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Full Length Research Paper

Agronomic efficiency of *Bradyrhizobium* in peanut under different environments in Brazilian Northeast

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Several legumes have natural ability to associate with nitrogen-fixing bacteria known as rhizobia. The efficiency of this association depends on the plant and bacterial genotype and the edaphoclimatic conditions. Peanut is a tropical legume able to associate with a wide range of rhizobia and the selection of efficient bacteria is important to increase the nitrogen fixation in this crop. In order to investigate the agronomic efficiency of two *Bradyrhizobium* strains, two peanut genotypes were used in field trails carried out in three environments located at Brazilian Northeast. The genotypes (BR1 and L7 Bege) were submitted to rhizobial inoculation (SEMIA 6144 or ESA 123, both *Bradyrhizobium* strains), and chemical nitrogen fertilization in randomized block design experiments. The following traits were analyzed: flowering (F), main axis height (MAH), number of nodules/plant (NN), number of pods/plant (NP) and weight of pods (WP). Differential responses were found in all to treatments to NN, NP and WP, in the three environments studied. Overall, ESA 123 showed good agronomic performance inducing higher pod production. The results support the evaluation of the *Bradyrhizobium* in further experiments aiming at its recommendation to commercial inoculants in Brazilian Northeast region.

Key words: Biological nitrogen fixation, inoculant, fertilization, symbiosis, rhizobia.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a plant of South America, and considered as one of the main oilseeds grown in Brazil and worldwide. It is currently the fourth largest crop of oilseeds, being grown in more than 100 countries, with about 45 million tons, where 67% of world production is concentrated in China, India, Nigeria, United States and Sudan (FAOSTAT, 2015; USDA, 2016). In Brazil, peanut crop is grown in different climates in Southeast, South and Northeast regions, by using robust cultivars in order to meet up with the food market (CONAB, 2015).

In Northeast region, peanut is cropped mainly by small

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farmers that adopt earliness and upright genotypes in agroecological systems. Peanut plants respond positively to nitrogen fertilization and to rhizobial inoculation (Melo, 2013). The N supply in plants is often provided by chemical fertilizers, some of them have low efficiency due to its natural transformations in soil (e.g. denitrification and volatilization), lead to losses that can achieve up to 70% of the N applied (Mortvedt et al., 1999; Signor and Cerri, 2013). Biological nitrogen fixation (BNF) is an alternative to nitrogen supply to several plants, especially legumes due to its association with rhizobia present in roots (Peix et al., 2015). In agriculture, this relationship could be exploited by production and use of inoculants containing efficient strains of rhizobia, and applied directly in seeds or soil (Nogueira and Hungria, 2013).

Peanut is a legume able to nodulate with a wide range of native rhizobia, which often is high competitive and low efficient to N fixation (Santos et al., 2007; Thies et al., 1991). This is why it is difficult to establish an effective association between peanut and rhizobia (Borges et al., 2007). Studies in this direction should be encouraged in order to identify high competitive and efficient strains to stimulate the use of inoculants in peanut, by growers. Currently, SEMIA 6144 is the unique Brazilian commercial *Bradyrhizobium* strain recommended to peanut, although several studies involving efficiency of strains collected from different environments have been done (Hoffman et al., 2007; Lyra et al., 2013; Torres-Júnior et al., 2014).

Rhizobia efficiency is dependent on several factors, such as soil fertility, weather and genotype x strain interaction (Marinho et al., 2014). The Brazilian Northeast has a large variation of soil and climate, and the predominant climate in the region is the dry and hot Semiarid, in which soils are often shallow and low fertile due to irregular water availability. These conditions are not favorable to proliferation of rhizobia inoculated in soil, although *Bradyrhizobium* has broad variability (Santos et al., 2005; Hoffman et al., 2007). Then, the identification and further recommendation of efficient rhizobia strains represent a wide benefit to peanut growers established in Northeast region.

Here, the agronomic efficiency of two peanut genotypes inoculated with two *Bradyrhizobium* strains in three different environments of Brazilian Northeast was evaluated.

MATERIALS AND METHODS

Genetic resources and rhizobia growth

Two bacterial strains were tested in the present study. The *Bradyrhizobium elkanii* SEMIA 6144 is used in commercial inoculants to peanut in Brazil. The isolate ESA 123 is a *Bradyrhizobium* sp. obtained from a trap-host experiment using soils from peanut production site in Barbalha, CE, in Brazilian Semiarid region. This bacterial isolate was previously characterized and selected by Cunha (2014).

Both bacterial isolates were grown in yeast extract-mannitol (YM) liquid medium (Vincent, 1970) in constant stirring of 120 rpm during 6 days, up to the end of exponential growth phase. An aliquot of 50 mL of the bacterial broth were added to plastic bags containing 200 g of sterilized peat, mixed by hand carefully and stored at 10°C for further inoculation in seeds at sowing (7 days after the inoculant preparation).

Two earliness and upright peanut genotypes (BR1 and L7 Bege) were used in assays. BR 1 is a cultivar developed by Peanut Breeding Program, coordinated by Brazilian Company of Agricultural Research (Embrapa) and recommended to semiarid region (Santos et al., 2010) and L7 Bege is a top line, derived from a drought tolerant cultivar (Senegal 55 437), with broad adaptability to Northeast region (Vasconcelos et al., 2015).

Field trails

The trials were carried out in field, during rainy season of 2014, in three different environments in Northeast region (Table 1). Composite sample (6 sub-samples) of each soil was collected at 0-0.2 m depth to evaluate chemical characteristics according to Silva (2009) (Table 2). Based on the results, soils were corrected with 1.5 Mg ha^{-1} of dolomitic limestone one month before the experiments set up. In addition, 60 kg ha^{-1} of simple superphosphate and 30 kg ha^{-1} of potassium chloride were used.

Each plot had five rows with 5 m, spaced in 0.7 m and data were collected from three central lines of each plot. Plants were spaced in 0.2 m. Three seed were sowed per hole, and further thinned to 2 plants per hole, at 15 days after emergence (DAE).

The treatments adopted were: a) inoculation of seeds with SEMIA 6144, b) inoculation of seeds with ESA 123, c) nitrogen fertilization (80 kg ha^{-1} of ammonium sulphate splitted in two applications, at the sowing and 30 DAE) and d) absolute control (without inoculation or nitrogen fertilization). The inoculation was carried out soon before the sowing using 250 g of inoculants to 10 kg of seeds. The inoculants, seeds and 150 mL of a sticking agent (supersaturated sucrose solution, at 70% w/v), were putted in a plastic bag and mixed by hand. The inoculated seeds were dried at the shadow for 30 min prior to sowing.

A completely randomized blocks design was adopted, in a factorial scheme of 2 (plant genotypes) × 4 (inoculation treatments) × 3 (environments). At 88 DAE, the plant height were measured and further harvest. For the harvest, the roots were separated from the shoots and carefully washed in tap water and the nodules were detached and counted. The pods were separated from the plants, dried under the sun for 4 days, and then counted and weighted.

Five agronomical traits were evaluated: flowering (F), main axis height (MAH), number of nodules/plant (NN), number of pods/plant (NP) and weight of pods (WP). Data were submitted to analysis of variance using the Sisvar 5.3 software (Ferreira, 2009). Tukey test ($p<0.05$) was adopted to mean comparisons, among genotypes (G), fertilization treatments (T), environments (E) and interaction effects.

RESULTS AND DISCUSSION

The treatments evaluated in this study induced to different responses in peanut genotypes to number of nodules/plant (NN), number of pods/plant (NP) and of weight of pods (WP), in all environments tested (Table 3). Effects of E x T and G x T interactions were also found meaning that the treatments promoted differences in agronomic traits of peanut genotypes grown in different sites. Flowering (F) and main axis height (MAH) were not influenced neither by treatments nor by environments

Table 1. Climatic characteristics of environments in Northeast region.

Site	Coordinates	C	RDC (mm)	T* (°C)	RH* (%)	Soil
Abreu e Lima, PE	07°54'43"S; 34°54'10"W, 19 m	T	1100	29	66	Neosol
Campina Grande, PB	07°13'50"S; 35°52'52"W, 551 m	S	409	22	84	Vertisol
Barbalha, CE	07°18'40"S; 39°18'15"W, 414 m	S	763	26	78	Vertisol

C- Climate: T - tropical, S – semiarid; RDC - total rainfall during peanut cycle; T - temperature; RH - relative humidity, *average during cycle.

