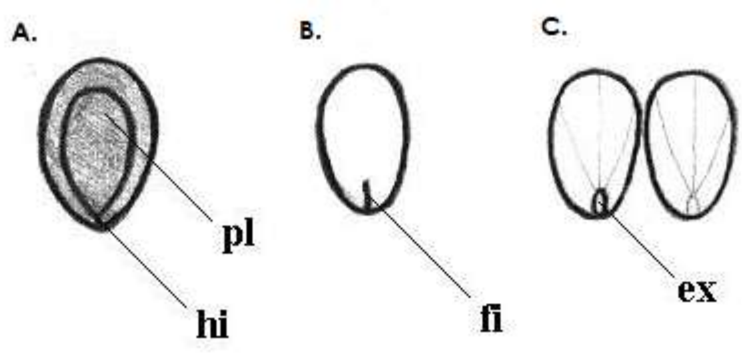

Figure 3. Aspects of the external morphology of seeds of Acacia farnesiana. A: seed, B-C: embryo (PL - pleurograma; HI - hilo; FI - cleft; EX - hypocotyl-radicle axis). Fonte: Kelina Bernardo Silva.

crass flats, obovate form, opposite and equals. On the base of the cotyledons, there is a small crack, with approximately 1 mm in length (Figure 3B). The axis of the hypocotyl and radicle is short, straight, and does not go

Table 2. Physical characteristics of seeds of *Acacia farnesiana*.

Variable	Average	Standard deviation	CV(%)	Variation range
Length (mm)	6.97	0.52	7.44	4.69-8.20
Width (mm)	5.43	0.44	8.15	3.95-7.11
Thickness (mm)	3.49	0.29	8.24	2.90-4.50
Weight (g)	0.11	0.01	13.00	0.062-0.152
Number of seeds per fruit	105.6	-	-	-

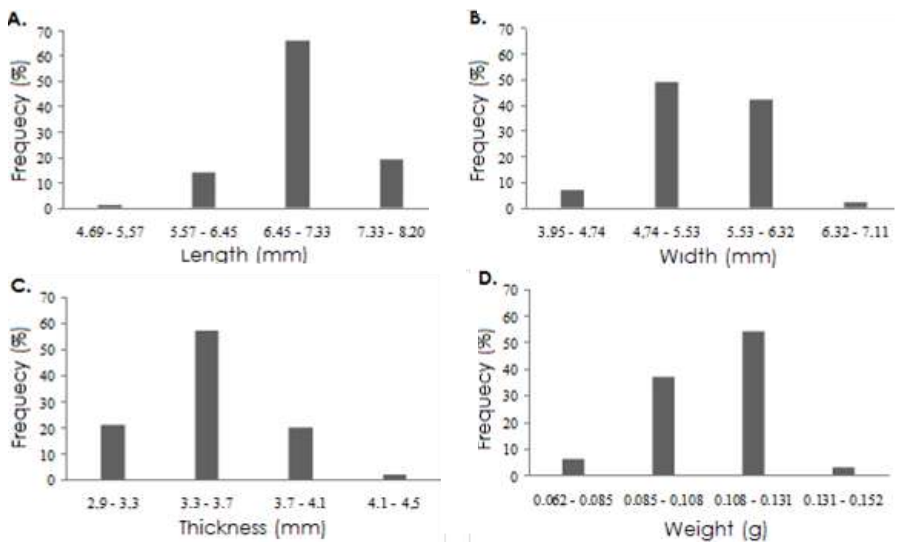

Figure 4. Frequency distribution regarding length, width, thickness, and weight of seeds of *Acacia farnesiana*.

up until the cotyledons (Figure 3C). The seedling is not visible and is devoid of mature seed endosperm, with all the material stored in the cotyledons, so that they occupy the entire length of the seed.

Variations in color, shape, and seed size were described to some species from the caatinga (Araújo et al., 2007); however, according to Araujo et al. (2007) there is little information about morphological variations of the seeds from the caatinga, making difficult a broader discussion about the importance of these changes for successful seed germination and seedling establishment.

The average length is 6.97 mm (range 4.69 to 8.20 mm); average width of 5.43 mm (range 3.95 to 7.11 mm); average thickness of 3.49 mm (range 2.90 to 4.50 mm); average weight of 0.11 g (ranging from 0.062 to 0.152 g) and thousand seed weight of 105.6 g (Table 2). These results are similar to those obtained by Battilani et al. (2011) who studied the morphology of Guibourtia hymenifolia (Moric.) J. Leonard.

The results of frequency analysis of the seeds (Figure 4A to D) indicated predominance of 66% with lengths ranging from 6.45 to 7.33 mm, 49% width ranging from 4.74 to 5.53 mm, 57% thickness ranging from 3.3 to 3.7 mm and 54% weight ranging from 0.108 to 0.131 mm.

In most of the habitats, seed size varies from ten orders of magnitude, although within the same species this size represents less than half of this variation (Brito et al., 2014). Species with large seeds have greater persistence and establishment of a broad range of environmental conditions, while species with small seeds are more perturbation dependent. Large seeds increase seedling survival. However, in order to understand that in relationship it is necessary to have knowledge of how the reserves of seed are used during germination and early seedling establishment (Nogueira et al., 2010).

The weight of the seed has a strong influence on the plant establishment, since the heavier seeds often originate seedlings with larger initial length and better survival in a low light environment (Parker et al., 2006). On the other hand, to Nogueira et al. (2010) small seeds have greater ease in obtaining water for germination than large seeds, due to the higher surface/volume ratio. Thus, because of its size, seeds of *A. farnesiana* have advantages for semi-arid conditions of the Brazilian Northeast, since water availability is restricted to a short rainy season.

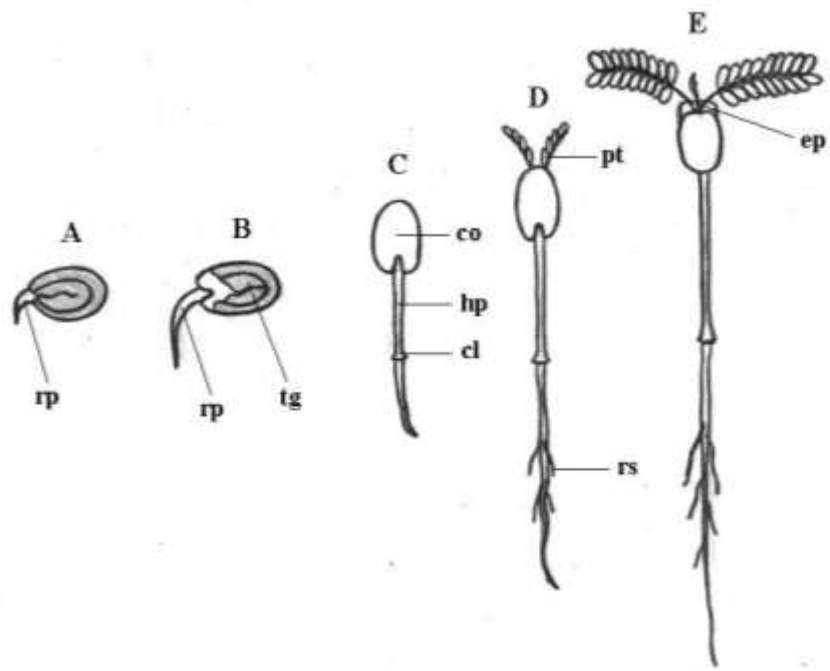

Figure 5. Morphological aspects of seed germination and seedling development of *Acacia farnesiana*. A - Beginning of root protrusion, B - Elongation of the main root, C - Development of the hypocotyl, D - Protophilus and emergence of secondary roots; and - formed seedlings (RP - taproot; TG - tegument CO - cotyledons; HI - hypocotyl; CL - lap; RS - secondary root; PT - foliage leaf; EP - epicotyl). Fonte: Kelina Bernardo Silva.

Germination characterization

The swelling of the seed can be observed 24 h after seeding, and on the third day the root protrusion in the micropyle region is observed, which is thin, conical, glabrous, and white in color, having an expressive development (Figure 5A to B). Subsequently, with the loosening of the integument, arises the paracotyledons with a yellowish green color, obovate shape, opposite, glabrous, crass, with a visible crack in the base, which are the first photosynthetic organs of the seedling (Figure 5C).

Initially, the hypocotyl is short, straight, bright green in color, glossy and, as it grows, there is a differentiation of the root due to the increased width, shown by the larger diameter of the hypocotyl, where the cervix is located (Figure 5C). From the sixth day after sowing, the paracotyledons are semi-open, appearing among them protophilus of the first order (Figure 5D); epicotyl is very small and barely noticeable; still at this stage, the first secondary roots emerge just below the cervix (Figure 5D).

On the seventh day after sowing, the protophilus of the first order are fully expanded and appositive, and may terminate the germination phase; the seedling is pivoting brownish, with average length of 105 mm root. The hypocotyl is bright green, cylindrical, smooth, glabrous, and straight, with average length of 55 mm; during this stage the paracotyledons have dark a green coloration, are crass, dull and persistent, with cordate base, obtuse apex, and hole board and obovate shape. The epicotyl is short, straight, cylindrical, thin, bright green, and glabrous. At the apex is the apical, while the first couple of protophilus are opposites, both being composed of a pinna. The pinnae are par pinnate, with 9 pairs of green leaflets-dark and membranous consistency; each leaflet with a base and obtuse apex, sepsis without apparent nervous and entire margin (Figure 5E).

The germination of *A. farnesiana* seeds is considered by Silva et al. (2008) as epigeal and phanerocotylar, and the most common form of germination for most legumes.

Morphological characterization of seedlings

From the 15th day after sowing, change of phyllotaxis in seedlings of *A. farnesiana* is observed, with the presence of the first pair of pinnate, which are opposed and unique, and are followed by pinna position switches, two in number; at the base of the pedicel of the pair of pinnae two prominent stipules (visible and opposite) are formed. The epicotyl is straight, thin, glabrous and reddish, and at this stage, the primary root has tender consistency and secondary roots become abundant; paracotyledons in the

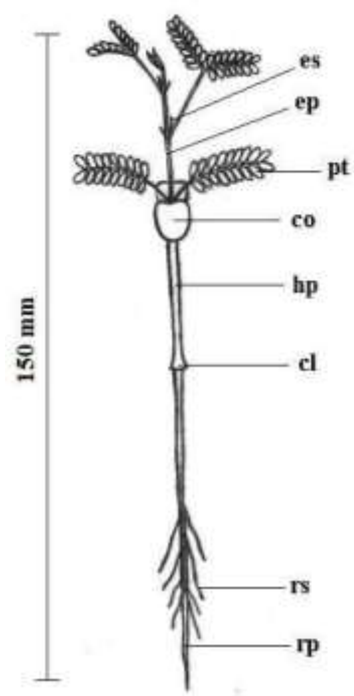

Figure 6. Morphological external seedling of *Acacia farnesiana*. (RP - taproot; CO - cotyledons; HI - hypocotyl; CL - lap; RS - secondary root; PT - foliage leaf; EP - epicotyl; ES - stipules).

seedlings are persistent and measure approximately 150 mm long (Figure 6).

Conclusions

The fruits, seeds, germination, and seedlings of A. farnesiana have quite homogeneous morphological characters and can be used in studies aimed at identifying lots of seeds, germination in the laboratory, and recogni-tion of the seedlings in early stages of development.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(31), pp. 2920-2936, 4 August, 2016
DOI: 10.5897/AJAR2016.11127
Article Number: 3364CB659823
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Special liming materials for acidity control of soils with variable charge

Jessica Alves dos Santos[1]*, Adriel Ferreira da Fonseca[1], Domenico Zocco[2] and Igor Vieira[3]

[1]State University of Ponta Grossa, Av. Carlos Cavalcanti, Ponta Grossa, Paraná, Brazil.
[2]Omya International AG, P. O. Box 335, Baslerstrasse 42, 4665 Oftringen, Switzerland.
[3]Omya do Brasil Ltda, Av. Angélica, 2220, 2° andar, São Paulo, São Paulo, Brazil.

Received 13 April, 2016; Accepted 8 July, 2016

Soil acidity has been the major limiting factor to farming activities in the tropics and subtropics. The objective of this study was to verify the efficacy of special liming materials, when compared with the dolomitic limestone, regarding the correction of soil acidity with variable charge in controlled conditions. Samples of a Typic Dystrudept and Rhodic Hapludox were collected from a of layer 0-20 cm, and used to carry out two experiments in a completely randomized design of 4 x 4 x 8 factorial design. Four liming materials were studied: dolomitic limestone (DL), granulated micronized calcite (GMC), granulated micronized dolomite (GMD) and carbonated suspension (CS). After they have been characterized, each liming material was added to the soils using doses that aimed to increase the base saturation (V) to 50, 70 and 90% and a control treatment was included. The treated soil samples were incubated at 23 ± 2°C and 60% soil water retention capacity for eight periods (0, 7, 15, 30, 45, 60, 75 and 90 days). The attributes active (pH), potential (H+Al) and exchangeable (Al^{3+}) acidity and V were evaluated. The special liming materials GMC and CS were efficient enough to reduce the active potential and exchangeable acidity, and increase V in soils with variable charge.

Key words: Micronized liming materials, carbonated suspension, soil reaction, Inceptisol, Oxisol.

INTRODUCTION

The need to increase food production has implied the need to improve plant growth in soils with fertility restrictions. In the tropics and subtropics, predominance of soils with variable charge, the major limiting factors to the farming production have been acidity and aluminum (Al) toxicity (Castro and Crusciol, 2013; Vendrame et al., 2013). Correcting the acidity of these soils does not limits only the neutralizing capacity, the exchangeable aluminum (Al^{3+}), but also the pH increase which results in the consumption of protons from the surface functional groups (mainly silanol, aluminol, iron oxide-OH and aluminum oxide-OH radicals, carboxyl and phenolic groups of the soil organic matter), generating negative electrical charge (Sparks, 2003).

After the correction, the soil tends to acidify due to the following factors: (i) rainfall, (ii) basic cations leaching

*Corresponding author. E-mail: jessicaalvesdossantos@bol.com.br. Tel: +55 42 3220-3789.

Table 1. Characteristics of the two soils (Inceptisol and Oxisol) studied.

Soil	Source material	Geographic coordinates	Altitude (m)	Native vegetation		Management history	
Inceptisol[1]	Itararé sandstone3	S 25º24'37.8'' W 49º58'22.8''	900	Araucaria Forest Montana		No-till for 15 years in crops succession between black oat and soybean during 2007/08 to 2011/12	
Oxisol[2]	Ponta Grossa Shale3	S 25º0'28.26'' W50º15'09.31''	800	Araucaria Forest Montana		Without cropping	
	$pH_{(CaCl_2)}$	H+Al	Al	Ca	Mg	K	CEC[4]
		mmol$_c$ dm^{-3}					
Inceptisol[1]	4.3	103.3	11.3	30.8	9.3	2.3	145.6
Oxisol[2]	3.8	151.6	26.0	6.0	7.0	1.5	166.1
	V[5]	OC[6]	Clay	Silt	Sand	BD[7]	S[7]
	%	g dm^{-3}		g kg^{-1}		g cm^{-3}	cm^{3}cm^{-3}
Inceptisol[1]	29.0	21.0	200.0	255.2	544.8	1.24	1.5
Oxisol[2]	8.7	33.0	736.0	174.2	89.8	0.99	1.0

[1]Typic Distrudept; [2]Rhodic Hapludox; [3]According to Mineropar (2001); [4]CEC: cation exchange capacity; [5]V: base saturation; [6]OC: organic carbon by Walkey-Black method; [7]BD: bulk density, and S: water saturation, according to EMBRAPA (1997). The other soil attributes were performed according to regional procedures (Pavan et al., 1992).

(calcium - Ca^{2+}, magnesium – Mg^{2+}, potassium– K^+ and sodium– Na^+), (iii) uptake and exportation of these cations by the plants, (iv) hydrolysis reactions in the clay-humic plasma, (v) addition of soluble salts and fertilizers (mineral and organic) in the soil-plant system (Nagy and Kónya, 2007; Havlin et al., 2014). Therefore, suitable management and acidity control of soils with variable charge are basic principles of sustainable agriculture (Kirkham et al., 2014) and food safety in underdeveloped countries (Spiertz, 2012; Curtis and Halford, 2014).

Limestone is the most common used liming material to control soil acidity. However, this limestone present low solubility in water (1.4 mg L^{-1}) and needs to be previously applied in advance (around three months), to produce satisfactory agronomic results (Raij, 2011). Therefore, it is important to study special liming materials that present enhanced reactivity in short-term and with the possibility of being used more efficiently in conservationist agriculture systems (in which the soil is not revolved, for example, no-tillage system).

The most important factors to determine the efficacy of liming materials to control soil acidity are: release neutralizing (OH^- or HCO_3^-), particle size and specific surface area, original material crystalline structure and calcium content ($CaCO_3$) (Havlin et al., 2014). In such context, special liming materials are ranked for their particle size, that is, they are finer, than the regular limestones (from particle size of 300 to 840 µm) and larger specific surface, favoring their reactivity in the soil. All these parameters are fundamental to determine their reactivity and efficacy once applied to the soil. This issue is important but there is lack of information on the reactivity and efficacy of special liming materials in soils with variable charge. Moreover, there are few studies on soil acidity control in Inceptisol (mainly in Typic Distrudept) when compared with the number of papers published that emphasize on Oxisols acidity. The Typic Distrudept has distinct characteristics and the representation to grain and forage production in the south of Brazil (EMBRAPA, 2006).

This study compares the efficacy of special liming materials to that of dolomitic limestone, on their correction of soil acidity with variable charge (Typic Distrudept and Rhodic Hapludox) and estimated bases saturation (V) after incubation in controlled conditions for up to 90 days.

MATERIALS AND METHODS

Samples from the 0-20 cm layer of two soils with variable charge (Typic Distrudept and Rhodic Hapludox) were collected in the region of Campos Gerais of Paraná, Brazil, and their characteristics are presented in Table 1. After they have been collected, the samples were dried in oven with air forced circulation at 40°C for 48 h, ground and sieved in a 2.0 mm mesh sieve. Each soil represented one experiment.

The design used, for both experiments, was completely randomized in a 4x4x8 factorial design with four replications. Four liming materials were analyzed: dolomitic limestone (DL), granulated micronized calcite (GMC), granulated micronized dolomite (GMD) and carbonated suspension (CS). The results of physical and chemical characterization (Table 2 and Figure 1) of liming materials were performed according to França and Couto (2007) and MAPA (2007). For each liming materials, there was a

Table 2. Chemical and physical attributes of liming materials (dolomitic limestone – DL, granulated micronized calcite – GMC, granulated micronized dolomite – GMD and carbonated suspension – CS) studied.

Liming materials	CaO[1]	MgO[2]	RE[3]	NP[4]	ECC[5]	CCE[6]
	g kg^{-1}					
DL	265.9	257.6	832.0	1079.7	898.7	1117.3
GMC	462.2	15.5	1000.0	962.7	962.7	861.5
GMD	345.9	121.5	1000.0	1006.5	1006.5	919.4
CS	361.1	8.30	1000.0	770.0	770.0	663.4

[1]CaO: calcium oxide; [2]MgO: magnesium oxide; [3]RE: relative efficiency of the liming materials and [4]NP: neutralizing power: analytical determinations performed according MAPA (2007); [5]ECC: effective calcium carbonate obtained by the equation [(NP*RE)/100]; [6]CCE: Calcium carbonate equivalent which represents the neutralization value of the material compared to pure $CaCO_3$ obtained by the equation [(CaO x 1.79)+(MgO x 2.48)].