Table 2. Chemical characteristics of the soil sampled at experimental fields before the experiments implementation.

Site	[1]pH	[2]OM	[3]Al^{+3}	[3]Ca^{+2}	[3]Mg^{+2}	[4]K^{+}	[4]P
		g kg^{-1}	mmol$_c$.dm^{-3}				mg.dm^{-3}
Abreu e Lima, PE	6.6	13.8	nd[5]	27.4	12.0	1.8	52.6
Campina Grande, PB	8.2	6.3	nd	46.1	8.4	3.2	72.3
Barbalha, CE	6.3	11.9	nd	67.9	30.9	2.2	12.1

[1]pH in water (1:2.5); [2]OM- organic matter (Walkley and Black method); [3]extracted with KCl (1 mol.L^{-1}); [4]Mehlich 1 method; [5]not detected.

Table 3. Synthesis of variance analysis for peanut traits obtained from different treatments, in the three environments.

Variation source	DF	Mean Square				
		F	MAH	NN	NP	WP
Environment (E)	2	1718^{ns}	14051^{ns}	143703*	3516*	4183*
Genotype (G)	1	1.50^{ns}	63.42^{ns}	158559*	495*	3579*
Treatment (T)	3	2.18^{ns}	52.03^{ns}	2658.9*	92.22*	491*
E x T	6	0.37^{ns}	29.80^{ns}	4613^{ns}	35.98^{ns}	124*
G x T	3	0.63^{ns}	27.39^{ns}	8341^{ns}	12.26^{ns}	102*
G x E	3					
E x G x T	6	1.02^{ns}	17.56^{ns}	50.84^{ns}	30.03^{ns}	68.19^{ns}
E x T1	2	406*	3676.8*	12550.1^{ns}	691.6*	1281.5*
E x T2	2	427*	3756.3*	52628.9*	567.6*	258.6*
E x T3	2	435*	3374.6*	48693.1*	1270.3*	1516*
E x T4	2	450*	3332.9*	43672.3*	1095*	1501.4*
G x T1	1	0.66^{ns}	122.4*	56025.1*	87.7^{ns}	1225.9*
G x T2	1	0.04^{ns}	20.61^{ns}	4166.9^{ns}	51.6^{ns}	1293.3*
G x T3	1	2.66*	1.69^{ns}	71652.7*	169.2*	1148.4*
G x T4	1	0.04^{ns}	0.95^{ns}	51738.8*	223.5*	218.8*
Block	3					
Error	69					
Total	95					
Mean		24.85	45.50	253.61	22.13	40.38
CV (%)		2.5	10.9	29.5	22.7	18.1

DF - Degrees of freedom, CV - coefficient of variation, *statistically significative by F test (p<0.05), ns - non significative, F - flowering (DAE), MAH - main axis height (cm), NN - number of nodules/plant, NP - number of pods/plant and WP - weight of pods (g), T1- No N-fertilization, T2- N-chemical, T3- ESA 123, T4- SEMIA 6144.

tested.

These traits show low variation in BR 1 and L7 Bege, both upright and earliness genotypes, with full pod maturation between 85-90 days (Santos et al., 2013).

The means of treatments and E x G, E x T and G x T interactions are shown in Table 4. It was found that high

Table 4. Mean of isolate and interaction factors of traits in peanut grown under different treatments, in the three environments.

Environments (E)	F (DAE)	MAH (cm)	NN nod/plant	NP pods/plant	WP g/plant
E1- Abreu e Lima, PE	23.81	46.32	284.92[a]	20.69[b]	36.19[b]
E2- Campina Grande, PB	25.47	44.96	299.24[a]	21.13[b]	38.07[b]
E3- Barbalha, CE	25.15	45.23	176.67[b]	25.10[a]	46.87[a]
Genotype (G)					
G1- BR 1	25.01	46.12	212.97[b]	21.03[b]	35.86[b]
G2- L7 Bege	24.70	44.49	294.25[a]	23.57[a]	44.85[a]
Treatment (T)					
T1- No N-fertilization	25.92	45.62	239.12[c]	18.87[b]	31.50[b]
T2- N-chemical	23.86	47.14	253.06[b]	21.55[ab]	40.52[ab]
T3- ESA 123	24.71	44.82	263.24[a]	23.86[a]	45.02[a]
T4- SEMIA 6144	24.89	43.63	259.01[ab]	23.75[a]	44.42[a]
Interactions					
Environments (E) × Genotypes (G)					
E1 x G1	24.62	43.13	218.05[bc]	19.10[bc]	34.97[c]
E2 x G1	25.35	45.31	273.82[b]	18.04[bc]	37.99[b]
E3 x G1	25.43	45.11	147.02[c]	26.93[a]	40.77[b]
E1 x G2	23.00	47.52	351.78[a]	22.28[b]	38.87[b]
E2 x G2	25.18	44.23	324.65[a]	19.21[bc]	41.96[b]
E3 x G2	25.47	45.24	206.31[bc]	27.20[a]	47.68[a]
Environments (E) × Treatments (T)					
E1 x T1	25.15	44.73	246.46[b]	17.56[c]	32.05[c]
E2 x T1	25.00	45.49	274.54[ab]	19.26[c]	33.34[c]
E3 x T1	26.00	45.06	196.35[c]	18.73[c]	36.12[bc]
E1 x T2	25.50	44.39	305.80[a]	21.44[b]	38.06[bc]
E2 x T2	25.12	44.82	293.72[ab]	22.03[b]	39.12[bc]
E3 x T2	24.00	45.23	159.66[c]	25.83[b]	42.35[b]
E1 x T3	23.25	46.15	309.46[a]	22.74[b]	44.20[b]
E2 x T3	26.00	45.49	307.10[a]	21.16[b]	44.39[b]
E3 x T3	26.05	46.84	173.17[c]	36.35[a]	50.33[a]
E1 x T4	24.55	46.04	277.95[ab]	21.03[b]	40.35[bc]
E2 x T4	24.25	45.58	321.58[a]	25.05[b]	43.14[b]
E3 x T4	25.37	46.15	177.50[c]	33.37[a]	46.18[b]
Genotypes (G) × Treatments (T)					
G1 x T1	25.08	45.88	190.80[d]	16.94[c]	33.85[c]
G1 x T2	24.62	46.07	239.88[c]	19.86[bc]	35.98[c]
G1 x T3	24.13	45.59	208.60[cd]	20.77[bc]	40.89[ab]
G1 x T4	25.25	44.44	212.58[c]	21.91[b]	38.84[b]
G2 x T1	25.05	44.37	287.43[b]	21.76[b]	37.75[b]
G2 x T2	24.53	46.21	266.24[bc]	22.88[b]	43.96[a]
G2 x T3	25.17	44.86	317.88[a]	26.72[a]	45.93[a]
G2 x T4	25.33	44.83	305.44[a]	25.96[a]	42.86[ab]

Means with the same letter in the column do not differ by the Tukey test (p<0.05). F- flowering (DAE), MAH - main axis height (cm), NN- number of nodules/plant, NP- number of pods/plant, WP- weight of pods (g).

nodulation of rhizobia isolates at Abreu e Lima and Campina Grande, both benefited by warm weather in these environments. In Barbalha, however, nodulation was expressively reduced possibly due to wide adaptation of native rhizobia population to semiarid environment (E3 x T1), contributing to inhibition of occupation of the nodulation sites by SEMIA 6144 and ESA 123. Based on reports available in literature, nodulation in several legume-rhizobia systems are negatively influenced by high temperature and low soil moisture (Kahindi et al., 1997; Kulkarni et al., 2000), whose characteristics are often found in Brazilian Semiarid. However, some native isolates may overcome the unfavorable conditions and improve its nodulation capacity contributing to plant establishment and production (Hungria and Vargas, 2000; Marinho et al., 2014). It could explain the behavior of ESA 123, an isolated selected from Barbalha (Cunha, 2014), that contributed to increase the pod production of plants. This behavior was not found in SEMIA 6144 that was isolated from peanut production belt, in Southern region of Brazil.

Despite low nodulation seen in Barbalha, the number and weight of pods were higher than in others environments, especially in ESA 123 treatment (E3 x T3), indicating that even with climatic limitations, this isolate was more responsive to improve the production of both peanut genotypes (G1 x T3 and G2 x T3).