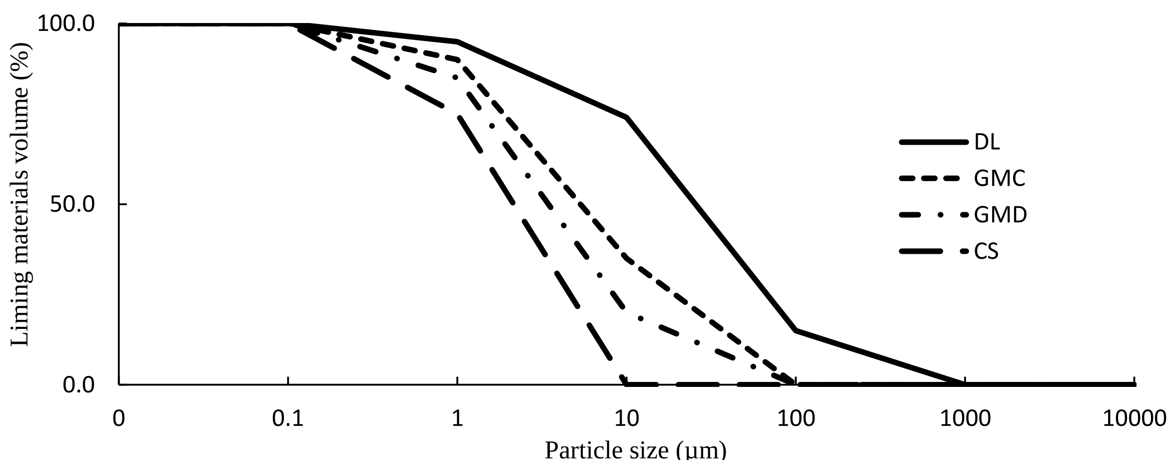

Figure 1. Particle size of the liming materials under study: dolomitic limestone (DL), granulated micronized calcite (GMC), granulated micronized dolomite (GMD) and carbonated suspension (CS). The liming materials attribute was obtained according to procedures of França and Couto (2007).

control treatment. Also, three doses were studied aiming to increase V to 50, 70 and 90%. The liming materials were duly homogenized in the soil and incubated for 0, 7, 15, 30, 45, 60, 75 and 90 days, at constant temperature of 23 ± 2°C and soil humidity conditions aiming at 60% of soil water retention capacity [169.4 and 112.0 ml of deionized water (average electrical conductivity: 0.5 µS cm^{-1}) was added to the Typic Distrudept and Rhodic Hapludox, respectively]. Each experimental unit consisted of 500 g soil. The lime requirement (LR) was obtained with the equation according to Raij et al. (1996):

$$LR = [CEC (V_2 – V_1)]/(10 x ECC) \quad (1)$$

LR: lime requirement (Mg ha^{-1}) for layer 0-20 cm; *CEC*: cation exchange capacity (mmol$_c$ dm^{-3}); V_1: base saturation (%) obtained; and V_2: base saturation (%) aimed.
The ECC was estimated with the equation according to Raij (1977):

$$ECC = (NP \times RE)/100 \quad (2)$$

ECC: effective calcium carbonate – %; *NP*: neutralizing power – calculated with the equation [CaO (%) x 1.79 + MgO (%) x 2.48] and *RE*: relative efficiency of the liming.

For the special liming materials, 100% RE was adopted, due to the fact that they present very fine particle size (mean particle size < 10 µm) when compared with a limestone which is considered to have fine particles (mean particle size < 300 µm) (Figure 1). The doses estimated to increase V to 50, 70 and 90% are presented in Table 3.

After each incubation period is finished (0, 7, 15, 30, 45, 60, 75 and 90 days), the experimental units of both soils were removed from the incubation room, taken to the laboratory, dried in oven at 40°C with air forced circulation, they were then ground and sieved in a 2.0 mm mesh sieve. In the sequence the following attributes were determined: active (pH), potential (H+Al) and exchangeable (Al^{3+}) acidity employing the methods suggested by Pavan et al. (1992). The concentrations of exchangeable cations (calcium– Ca, magnesium– Mg and potassium– K) were used to estimate the V values.

Data were submitted for statistical analysis employing the computer program SAS Version 9.1.2 (SAS, 2004). The program suggested transformations to the square root of the potential acidity (H+Al) and V attributes for both soils under study. Three factors were considered in the statistical model: (i) four soil acidity liming

Table 3. Doses of each liming material applied [dolomitic limestone (DL); granulated micronized calcite (GMC); granulated micronized dolomite (GMD) and carbonated suspension (CS)] to increase the soil base saturation (V) to 50, 70 and 90%.

Doses	Liming materials							
	DL	GMC	GMD	CS	DL	GMC	GMD	CS
	g of liming material per kg of soil				dose of liming materials (Mg) corresponding per hectare			
Typic Distrudept								
50%*	1.38	1.28	1.23	1.60	3.41	3.18	3.05	3.98
70%*	2.68	3.72	2.39	4.65	6.65	6.21	5.94	7.76
90%*	3.99	3.72	3.56	4.65	9.89	9.23	8.83	11.54
Rhodic Hapludox								
50%*	3.83	3.57	3.42	4.47	7.63	7.13	6.82	8.91
70%*	5.68	5.30	5.07	6.63	11.33	10.58	10.12	13.22
90%*	7.54	7.03	6.73	8.80	15.03	14.03	13.42	17.54

The quantities of each liming material to estimate the need for liming were obtained with the equation (according to Raij et al., 1996): LR = [CEC(V2 – V1)/10*ECC], where: LR: lime requirement (Mg ha^{-1}) for layer 0-20 cm; CEC: cation exchange capacity ($mmol_c$ dm^{-3}); V1: base saturation (%) obtained; and V2: base saturation (%) aimed. The ECC was estimated through the equation (according to Raij, 1977): ECC = (NP x RE)/ 100, where: ECC: effective calcium carbonate – %; NP: neutralizing power – calculated through the equation [CaO (%) x 1.79 + MgO (%) x 2.48] and RE: relative efficiency of the liming.

materials: (DL, GMC, GMD and SC), (ii) four doses of each liming material applied (to increase V to 50, 70 and 90% besides the control treatment) and (iii) eight incubation periods were studied (0, 7, 15, 30, 45, 60, 75 and 90 days). The effect of predictive variables (doses of liming materials) was adjusted to the response variables (soil attributes) in each incubation period, using the regression models (linear or quadratic). Besides that, the profile analysis was used to compare the effects of each dose of soil acidity liming materials employed in the incubation periods.

RESULTS

Interactions between the liming materials and doses applied (F = 55.69 to pH, F = 122.32 to H+Al, F = 87.21 to Al and F = 154.56 to V; P < 0.0001); liming materials and incubation periods (F = 4.22 to pH, F = 8.60 to H+Al, F = 35.08 to Al and F = 15.25 to V; P < 0.0001); doses applied and incubation periods (F = 7.39 to pH, F = 13.02 to H+Al, F = 35.19 to Al and F = 38.41 to V; P < 0.0001); and liming materials, doses applied and incubation periods (F = 3.10 to pH, F = 7.69 to H+Al, F = 13.58 to Al and F = 11.28 to V; P < 0.0001) were observed in Typic Distrudept.

Also observed were interactions in Rhodic Hapludox between the liming materials and doses applied: (F = 192.39 to pH, F = 205.92 to H+Al, F = 43.34 to Al and F = 368.53 to V; P < 0.0001); liming materials and incubation periods (F = 25.27 to pH, F = 48.59 to H+Al, F = 32.29 to Al and F = 85.24 to V; P < 0.0001); doses applied and incubation periods (F = 15.03 to pH, F = 118.50 to H+Al, F = 467.62 to Al and F = 95.08 to V; P < 0.0001); and liming materials, doses applied and incubation periods (F = 15.39 to pH, F = 22.40 to H+Al, F = 8.81 to Al and F = 37.77 to V; P < 0.0001).

In typic distrudept

All the special liming materials reduced active (pH) and potential H+Al (Figure 2) and exchangeable Al^{+3} acidity (Figure 3), in all the doses under study when the incubation period increased. This fact may be ascribed to the interaction between periods of incubation, liming materials and doses.

The GMC reduced active acidity (pH) (Figure 4) after 0, 30, 60 and 90 days and the potential acidity (H+Al) (Figure 5) after 15 and 30 days of incubation, in doses to increase V to 70 and 90% respectively. However, CS was more efficient in the reduction of potential acidity (H+Al) (Figure 5) when applied in the dose aiming to increase V to 90%. The exchangeable acidity (Al^{+3}) (Figure 6) was neutralized after the application of the special liming materials GMC and CS in doses to increase V to 70 and 90%. GMD was the only liming material under study that was inefficient to reduce exchangeable acidity (Al^{3+}) on 7, 15, 30, 45, 60 and 90 days after being applied (Figure 6).
The exchangeable acidity (Al^{3+}) was kept low with the application of GMC doses to increase V to 70 and 90% during the 90 days of incubation. This fact was not observed with the special liming materials GMD (Figure6). Therefore, special liming materials (GMC and CS) presented higher efficacy, in short-term (90 days), when compared with the DL.

All liming materials under study increased the base saturation of Typic Distrudept (initial V: 29.0%). However, only GMC, applied in the dose to increase V to 70% (Figure 3) resulted in a value close to the aim (V: 70%) in the first 30 days of incubation (Figure 7). After 60 days of incubation, GMC and CS, used in doses to increase V to