Taking in account the behavior of genotypes as to nodulation, it was found that L7 Bege was more benefitial to rhizobia inoculation, especially with ESA 123, an isolate also adapted to semiarid region. This top line is a Valencia-high yield, obtained by crossing with a Brazilian high yield (IAC Tupã) and an African drought resistant (Senegal 55437) cultivar. As seen in Table 4, the nodulation, number of pods and weight of pods were higher in L7 Bege than BR 1, in all environments.

Although, the results obtained with BR 1 have been less expressive than those with L7 Bege, promising results were also achieved, strengthening the benefits of rhizobial inoculation in management of this cultivar. The inoculation with SEMIA 6144 achieved an increasing of 8.0 and 14.7%, in relation to nitrogen fertilization and control, respectively. With ESA 123, the rates were 13.6 and 20.8%, respectively. In others studies, positive responses of BR1 to inoculation with SEMIA 6144 were found in pot experiments (Melo, 2013; Torres-Júnior et al., 2014), but the performance of this association were not evaluated, up to now, for field conditions. These results are quite relevant because a positive interaction of BR 1 with a recommended strain is very important for the spread of adoption of inoculation practice by peanut farmers.

The performance seen here with ESA 123 in both peanut genotypes and in three environments was very satisfactory because it indicates the potential of this strain for further use in others assays, aiming at recommendation of commercial inoculants. In Brazil, the selection of new rhizobia to legume crops is carried out under the determinations of the Ministry of Agriculture, Livestock and Food Supply (MAPA, 2011). The stability of agronomic efficiency in different environments is one of the most important criteria in selection procedures. Studies based on selection of new *Bradyrhizobium* strains to commercial legumes have been carried out in Brazilian Northeast region, in last years (Marinho et al., 2014). Even so, there is still lack of knowledge regarding strain selection for peanut, and this work offers broad information on the behavior of upright genotypes evaluated under inoculation of a new *Bradyrhizobium* strain in different environments of Brazilian Northeast region.

Conclusions

The strain ESA 123 showed agronomic benefits of peanut in different environments of Brazilian Northeast region. The results indicate ESA 123 for standardized experiments in different locations aiming further at recommendation of commercial inoculants of peanut in Brazil.

Conflict of interest

The authors have not declared any conflict of interest.

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Cadmium effects on sunflower growth and mineral nutrition

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A hydroponic greenhouse study was carried out to evaluate the effects of increasing cadmium (Cd) concentration on plant growth, mineral nutrition and Cd distribution of H-250 sunflower genotype. Exposure to increasing Cd concentrations reduced plant biomass by 40, 34, 47 and 42% of the total, leaves, stem and roots dry weights as compared to the control. Regardless of the treatment most of Cd uptake by the genotype was allocated in the root, followed by leaf and stem. The higher bioconcentration factors values in both above ground and underground plant tissues and low transfer factor value indicated that this genotype may be an alternative for use in phytostabilization programs. The results also showed that increasing Cd concentration disrupted plant homeostasis as it increased the concentration of some nutrients and had adverse effect on others, impacting plant growth. In this context, the results suggest that the low magnesium, iron and manganese concentrations in the leaves were the main cause for plant biomass reduction and leaf chlorosis and necrosis, as each one of these elements plays a key role on the chlorophyll molecule and on photosynthesis process.

Key words: Bioremediation, bioaccumulation factor, heavy metal stress, *Helianthus annuus*, transfer factor.

INTRODUCTION

Cadmium (Cd) is a non-essential trace metal that presents potential threat for humans, plants, microorganisms and environment (Dong et al., 2007). It is considered to be one of the most toxic heavy metals in soil and water environments in the world (Järup and Åkesson, 2009). Exposure of humans to Cd is an important issue. Based on the combined considerations of frequency, toxicity and potential for human exposure, Cd is ranked as the fourth metal In the List of Hazardous Substances (ATSDR, 2014). Environmental Cd contamination is a serious concern due to its high solubility in water Cd and because it can easily enter in

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the food chain mainly by plant uptake and accumulated in plants and animals to concentrations that may pose carcinogenic, teratogenic, and mutagenic effects (Nazar et al., 2012). The Food and Agriculture Organization/World Health Organization (FAO/WHO, 1972) established a maximum weekly intake of 7 µg Cd per kg of body weight. Human activities, such as metallic industries, herbicides, concentrated phosphate fertilizers and industrial and municipal waste have contributed to the increase of Cd concentrations in the environment (Benavides et al., 2005).

To cope with metal toxicity or to maintain the level of essential metal within physiological ranges, plants have evolved complex cellular mechanisms of accumulation or exclusion to regulate uptake of nonessential metals. The main characteristic of the plants that accumulate metals is to translocate most of them to the aboveground tissue. These plants present bioconcentration factor - BCF (the ratio of metal concentration in shoot to soil), and transfer factor - TF (the ratio of metal concentration in shoot to root) greater than one. In contrast, excluders plants limit soil-root and root-shoot transfers, and therefore BCF and TF are lower than one (Malik et al., 2010; Yoon et al., 2006).

Exposure of non-accumulator plant species to Cd can inhibit plant growth and can cause plant stunting, chlorosis, apical meristem necrosis, brownish colour and plant death (Smeets et al., 2005). The metal toxicity may also alter many plant physiological processes including the nitrogen and carbohydrates metabolism, photosynthesis, mineral nutrients assimilation, and the plant water relationship (Gajdos et al., 2012). Cadmium induced plant tissue toxicity may be accompanied with reduced uptake of essential plant mineral nutrients. Increasing Cd concentration, in the external solution, may reduce the plant uptake of Ca^{2+}, Fe^{2+}, Zn^{2+}, and Mn^{2+} due to competition for the same membrane transporters. Some of these nutrients are also enzyme cofactors, involved in plant defense against oxidative stress, increasing the rate of biochemical reactions and physiological processes in plants (Nazar et al., 2012; Sarwar et al., 2010).

Sunflower (*Helianthus annuus* L.), a fast growing crop (Gajdos et al., 2012), is the fourth largest oil producer after soybean, canola and cotton (USDA-FAS, 2015) and presents a great economical potential for biodiesel production. The genotype diversity of sunflower confers to the specie a great plasticity in response to various growth environmental conditions (Capone et al., 2012) including the environmental stress. Sunflower has been cultivated as food and feed crop as well as for bioenergy production. The ability of the specie to remove organic and metal pollutants has also been evaluated in phytoremediation studies (Lopes Júnior et al., 2014; Zou et al., 2008). Selection of Cd-tolerant and Cd-accumulator sunflower genotypes can be achieved evaluating the Cd concentration in their organs. The pattern of metal uptake and distribution of the specie vary with the level of contamination (Simon et al., 1998) and cultivars (Ansari et al., 2009; Zou et al., 2008). It is hypothesized that sunflower can be cropped up to a maximum Cd available concentration. The present study was undertaken, aiming better understanding in the growth, metal accumulation and partitioning, and mineral nutrition of H-250 sunflower genotype exposure to increasing Cd concentration. This information is relevant to indicate the maximum Cd concentration for this genotype cultivation.

MATERIALS AND METHODS

Experiment setup

Seeds from H-250 sunflower genotype were sown in 200 mL plastic cups containing washed sand and irrigated daily with distilled water. Five-day-old seedlings selected for uniformity in size and form were acclimatized in pots containing half-strength Hoagland and Arnon (1950) nutrient solution at pH 6.0 ± 0.5. Seven days later, they were transferred to containers containing 10 L of this same nutrient solution containing five different cadmium doses (0; 2.5; 5.0; 7.5 or 10.0 µM), from here on referred to as Cd_0, $Cd_{2.5}$, $Cd_{5.0}$, $Cd_{7.5}$ and Cd_{10}, applied as $Cd(NO_3)_2.4H_2O$. Preliminary studies using various Cd concentrations (from 0 to 50 µM) have shown that concentrations of up to 10 µM are sublethal, but induce significant effects on short-term exposure. The nutrient solutions were aerated for 15 minutes every three hours by an air compressor, model GF180 (Resun Group CO, Shenzhen, Guangdong, CN). The volume of nutrient solutions was completed daily with distilled water. The mean values of temperature, air relative humidity and photosynthetic active radiation (at noon) in greenhouse were 27°C, 65% and 1200 µmol m^{-2} s^{-1}, respectively. The plants were harvested seven days after transplanting.