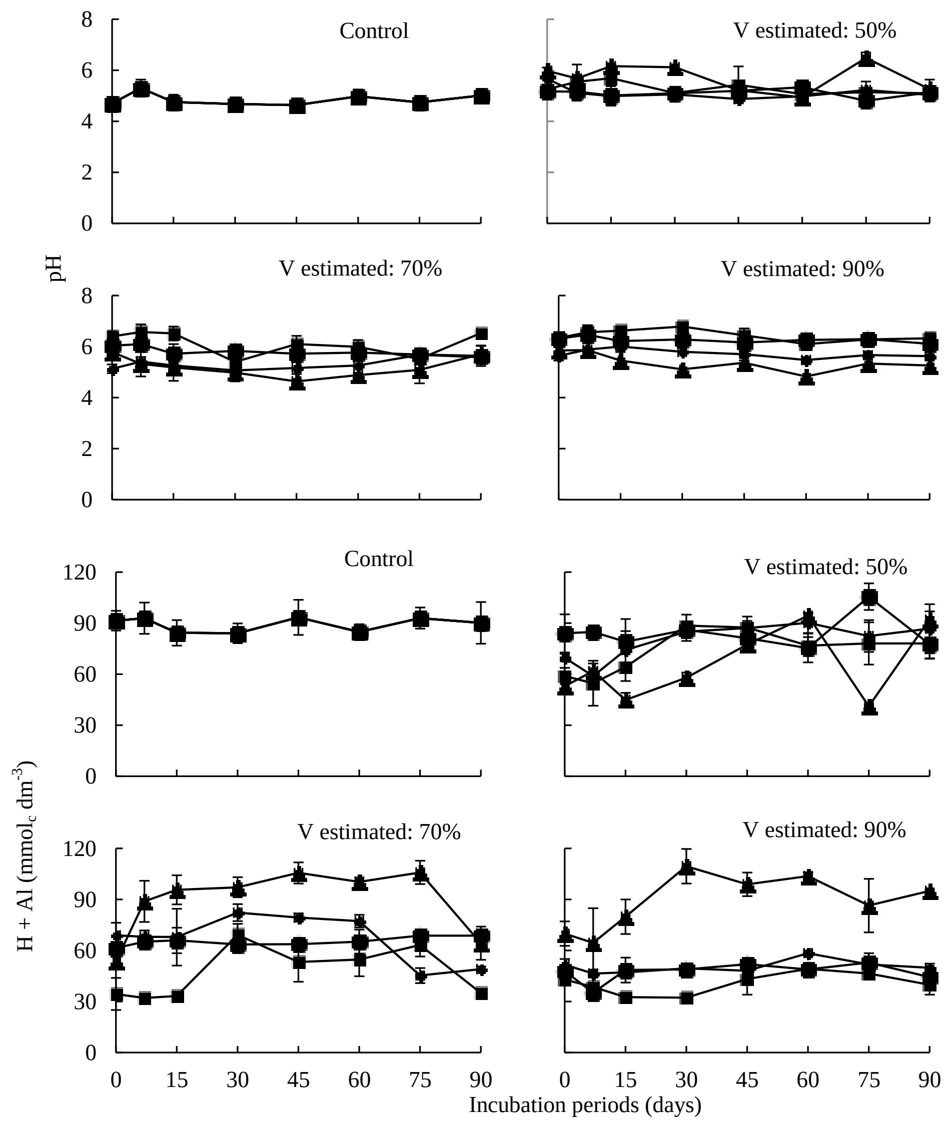

Figure 2. Active (pH) and potential (H + Al) acidity values on a Typic Distrudept (n = 4 ± standard deviation) during 90 days of incubation for the control treatment and after application of the liming materials aiming base saturation (V) to 50, 70 and 90%. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Coefficient of variation: 4.8 and 5.0 % to pH and H+Al, respectively.

70 and 90%, reacted similarly, keeping the average values very close. The special liming materials, GMC and CS are more efficient to increase V to the estimated values. GMD showed data discrepancy of all attributes under study.

In Rhodic Hapludox

All liming materials, doses and periods under study reduced the active (pH) and potential (H+Al) (Figure 8) and exchangeable Al^{3+} (Figure 9) acidity. The GMC and the CS applied in doses to increase V to 70 and 90%, after 7, 15, 30, 45 and 90 days of incubation, resulted in active acidity reduction (Figure 8 and 10). When CS was applied in the dose to increase V to 90% (Figure 8) on 45, 60 and 75 days, it was observed to be the most efficient to reduce acidity (H+Al) (Figure 11). The exchangeable acidity (Al^{3+}) was neutralized by the dose used to increase V to 50% with GMC (Figure 9) in the early days (0, 7 and 15 days) of incubation (Figure 12) and this was constant with the other doses studied. The CS, when applied in doses to increase V to 70 and 90%(Figure 9) showed efficacy (Figure 13).

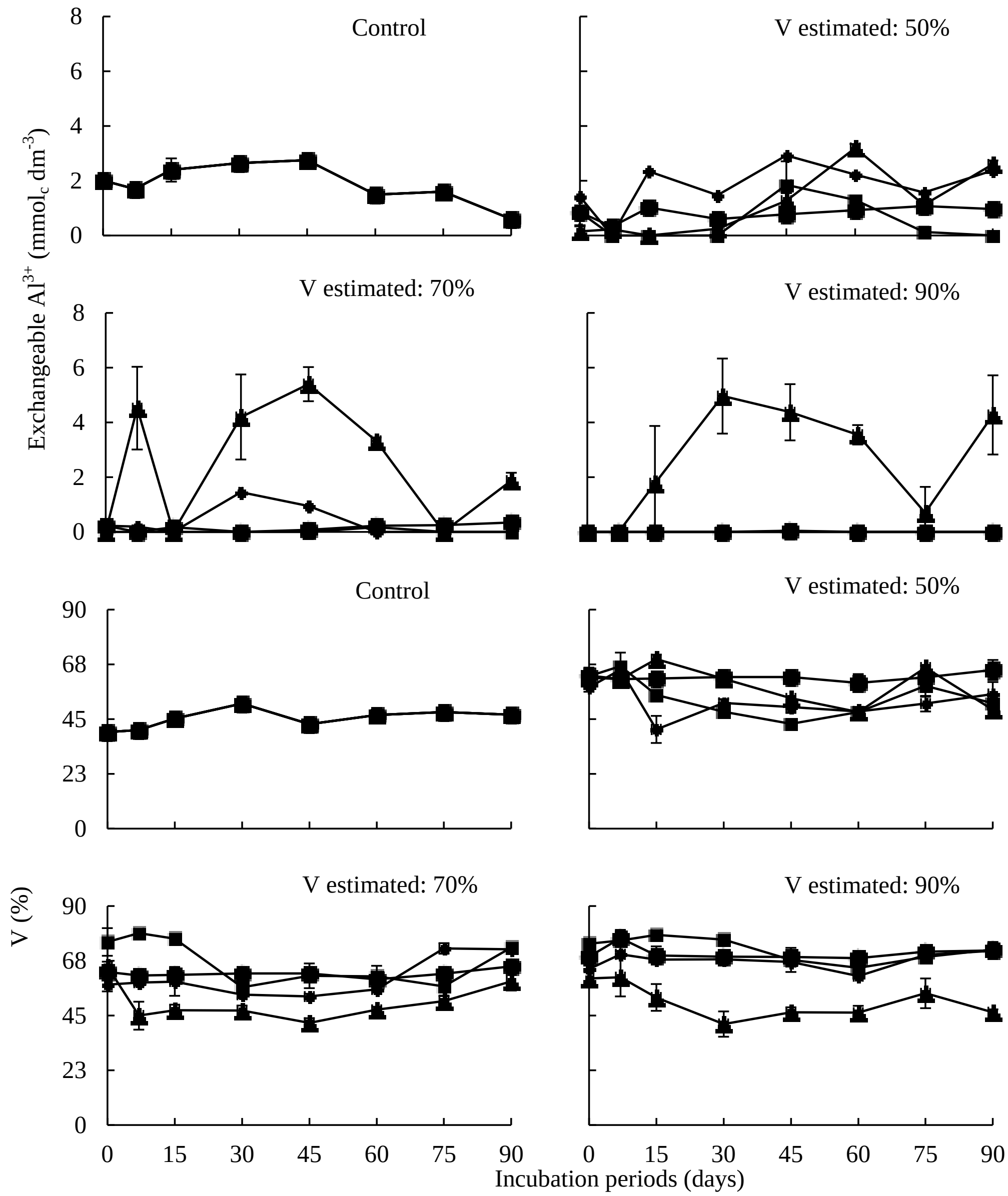

Figure 3. Exchangeable acidity (Al^{3+}) values and base saturation (V) values on a Typic Distrudept (n = 4 ± standard deviation) during 90 days of incubation for the control treatment and after application of the liming materials aiming base saturation (V) to 50, 70 and 90%. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Coefficient of variation: 35.2 and 2.3% to Al^{3+} and V, respectively.

DISCUSSION

Soil initial conditions and description of the liming materials studied

The soils

Typic Distrudept and Rhodic Hapludox were selected for this study because they exhibit high acidity and low base saturation (Table 1). The predominant mineralogy of the clay fraction was gibbsite and quartz for the Typic Distrudept, and kaolinite, goethite, hematite and quartz for the Rhodic Hapludox. Most of the special liming material volume have particle size of 1.91-6.58 µm (Figure 1), which is finer than the dolomitic limestone (high quality product in the market used as reference in this study). The specific surface area of the liming materials: DL, GMC, GMD and CS were 306.6; 1055.0;

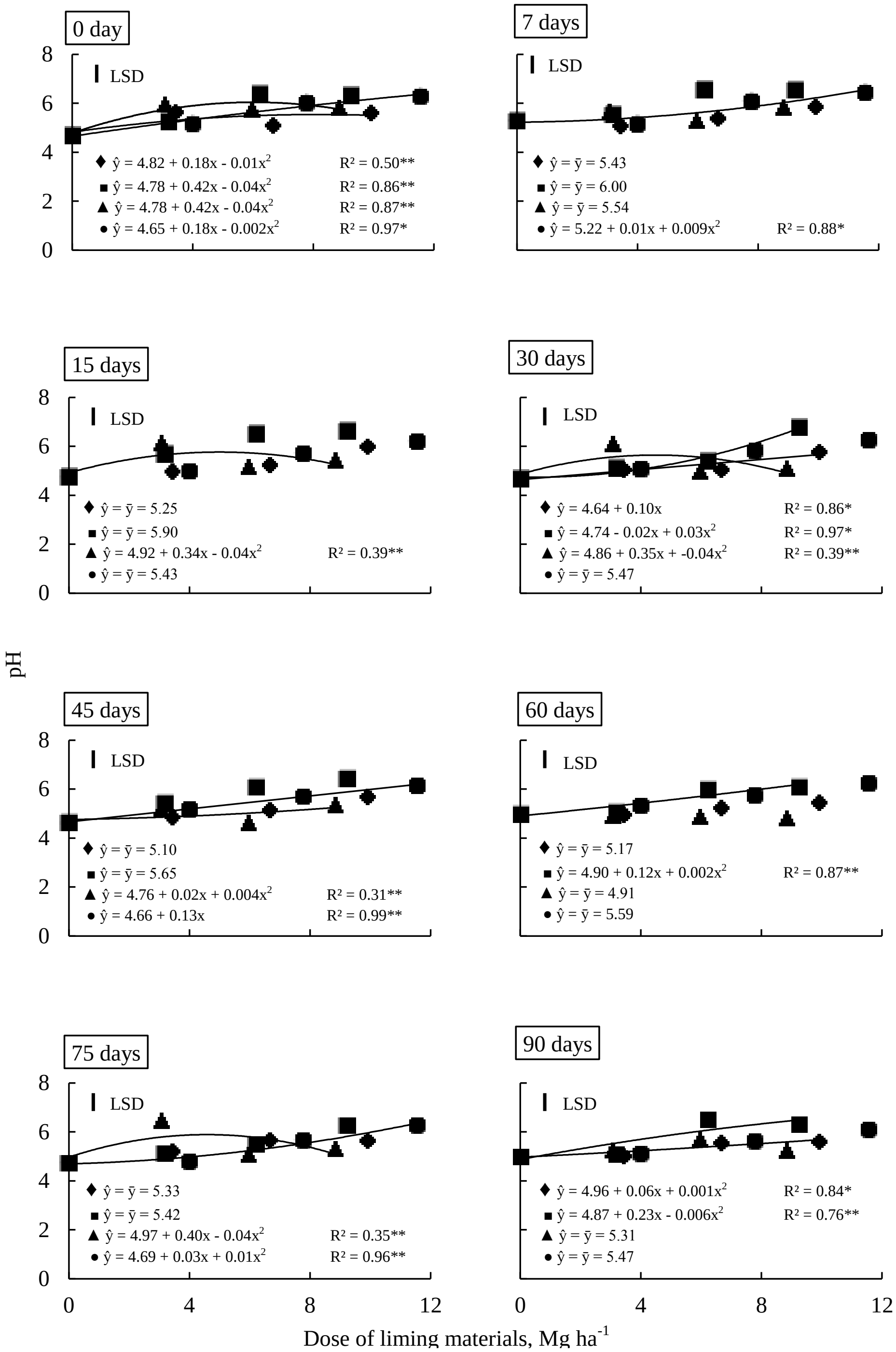

Figure 4. Active acidity (pH) values on a Typic Distrudept (n = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Vertical bars indicate the least significant difference (LSD). *: $P < 0.05$. **: $P < 0.01$.

1099.0 and 1559 m^2 kg^{-1}, respectively. This provides evidence of the special liming materials (GMC, GMD and CS) and their reactivity potential as compared to DL.

Changes on acidity of Typic Distrudept

The special liming materials reduced active (pH) (Figure

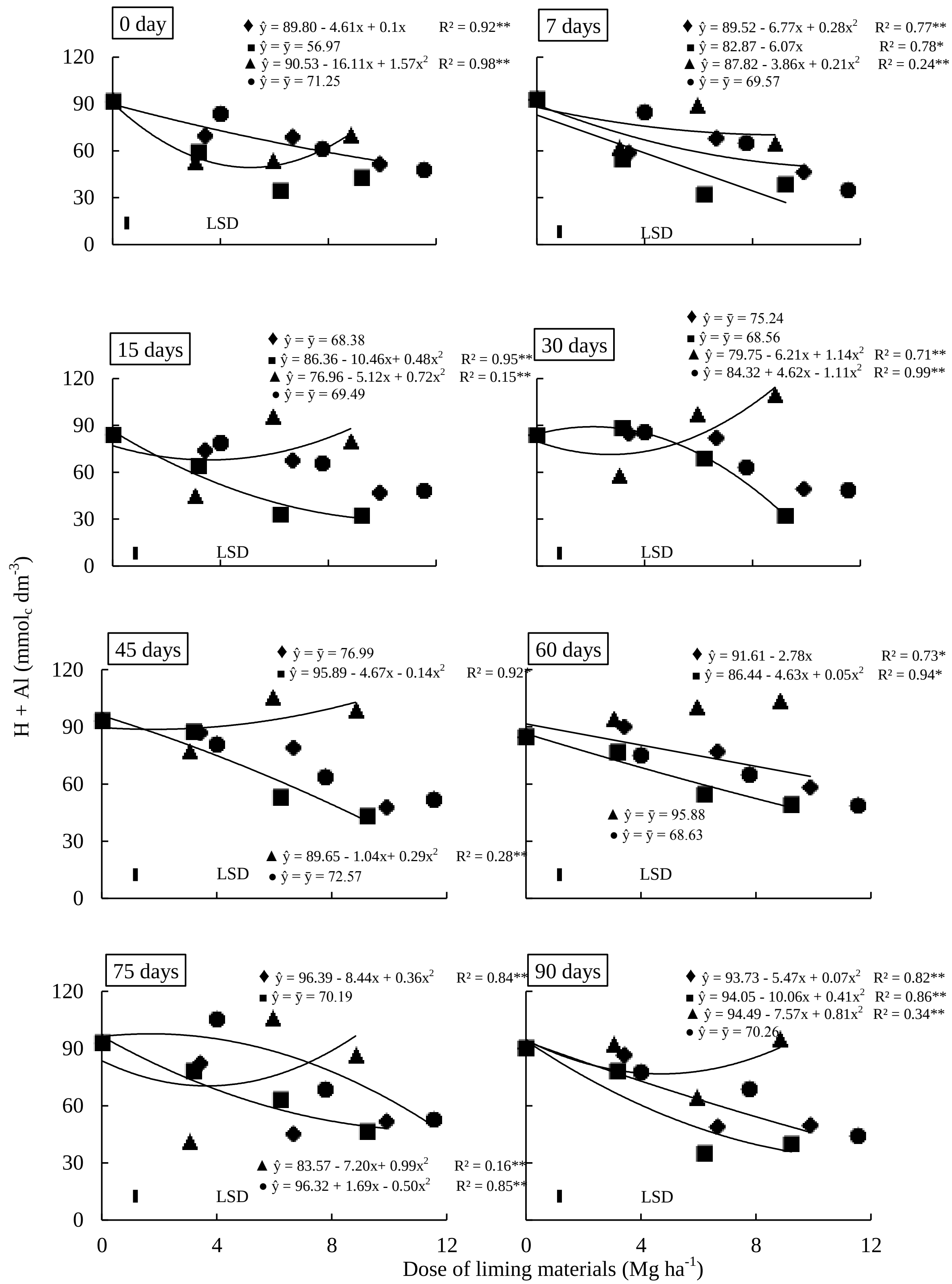

Figure 5. Potential acidity (H +Al) values on a Typic Distrudept (*n* = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Vertical bars indicate the least significant difference (LSD). *: $P < 0.05$. **: $P < 0.01$.

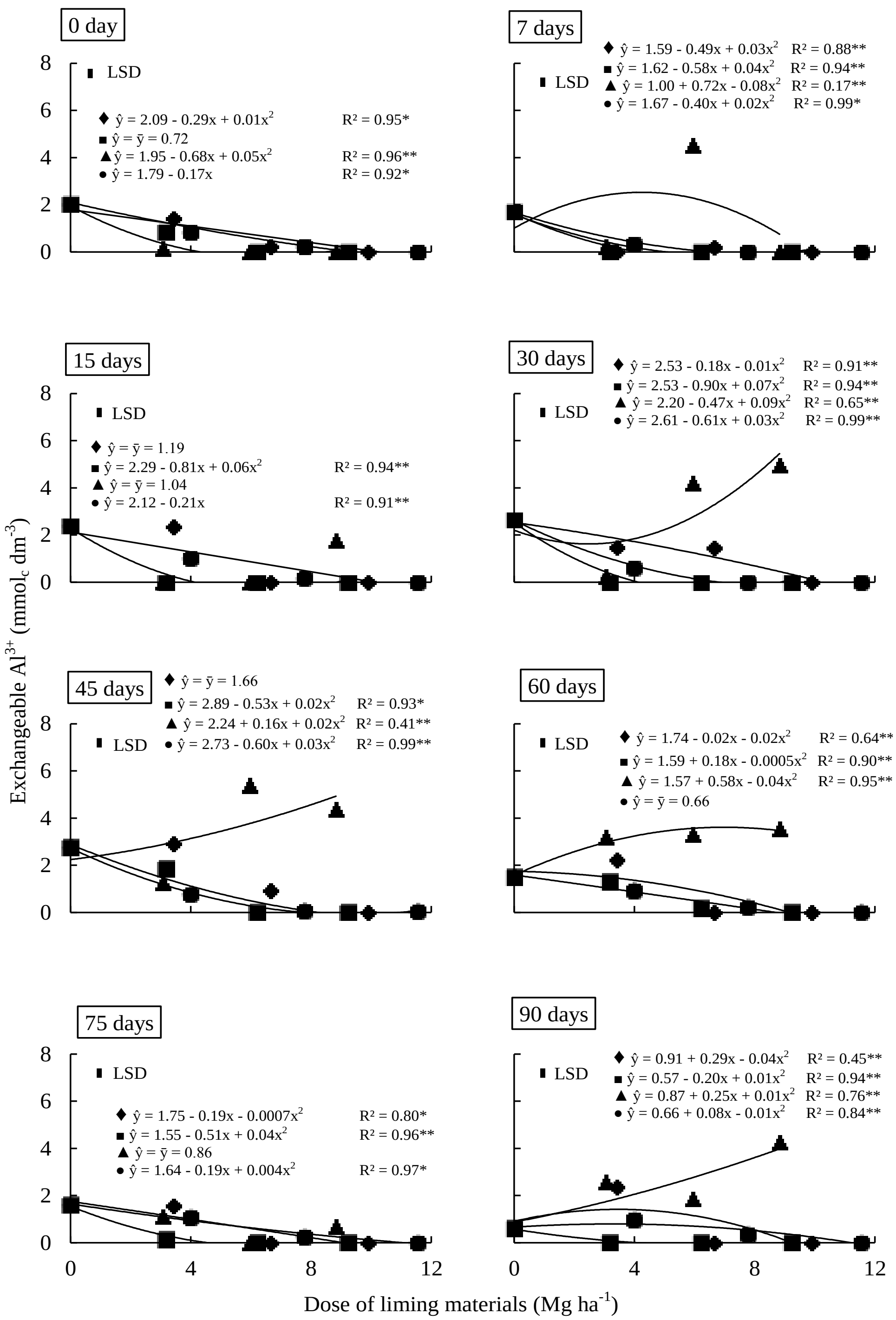

Figure 6. Exchangeable acidity (Al^{3+}) values on a Typic Distrudept (n = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone; (■) Granulated micronized calcite; (▲) Granulated micronized dolomite; (●) Carbonated suspension. Vertical bars indicate the least significant difference (LSD).*: P < 0.05. **: P < 0.01.