Growth measurements

The harvested plants were washed thoroughly with tap water, and then rinsed with deionized water. Sunflower plants were separated in leaf, stem and root and their fresh weight determined. Plant material was oven dried at 65°C for 72h and them the leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), and total dry weight (TDW) were determined. Leaf succulence (L_{SUC}) was calculated according to Mantovani (1999) as L_{SUC} = (LFW – LDW) / LA, where LFW is the leaf fresh weight (g), LDW the leaf dry weight (g $plant^{-1}$), and LA the leaf area (cm^2 $plant^{-1}$). Leaf area was measured using a WinDIAS image system, model W-C110-PC (Delta-T Devices Ltd, Cambridge, Cambs., UK). The dried plant material was ground to 20-mesh fineness using a Wiley mill, model STAR FT-50 (Fortinox, Piracicaba, SP, BR)

Cadmium and nutrient analysis

The plant parts were digested with 3.5 mL H_2SO_4 (96-98% w/w) and 2.0 mL 30% H_2O_2, as described by Jones (2001) for the analysis of the elements. The digested material was adjusted to 100 mL volume with deionized water. The total concentration of Cd, K, Ca, Mg, Mn, Fe, Zn and Cu in the solution were analyzed by Optical Inductively Coupled Plasma Spectrometry, model Optima 3300XL (Perkin Elmer, Norwalk, MA, USA). The determinations of N and P were performed by spectrophotometry by the methods of phenol-

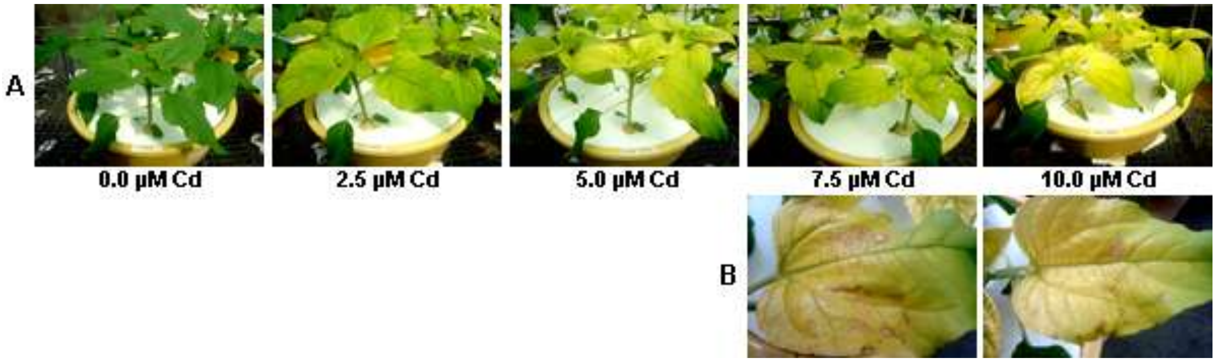

Figure 1. Sunflower leaves chlorosis (A) and necrosis (B) growing under increasing cadmium exposure concentration, in nutrient solution.

hypochlorite and molibdo-vanadate, respectively, as described by Faithfull (2002). The accuracy was confirmed using the standard reference material NIST-SRM-1572 (Gaithersburg, MD, USA). Cadmium bioconcentration factors of aboveground (BCF) and underground (BCF_R) plant tissues, and transfer factor (TF) were calculated using the following equations (Žaltauskaitė and Šliumpaitė, 2013):

$$BCF = Cd_{shoot} / Cd_{solution}$$
$$BCF_R = Cd_{root} / Cd_{solution}$$
$$TF = Cd_{shoot} / Cd_{root}$$

Where Cd_{shoot} and Cd_{root} are the cadmium concentrations in aboveground and underground tissues, respectively, and $Cd_{solution}$ is the cadmium concentration in nutrient solution.

Statistical analysis

The treatments were arranged as a completely randomized design with five doses of cadmium and four replications. All data are expressed as the mean of all replicates. Treatment effects were determined by analysis of variance according to the general linear model procedure of the Statistical Analysis System (SAS, 2003) at $p < 0.05$. Regression equations were then adjusted to the significant data.

RESULTS AND DISCUSSION

Exposure to nonessential elements, such as Cd, impacts plant homeostasis with reflex on plant growth, element partitioning, nutrient uptake, water relations (Hossain et al., 2010), enzyme activity (Ouariti et al., 1997; Van Assche and Clijsters, 1990), photosynthesis and respiration metabolisms (Mobin and Khan, 2007; Shi et al., 2010; Vassilev and Yordanov 1997), nutritional deficiencies and imbalances (Clemens, 2006; di Toppi and Gabbrielli, 1999).

Effect of Cd on sunflower development

After one week, youngest leaves of sunflower growing in all Cd spiked solutions showed symptoms of toxicity, as indicated by chlorosis (Figure 1). Cadmium concentration in the solution had a major impact on plant growth indicators. Exposure to increasing Cd concentrations linearly reduced the sunflower LDW (Figure 2A). For instance, exposure of 10.0 µM of Cd resulted in the reduction of LDW by ~0.73 g, which is equivalent to 0.057 g DW per µM Cd in solution. This effect was also observed on the SDW (Cd_0 1.06 to Cd_{10} 0.56 g DW, a reduction of 0.045g DW $µM^{-1}$ Cd-solution, Figure 2B); on RDW (Cd_0 1.22 to Cd_{10} 0.70 g DW or a reduction of 0.044 g DW $µM^{-1}$ Cd, Figure 2C) and, consequently, on TDW (Cd_0 4.42 to Cd_{10} 2.93 g DW or a reduction of 0.145 g DW $µM^{-1}$ Cd, Figure 2D). The LDW accounted for about 50% of the TDW. At the highest Cd-concentration (10 µM), the plant biomass reduction was higher in the stem (47%) and roots (42%) and lower in the leaves (34%) as compared to the control. Increasing Cd concentration did not alter the shoot to root ratio of the treatments.

Cadmium increasing concentration also reduced sunflower LA (13.13 cm^2 $µM^{-1}$ Cd, Figure 2E). Cadmium stress did not alter the water status of the leaves as indicated by the leaf succulence (L_{SUC}) that show no variation with increasing doses of cadmium in nutrient solution (Figure 2F). The leaf water concentration averaged 31.05 g H_2O cm^{-2} LA.

Cadmium promotes disturbances in vital physiological plant processes. Our data suggest that the photosynthetic activity restriction caused by chlorosis, necrosis and LA reduction may explain, at least in part, the Cd-induced plant growth reduction.

Cadmium concentration and partition in the plant

Cadmium concentration in the plant parts changed with Cd-solution (Figure 3). In leaf, ranged from 0.01 µmol g^{-1} DW at Cd_0 to 0.71 µmol g^{-1} DW at Cd_{10}; in stem varied from 0.03 µmol g^{-1} DW at Cd_0 to 0.71 µmol g^{-1} DW at Cd_{10}; and in root ranged from 0.03 µmol g^{-1} DW at Cd_0 to