2 and 4), potential (H+Al) (Figures 2 and 5) and exchangeable (Al^{+3}) acidity (Figure 3 and 6) and increased base saturation (Figure 3 and 7), in all doses under study when the incubation period increased. This

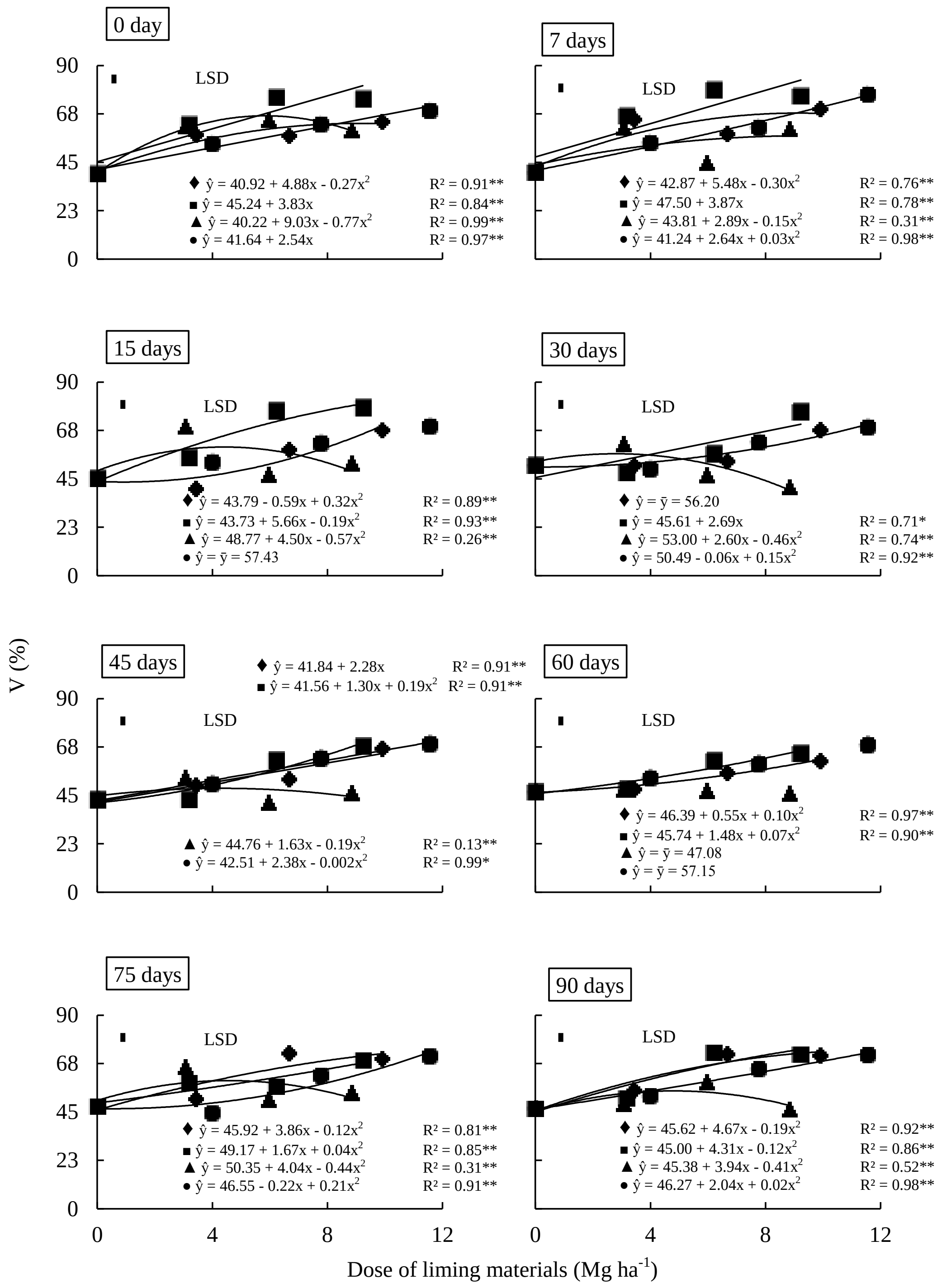

Figure 7. Bases saturation (V) values on a Typic Distrudept (n = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Vertical bars indicate the least significant difference (LSD). *: $P < 0.05$. **: $P < 0.01$.

fact may be ascribed to the interaction between periods of incubation, liming materials and doses. All liming materials reached the pH value ($CaCl_2$) 5.5, which has been considered ideal for most crops. Thus, materials

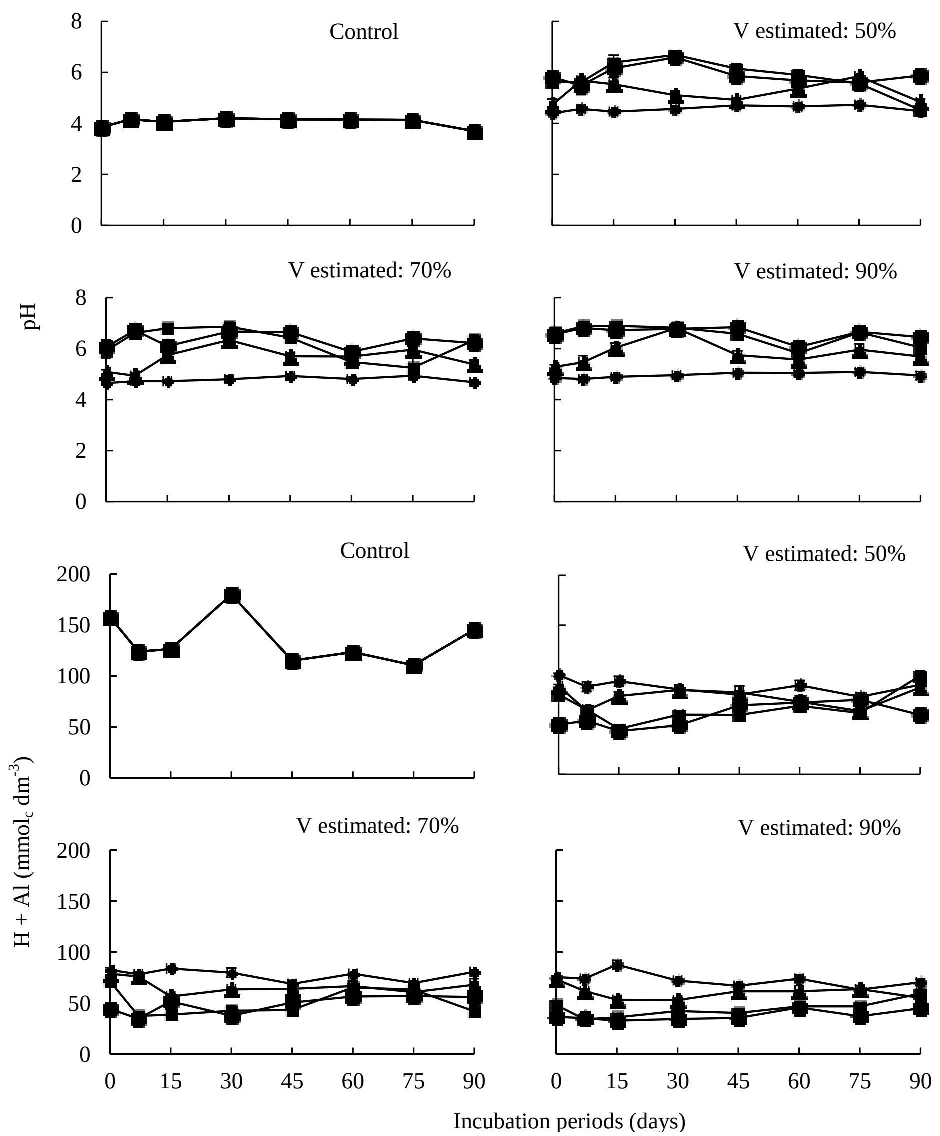

Figure 8. Active (pH) and potential (H + Al) acidity values on a Rhodic Hapludox (n = 4 ± standard deviation) during 90 days of incubation for the control treatment and after application of the liming materials aiming base saturation (V) to 50, 70 and 90%. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Coefficient of variation: 2.7 and 2.5% to pH and H+Al, respectively.

classified as special liming materials increase pH values (Oliveira et al., 2014) due to the release of OH^- and HCO_3^- and Ca content in the soil (Havlin et al., 2014) in accordance with the information in the current literature (Basak and Biswas, 2016).

The GMC reduced active (pH) and potential (H+Al) acidity. This show that the special liming material GMC reacted faster and neutralized the soil acidity. However, CS was more efficient to reduce potential acidity (H+Al) when applied in the dose aiming to increase V to 90%. Therefore, CS was efficient when applied in higher doses. Therefore, special liming materials (GMC and CS) presented higher efficacy, in short-term (90 days), when compared with the DL. This is a relevant fact, since studies on liming material incubation take into consideration the 90 days period (Alcarde, 2005) and, in this period, GMC and CS reacted much faster than DL.

Changes on acidity of Rhodic Hapludox

All liming materials, in their applied doses and in the periods used under this study reduced the active (pH) (Figure 8 and 10), potential (H+Al) (Figure 8 and 11) and

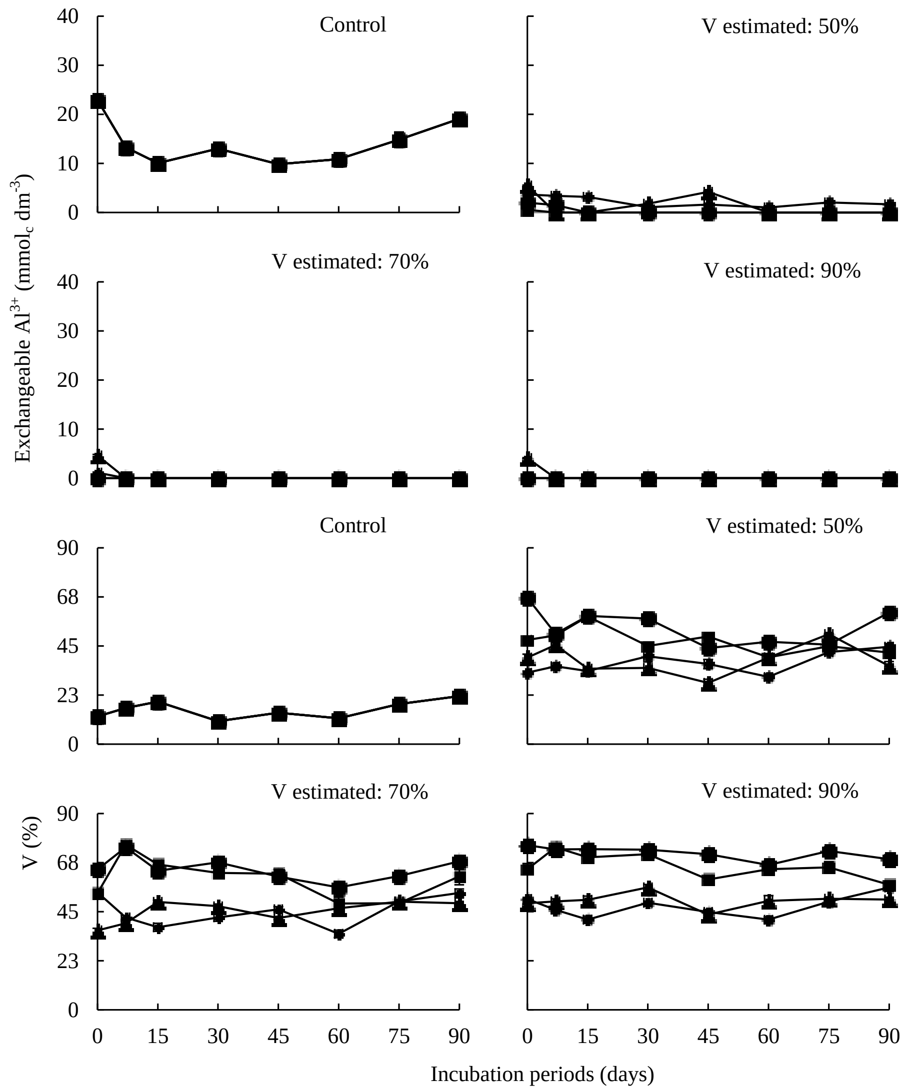

Figure 9. Exchangeable acidity (Al^{3+}) and base saturation (V) values on a Rhodic Hapludox (*n* = 4 ± standard deviation) during 90 days of incubation for the control treatment and after application of the liming materials aiming base saturation (V) to 50, 70 and 90%. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Coefficient of variation: 10.0 and 1.6% to Al^{3+} and V, respectively.

exchangeable Al^{3+} (Figures 9 and 12) acidity and increased V (Figure 9 and 13). This is because special liming materials are more reactive, presenting finer particles (Figure 1) and higher specific surface area, which provides better contact with the soil particles and consequently faster reaction (< 30 days), as compared to DL. Therefore, the use of GMC and CS in doses of 70 and 90% respectively, presented the highest efficacy to reduce active, potential and exchangeable acidity and increase V in the Rhodic Hapludox. The application of DL in Rhodic Hapludox, usually resulted in reduction in the active, potential and exchangeable acidity and increase in V (Caires et al., 2000, 2004; Fidalski and Tormena, 2005; Corrêa et al., 2007). However, DL presented lower efficacy in reducing active (pH), potential (H+Al) and exchangeable Al^{3+} acidity as compared to the special liming materials, GMC and CS throughout the periods observed in this study. GMD presented data discrepancy of all attributes under study in the Rhodic Hapludox. Therefore, the acidity control of soils with variable charge (Typic Distrudept and Rhodic Hapludox) and special liming materials can result in major soil quality and consequently, higher grain, meat and wood yield for the growing global population (Spiertz, 2012; Vendrame et

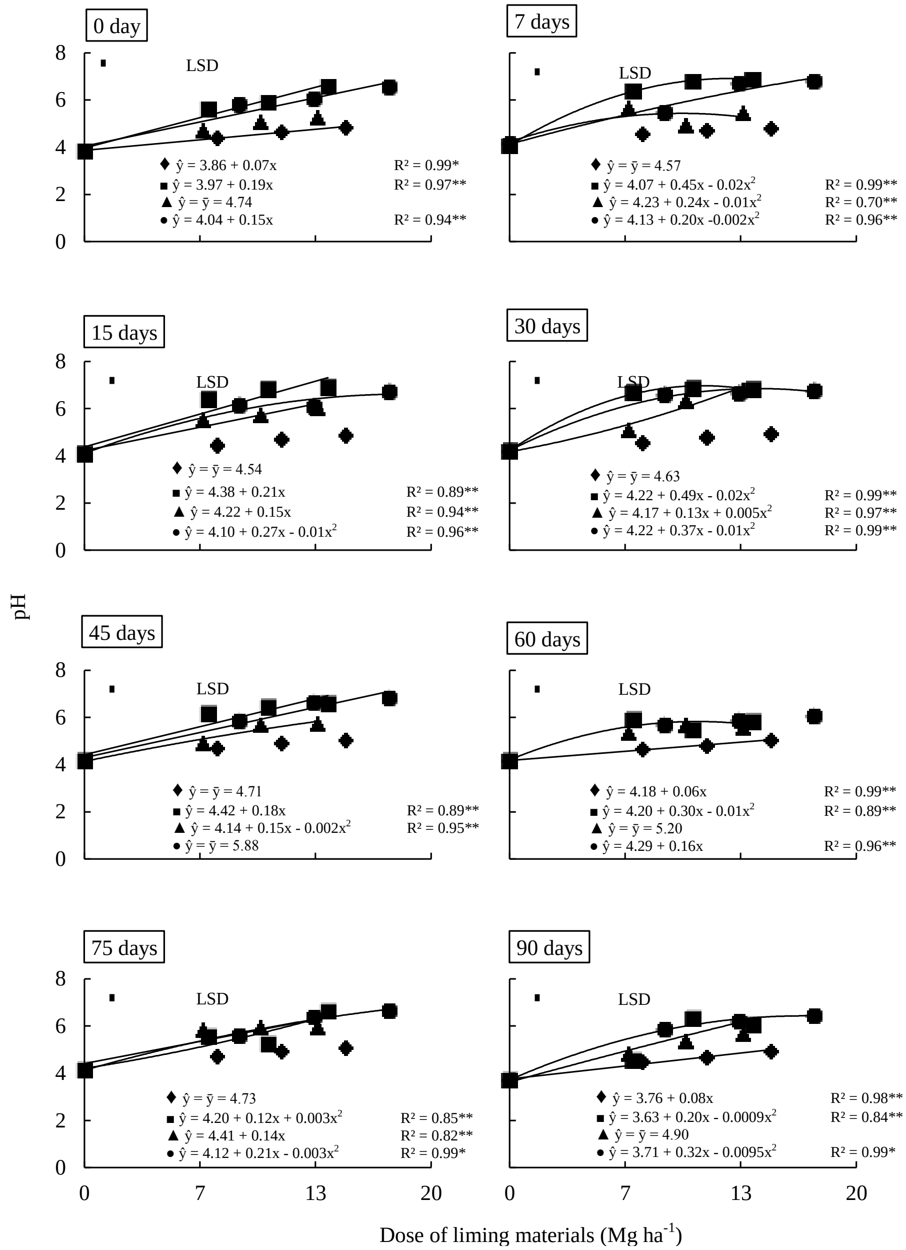

Figure 10. Active acidity (pH) values on a Rhodic Hapludox (n = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Vertical bars indicate the least significant difference (LSD).*: $P < 0.05$. **: $P < 0.01$.

al., 2013; Lasso et al., 2013; Curtis and Halford, 2014). However, field studies should be carried out to verify the residual effects of these special liming materials, as well as their effects on other soil fertility attributes; nutritional aspects and crop yield, particularly in conservationist management systems (with minimum soil revolving).

Conclusions

1) The special liming materials which were granulated micronized calcite and carbonated suspension were efficient to reduce active, potential and exchangeable acidity and increase base saturation in soils with variable

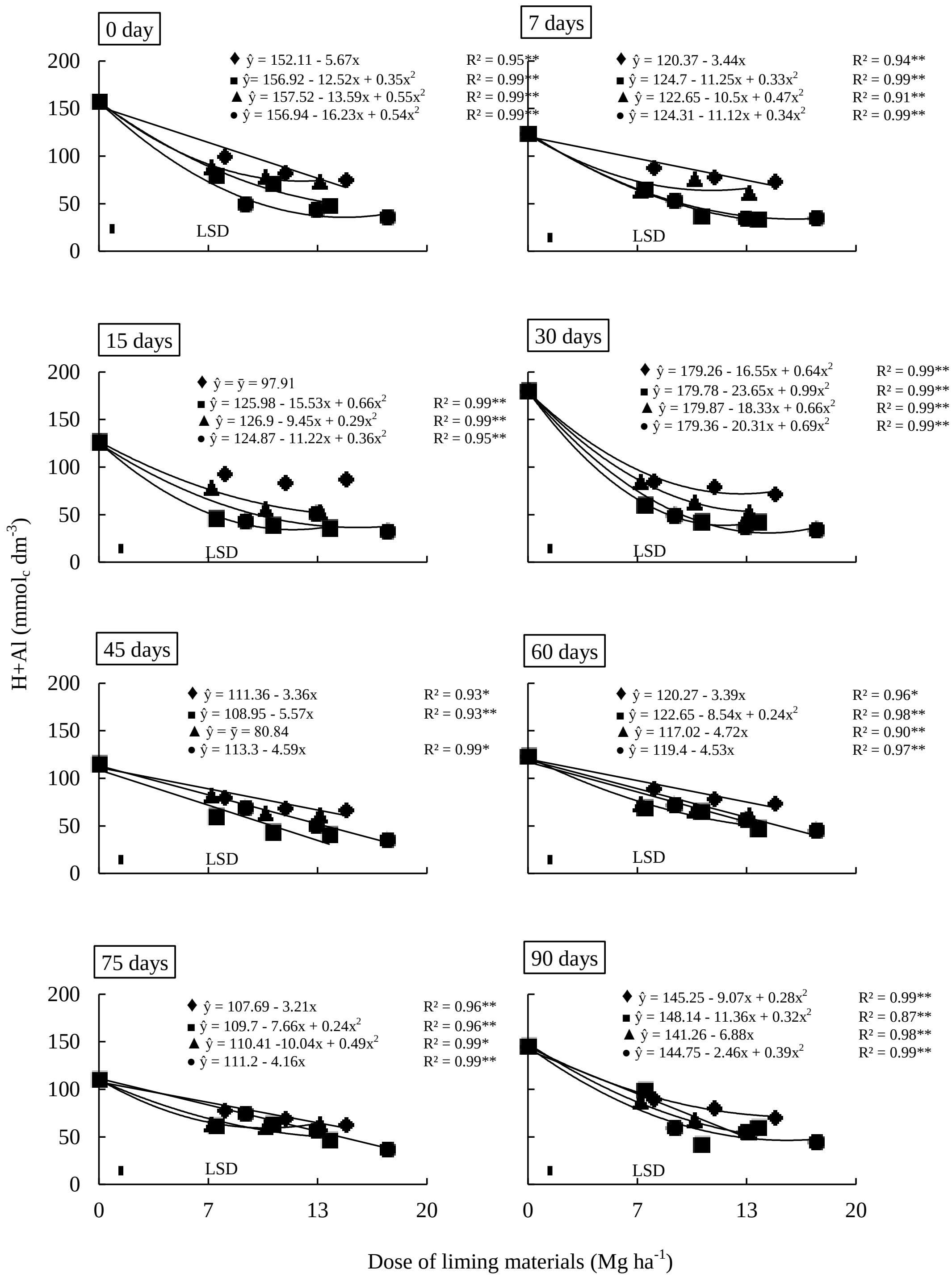

Figure 11. Potential acidity (H + Al) values on a Rhodic Hapludox (n = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Vertical bars indicate the least significant difference (LSD). *: $P < 0.05$. **: $P < 0.01$.