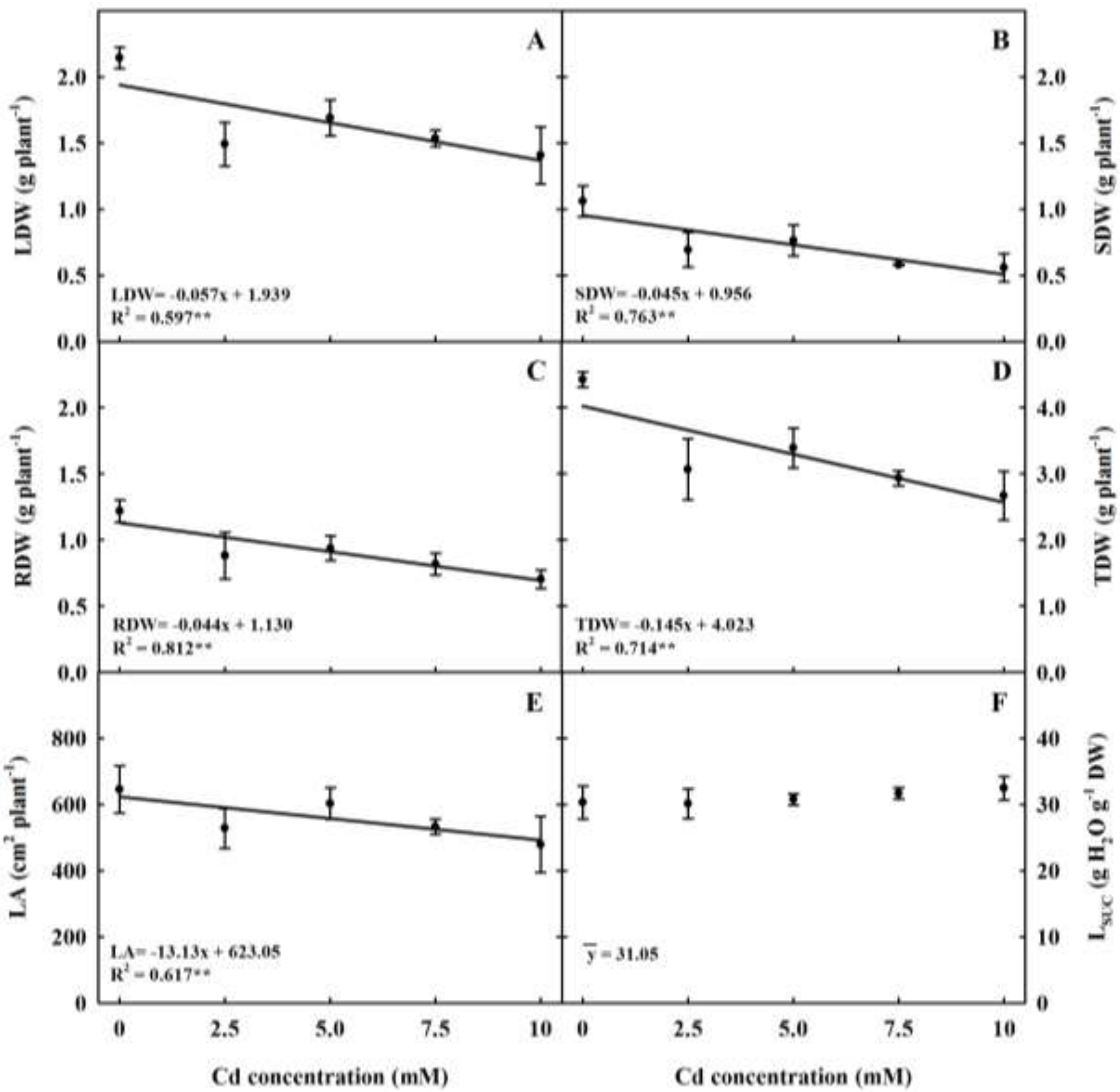

Figure 2. Leaf (LDW), stem (SDW), root (RDW) and total dry weights (TDW) leaf area (LA) and leaf succulence (L_{suc}) of sunflower plants growing in nutrient solution containing different cadmium concentrations. Values indicate the mean of four replicates ± S.D.

2.85 μmol g^{-1} DW at Cd_{10} (Figure 3A). To all plant parts it was observed a linear increase of Cd concentration with Cd solution (Figure 3A). Sunflower concentrated more Cd in the roots than in other plant parts in the Cd treatments. The highest difference in Cd root as compared to the other part of the plant occurred at $Cd_{2.5}$. For instance, at $Cd_{2.5}$ 71.0, 22.5 and 6.5% of the Cd were allocated in the roots, leaves and stem, respectively, as compared with 58.7, 29.7 and 11.6%, respectively in Cd_{10} (Figure 3B). The result of Cd partitioning in sunflower organs in this study was similar to others reported elsewhere (Lopes Júnior et al., 2014; Zou et al., 2008).

The higher concentration of Cd in the root was due to the immobilization of the metal in the roots caused by its limited translocation from the roots to the shoot. The higher Cd retention in sunflower root as compared to the other plant parts is attributed to a complex system, which involve the element adsorption, chelation, and compartmentalization, which in turns limits Cd translocation from roots to the shoot (Nocito et al., 2011). Cadmium passes from the external solution to the interior of the plant through the apoplastic transport where it accumulates (Lux et al., 2011). The apoplast is a dense region of negative charge due to the presence of carboxylic groups, which are critical to the retention of cations such as Cd^{2+} (Redjala et al., 2009). The roots act

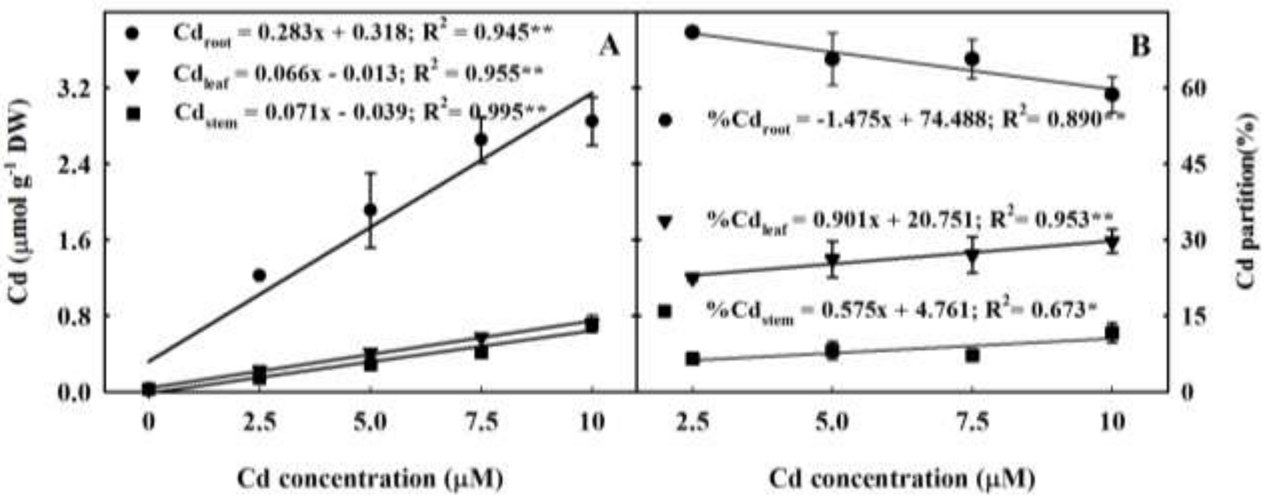

Figure 3. Cadmium concentration (A) and partition (B) in the leaf, stem and root of sunflower plants grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates ± S.D.

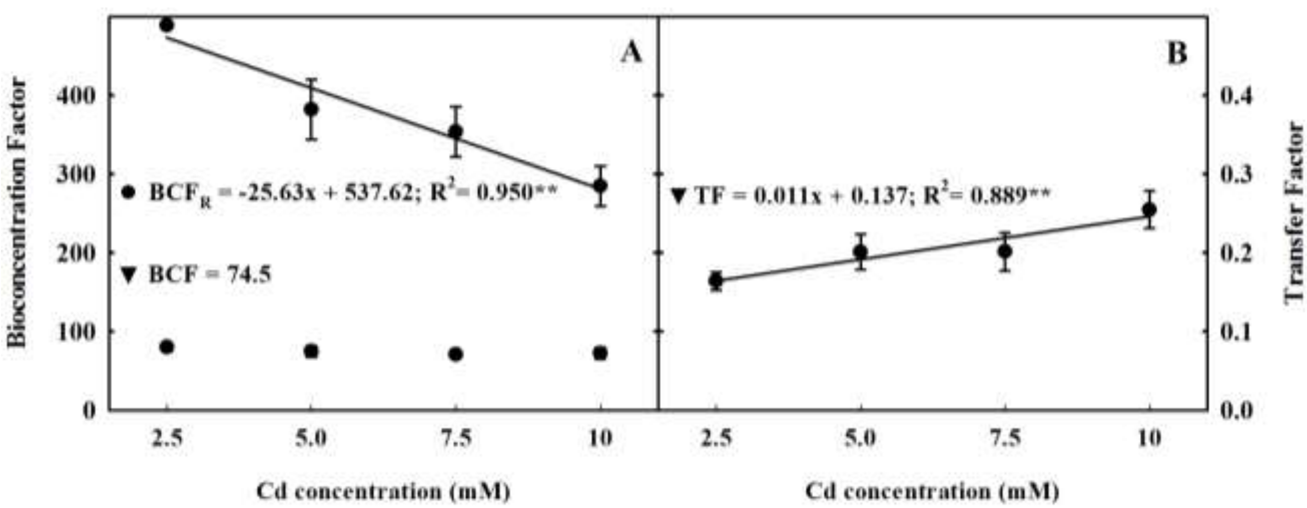

Figure 4. Bioconcentration Factor (BCF), Bioconcentration Factor of the root (BCFR) (A) and Transfer Factor (TF) (B) of sunflower plants grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates ± S.D.

as a barrier for immobilization of toxic ions, preventing their translocation to the shoot (Azad et al., 2011). The stem retention is also considered as a tolerance mechanism, by preventing that toxic elements disrupt cell metabolism in the leaves (Higuchi et al., 2013). The results of Cd partition in the plants parts show that the stem retention was not an effective mechanism for regulating the Cd transport from roots to the leaves.The ability of the H-250 sunflower genotype to concentrate Cd in the shoot tissues relative to Cd-solution (BCF) was constant around 74.5, over the range of the Cd concentration tested (Figure 4A). The most of Cd in the plant was located in the root, therefore BCF_R values ranged from 538 at $Cd_{2.5}$ to 281 at Cd_{10} (Figure 4A). The TF of the genotype were lower than one and ranged from 0.137 at $Cd_{2.5}$ to 0.247 in the plants growing at Cd_{10} (Figure 4B).

The ratio of metal concentration in the roots to growing media, bioaccumulation factor (BCF) has been used to estimate the ability of the plant concentrate the metal from the solution. On the other hand, the ratio of metal concentration in the shoots to the roots (TF) has been used to estimate metal translocation from the roots to the shoots. BCF and TF greater than one indicate that the metal is concentrated in the shoot relative to the growing media or that the metal is accumulating preferentially in shoot relative to the root. The BCF and BCF_R values found in this study were high, in contrast to the TF values which were low, suggesting that the sunflower plants can be used for the phytostabilization purpose. The limitation of Cd transfer from the root to the shoot may be seen as a defense mechanism used by plants to minimize the deleterious effects of excessive nonessential metals in the shoot and according to Hossain et al. (2010) it is considered a tolerance mechanism. While non-tolerant species and tolerant excluders tend to accumulate nonessential metals, as Cd in roots, accumulator plants evolved a more efficient mechanism to translocate metals from root to shoot.

Sunflower nutrient status

Cadmium toxicity alters plant macro and micronutrients uptake (Ciećko et al., 2004; Sarwar et al., 2010). The macronutrients N, P, K, Ca and Mg concentrations in sunflower leaf and root are shown in Figure 5. Increasing Cd concentration had a positive quadratic effect on leaf N (Figure 5A), P (Figure 5B) and K (Figure 5C). The estimated maximum sunflower leaf N (4.381 mmol g^{-1} DM), P (0.325 mmol g^{-1} DM) and K (1.577 mmol g^{-1} DM) concentrations occurred at $Cd_{5.9}$, $Cd_{9.3}$ and $Cd_{7.8}$, respectively. Thus, the estimated N, P and K concentrations were 10, 53 and 57% higher than the control, respectively. The concentration of N in the stem was not evaluated. Cadmium in solution also had positive linear effect on P stem and K root. The concentration of Cd-solution has no effect in the N and P concentrations in the root, and in K concentration in the stem.

The increase of N in the leaves is a clear indicative that the symptom of chlorosis presented by the plant was not determined by the deficiency of this nutrient. There are reports in the literature that N uptake decreases with the Cd concentration in nutrient solution (Guimarães et al., 2008; López-Millán et al., 2009). However the synergisms and antagonisms existent between the uptake of N and Cd by the plants seem to be related to nitrogen source (Ciećko et al., 2004). Studies have shown the existence of antagonistic interactions between $N\text{-}NH_4^+$ and Cd^{2+} uptake and synergistic interactions between $N\text{-}NO_3^-$ and Cd (Sarwar et al., 2010). As in this study the $N\text{-}NO^-_3$:$N\text{-}NH_4^+$ ratio in nutrient solution is 14:1, and $N\text{-}NO_3^-$ is responsible for at least 50% of total anions absorbed by plants (Marschner, 2012), the higher $N\text{-}NO_3^-$ plant uptake may have been used for balancing the electrical charge of the plant, thereby interfering with the regulation and absorption of other nutrients.

Although the Cd concentration did not impact the P concentration in the root, the P concentration in the sunflower leaves increased up to 53% as compared to the control as cadmium concentration in the nutrient solution increased. Similar result was found in a study with cedar plants (Paiva et al., 2001). Plants may maintain a higher P concentration in the leaves as a way

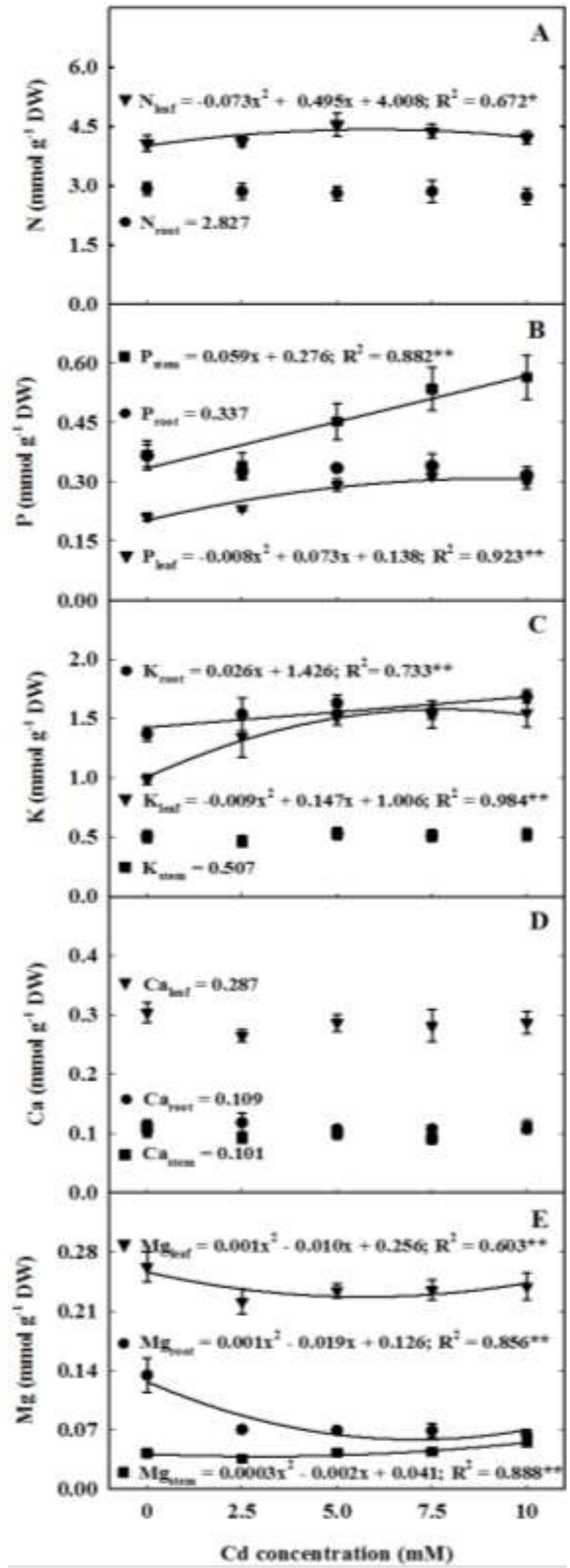

Figure 5. Concentration of nitrogen (A), phosphorus (B), potassium (C), calcium (D) and magnesium (E) in the root, stem and leaf of sunflower grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates ± S.D.

of reduce the free Cd and promoting the Cd-phosphate precipitate formation (Sarwar et al., 2010), which is sequestered in the vacuole and immobilized in the cell wall (Belleghem et al., 2006).