charge (Typic Distrudept and Rhodic Hapludox).
2) The application of granulated micronized calcite in a dose to increase base saturation to 70% in the Typic Distrudept and the carbonated suspension material in

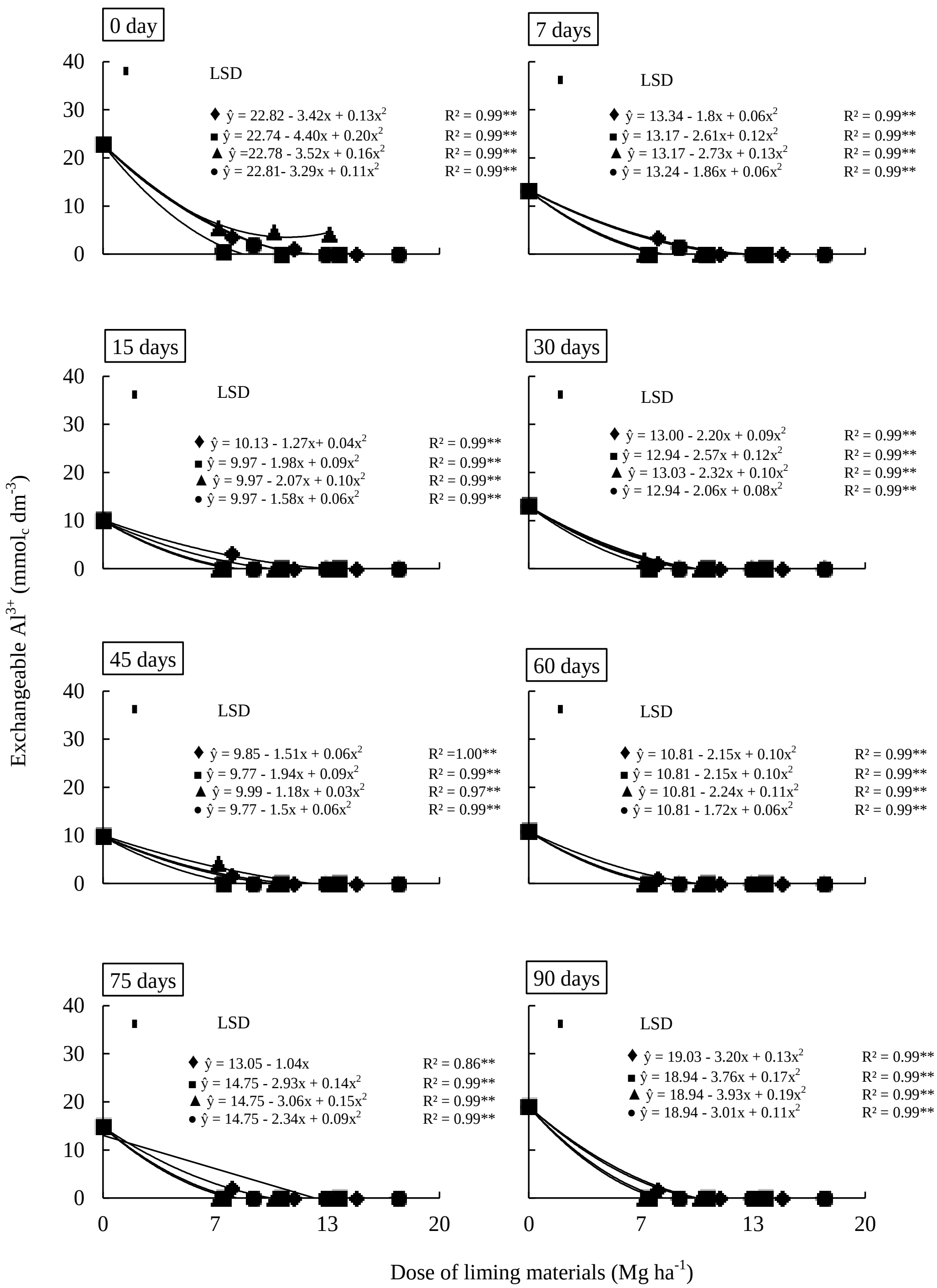

Figure 12. Exchangeable acidity (Al^{3+}) values on a Rhodic Hapludox (n = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Vertical bars indicate the least significant difference (LSD).*: $P < 0.05$. **: $P < 0.01$.

the dose to increase base saturation to 90% in the Rhodic Hapludox, were more efficient than the other doses.
3) The special liming materials which are granulated micronized calcite and carbonated suspension were more efficient than dolomitic limestone to control acidity (active, potential and exchangeable) in less than 30 days after

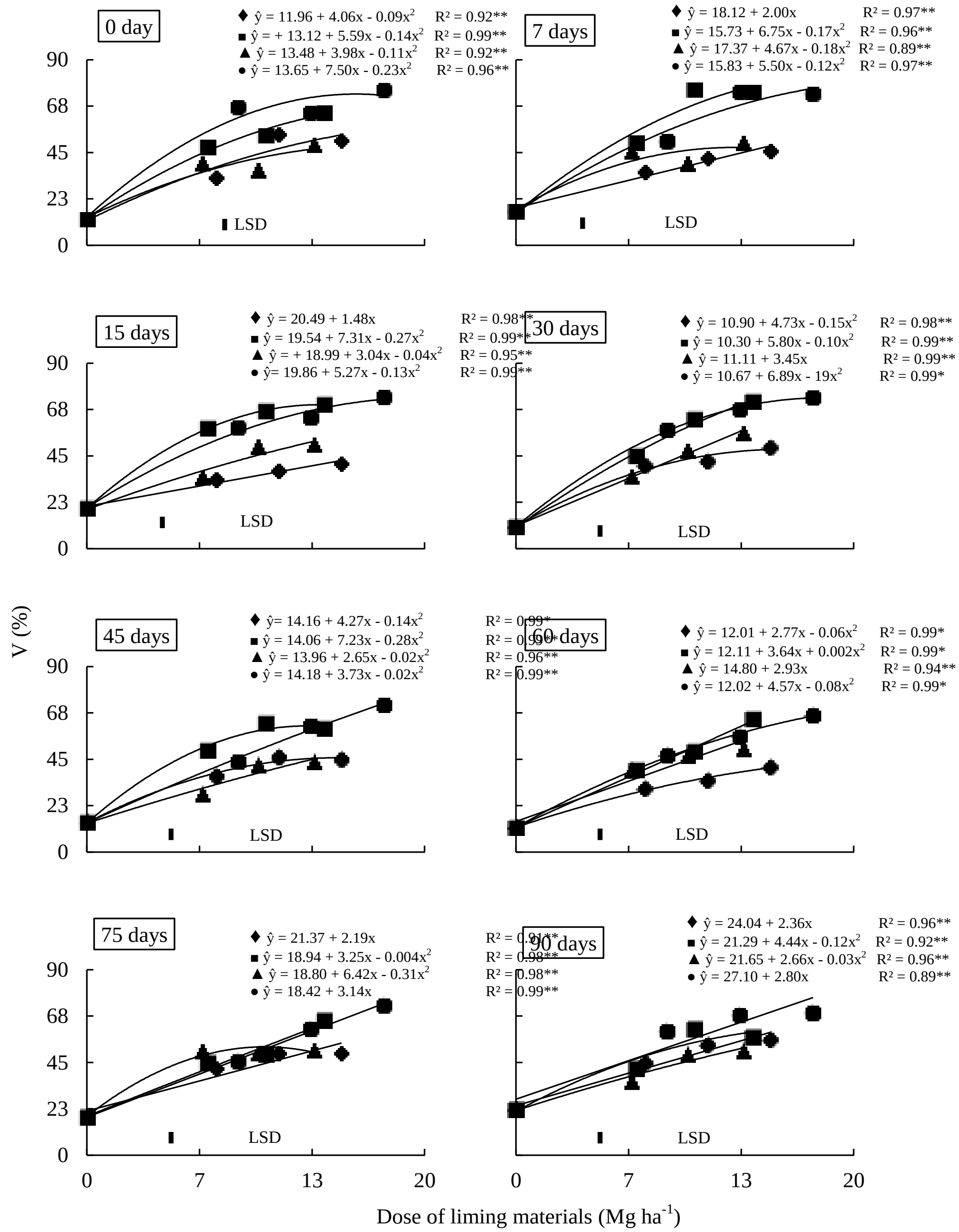

Figure 13. Base saturation (V) values on a Rhodic Hapludox (*n* = 4) during eight periods of incubation in the control treatment and in doses to increase the soil base saturation (V) to 50, 70 and 90% of each of the liming materials studied. (♦) Dolomitic limestone. (■) Granulated micronized calcite. (▲) Granulated micronized dolomite. (●) Carbonated suspension. Vertical bars indicate the Least Significant Difference (LSD). *: $P < 0.05$. **: $P < 0.01$.

they have been applied to the soil with variable charge.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author would like to show gratitude for the support of CNPq, CAPES, Omya do Brasil, and for the use of the research facilities of the Multi-User Laboratories Complex (C-LABMU/PROPESP/UEPG) and to all the students of

Agronomy Department (UEPG) who assisted in the soil sampling and chemical analysis.

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