Potassium plays a central role in the water relations (Meurer, 2006), therefore the increase in leaf and root K concentrations is consistent with the need of the plant to keep the turgor, mainly in higher Cd concentrations. The increasing K concentration in sunflower roots may have helped the maintenance of water absorption by the roots and its flux to the shoot. Additionally, the increasing K concentration in the leaves may have favored the stomata opening at higher Cd concentrations, thereby maintaining the plant gas exchange during stress. The increase in K concentration in sunflower leaves obtained in this study differed from those obtained for sunflower, (Simon, 1998), cedar (Paiva et al., 2001) and maize roots, (Ciećko et al., 2004). However, the Cd concentration in the solution, the time of the exposure to Cd, and the plant age of these studies were higher than that tested in our study.

The concentration of Cd-solution has no effect in the Ca concentration in the root, stem and leaf (Figure 5D). In contrast, increasing Cd concentration has negative quadratic effect in plant Mg (Figure 5E). The estimated minimum concentration in leaf (0.231 mmol g^{-1} DM), stem (0.038 mmol g^{-1} DM) and root (0.036 mmol g^{-1} DM) occurred, respectively, at $Cd_{5.0}$, $Cd_{3.3}$ and $Cd_{9.5}$.Iron, Mn, Zn, and Cu concentrations in sunflower leaves and roots are shown in Figure 6. Cadmium concentrations had positive effect on sunflower root Fe and Cu and leaf Zn but negative effect on leaf and stem Fe, leaf and root Mn, and stem and root Zn (Figure 6). The estimated minimum leaf Fe concentration (0.86 µM g^{-1} DW) was at $Cd_{7.65}$ µM Cd, representing a reduction of 87% when compared with Fe concentration of the control (6.75 µM g^{-1} DW) (Figure 6A). Leaf Mn concentration decreased linearly (0.375 µM g^{-1} DW) with Cd solution (Figure 6B). Leaf Fe and Mn concentrations were present in concentration reverse to that of Cd. Sunflower leaf Zn concentration increased with Cd-solution (Figure 6C). The estimated maximum leaf Zn concentration 1.12 µM g^{-1} DW occurred at $Cd_{7.4}$. Leaf Cu concentration had no change (0.929 µM g^{-1} DW) with Cd-solution (Figure 6D). The concentrations of Mn and Cu in the stem were below the level of detection.

Sunflower root concentration of Fe and Cu increased in 0.325 and 0.075 µmol g^{-1} DW, respectively for each unit of Cd-solution (Figures 6A and 6D). At Cd_{10} the root Cu concentration, 1.57 µmol g^{-1} DW, was twice the value of the control plants 0.819 µmol g^{-1} DW. In contrast, the root concentration of Mn and Zn decreased quadratically with Cd-solution (Figures 6B and 6C). The minimum Mn 18.10 µM g^{-1} DW and Zn 0.90 µM g^{-1} DW concentrations were observed at $Cd_{6.7}$ and $Cd_{7.3}$, respectively.

The cadmium toxicity symptoms (chlorosis and necrosis) as presented especially in younger leaves of H-250 sunflower genotype exposed to increasing Cd

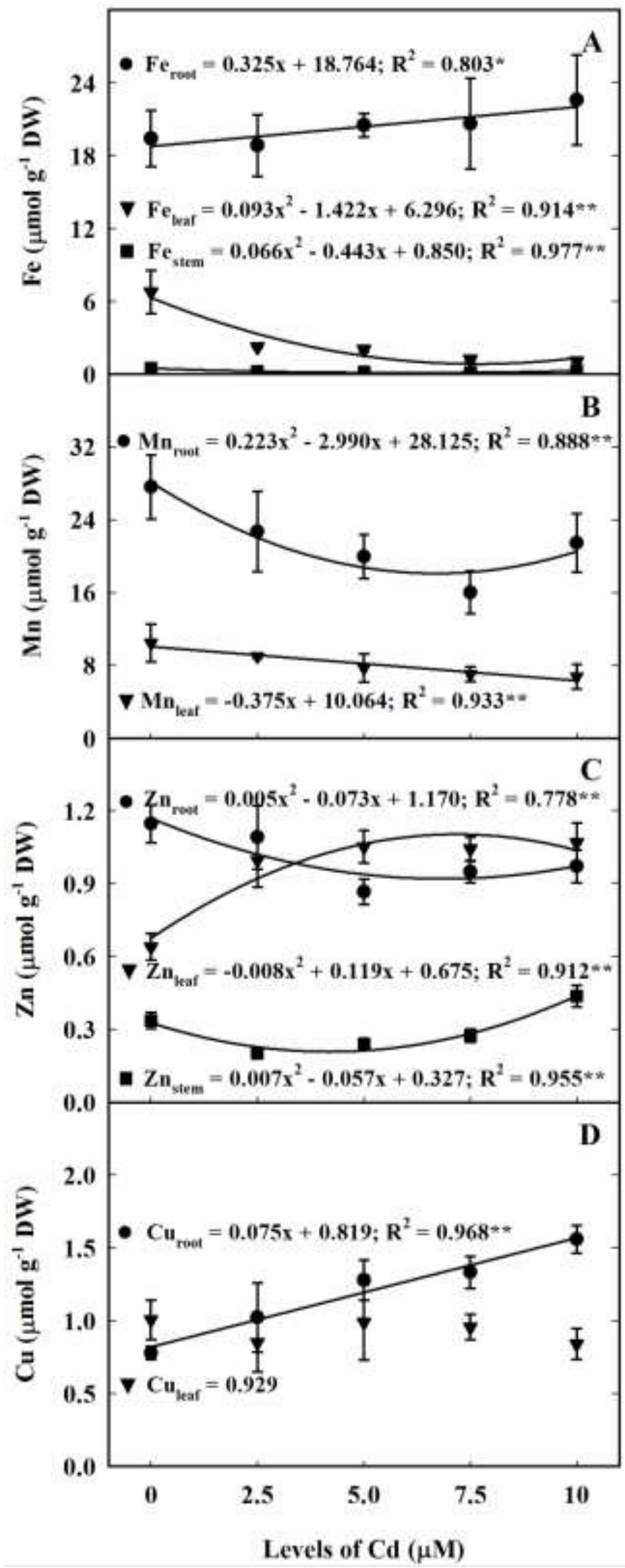

Figure 6. Concentration of Iron (A), manganese (B), zinc (C) and copper (D) in root, stem and leaf of sunflower grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates ± S.D.

concentrations have been reported elsewhere (Chaves et al., 2011; Zou et al., 2008). The increase of Cd in the nutrient solution may have an antagonist effect on the concentration of several elements since the Cd seems to enter in the plants, mainly via Ca^{2+} or Mg^{2+} channels or via other divalent transporters such as Fe^{2+}, Mn^{2+}, and Zn^{2+} (Clemens, 2006; Sarwar et al., 2010). In the present study, the chlorosis and necrosis symptoms were attributed to a combined adverse effect of Cd on leaf Mg, Fe and Mn concentrations.

The decrease of Fe concentration in the leaf in conjunction with the increase in the root also suggests the occurrence of Cd-induced disturbances in the Fe translocation from shoot to the root. Iron is frequently referred as the most affected nutrient by Cd exposure. According to Pál et al. (2006), leaf chlorosis and plant growth inhibition are the most noticeable symptoms caused by Cd stress. Considering that Fe plays a key role in the chlorophyll biosynthesis (Benavides et al., 2005), our results suggest that the decrease in the Fe concentration was a determining factor for plant chlorosis and biomass reduction.Our results also show a decrease of Mn concentrations in both leaves and roots. Manganese is a key element in restoring the chlorophyll molecule structure (Sarwar et al., 2010). In addition, Mn plays key role in the activity of enzymes that mediate the catalysis of the water-splitting reaction to produce oxygen and to provide electrons for the photosynthetic electron transport chain (Goussias et al., 2002; Nickelsen and Rengstl, 2013), and its deficiency directly affects the photosynthetic rate. Therefore, it is likely that the reduction of Mn concentrations observed in this study may be an additional factor inducing chlorosis symptoms and reducing plant growth.

Cadmium and Zn tend to compete for accumulation in plant shoots (Nocito et al., 2011). Zinc is an important nutrient in maintaining the biomembranes. Zinc also plays a crucial role as a functional and/or structural component of a number of enzymes, besides being essential for activity, regulation, and stabilization of protein structure (Mason, 2013). In this study, Cd induced a slight reduction in Zn concentration in the roots, but increased Zn concentration in the leaves, suggesting that the Cd-induced growth reduction did not seems to be related to changes in concentrations of this nutrient in the plant.

Cadmium stress induced an increase of 100% in root Cu concentration but did not affect the concentration of this element in the leaves. The increased root Cu concentration in Cd-stressed plants has also been reported in other studies (López-Millán et al., 2009; Obata and Umebayashi, 1997; Sandalio et al., 2001). The increase in root concentrations of Cu and Fe transition metals observed in this study may lead to increased hydroxyl free radical production through the Fenton reaction (Silva et al., 2013). Therefore, it could be hypothesized that Cd-induced oxidative stress was more pronounced in the roots than in the leaves. The observation that RDW was more affected than LDW by Cd-stress is an additional support to this hypothesis.

Conclusions

Our results show that H-250 is a Cd-excluder sunflower genotype, since it preferentially concentrates the metal in the roots. Thus, this genotype may be an alternative for use in phytostabilization programs. The results also showed that the increasing Cd concentration disrupted the plant nutrient homeostasis as it increased the concentration of some nutrients and had adverse effect on others, impacting plant growth. In this scenario, the results indicate that the low Mg, Fe and Mn concentrations in the leaves were the main cause for plant growth reduction and leaf chlorosis and necrosis, as each one of these elements plays a key role on the chlorophyll molecule and on photosynthetic process.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Morphology and biomass yield of forage cactus under mineral fertilization in organic soil

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The objective of this study was to evaluate the morphological characteristics and yield of the biomass of forage cactus in mineral fertilizers in organic soil. The experiment was conducted in vases, in the open in the experimental area of the Federal University of Campina Grande - UFCG. The experimental design was completely randomized in a factorial scheme 3×2 with four replications in which factors consisted of three palm varieties (Orelha de Elefante, Baiana and Miúda) and two managements fertilization (M1 - without application of fertilizer in the foundation and M2 - with NPK application on the foundation). The cultivars and the type of isolated fertilization significantly influenced the morphology and yield of the biomass of forage cactus under mineral fertilizers in organic soil. Treatment with NPK application had positive influence on the morphology and yield of cultivars biomass. The length, width, thickness and green mass cladodes weight of Orelha de Elefante and Baiana showed maximum yield. The interaction between the factors was significant only in the yield in kg^{-1} vase of forage cactus, with the NPK fertilization that led to maximum yield for cultivar Miúda.

Key words: Morphometry, *Opuntia*, *Nopalea*, green mass production, fertilization types.

INTRODUCTION

The spineless cactus is one of the main sources of livestock feed in the Northeast of Brazil; it serves as an important source of water, energy and minerals to the livestock (Almeida et al., 2012). This plant is notable for its juicy and roughage nature food which makes it highly digestible, and which is indeed of paramount importance for the formulation of feed for livestock (Morais and Vasconcelos, 2007).

According to Donato et al. (2014) several supplementary feeding alternatives have been used in the semi-arid region to manage the effect of water, energy and minerals limitations. Due to these limitations, plants which are tolerant to the effect of water stress, high temperature and radiation are planted more in the region.

The spineless cactus *Opuntia ficus-indica* (L.) Mill., stands out for its cactaceous, features which include numerous anatomical characteristics and morphological and physiological adaptation to the dry ecological condition which is prevalent in the semi-arid Northeast

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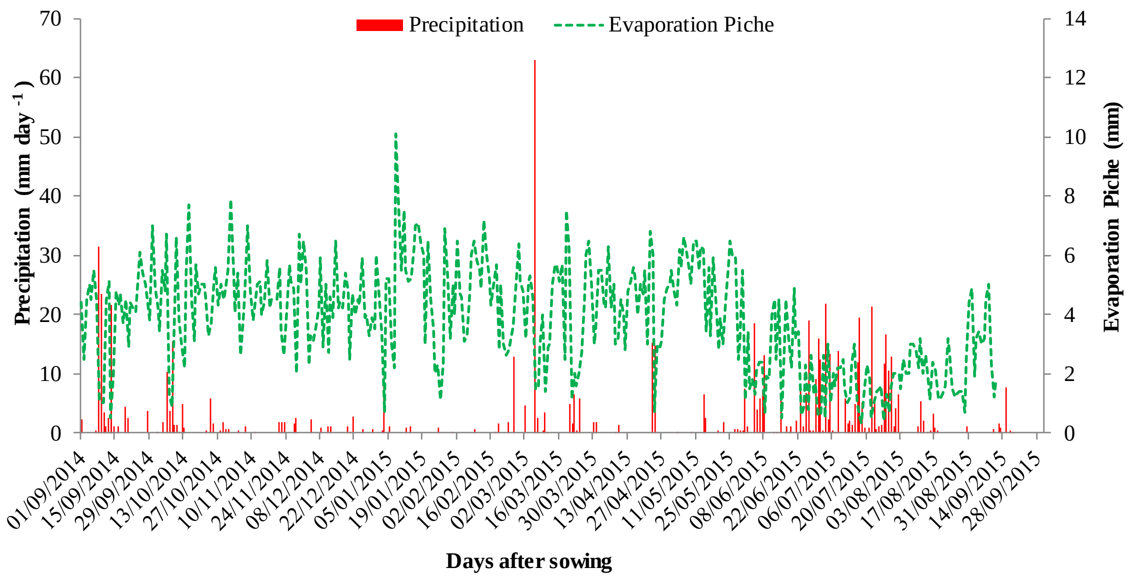

Figure 1. Rainfall and evaporation Piche during the experiment period.

(Oliveira et al., 2010).

According to Silva et al. (2010) the reason for the forage palm's highly efficient water use is due to their morphological and physiological characteristics, which allows high-capacity daily CO_2 capture and reduced water loss. Phenomenally at night, its gas exchange is termed as the crassulacean acid metabolism (CAM).

Management techniques such as fertilization, plant spacing, and crop management have been indicated as the most influential factors on the productivity of forage cactus (Alves et al., 2007). Consoli et al. (2013) in their study of Miúda growth obtained a yield of 12.9 Mg ha^{-1} $yearr^{-1}$. Cunha et al. (2012) also in their study of Miúda cultivar reported an average production of 180 t ha^{-1} in green matter forage cactus at 20 months, with a density of 40,000 plants per ha^{-1}.

Other factors such as greater availability of nutrients in the soil, mineral fertilizers are essential for obtaining high yields are also examined.

The aim with this study was to evaluate the morphological characteristics and yield of the biomass of forage cactus under mineral fertilizers in organic soil.

MATERIALS AND METHODS

The experiment was conducted in pots which were placed in an open area at the academic unit of agricultural engineering, linked to the Center for Technology and Natural Resources (CTRN) of the Federal University of Campina Grande (UFCG - PB), located in the geographical coordinates: 7° 15' 18'' South latitude, 35° 52' 40'' West longitude and average altitude of 550 m. According to the Köppen climate classification, adapted to Brazil (Coelho and Soncin, 1982), the climate is the Csa type, which is climate mesothermal, sub-humid, with periods of hot and dry season of about 4-5 months and rainy season from autumn to winter.

The daily data of rainfall and evaporation Piche of Campina Grande – PB as in Figure 1, from September 2014 to September 2015, which corresponds to the period of the research were obtained from the National Institute of Meteorology (INMET). According to Macedo et al. (2011) studying the intensity of the rainfall in the city of Campina Grande - PB using a time series of climate data an average rainfall of 804.9 mm was obtained; less rainfall was observed from October to December.

Figure 2 presents the maximum air temperature; average, minimum, and relative humidity in the experimental period from the National Institute of Meteorology (INMET). The average maximum temperature recorded is 30.6°C, the minimum 18.6°C and an annual average of 25.0°C (Cabral Junior et al., 2013). The average relative humidity of air is 83.0% (Alves et al., 2009; Guedes Filho et al., 2012).

The soil used in the study was classified as Typic Dystrophic (Embrapa, 2013), and the chemical characteristics in depth of 0-20 cm, are presented in Table 1.

The treatments consisted of the combination of two factors: Three cactus pear cultivars (Orelha de Elefante, Baiana and Miúda) and two fertilization management (M1 - without application of fertilizer in the foundation and M2 - with NPK application in the foundation).

The experimental design used was completely randomized, with four replications, so that the factors studied were arranged in a factorial design of 3 × 2. The six proposed treatments were arranged in 24 vases of 22.5 L each, with space of 0.5 m between plants and 1.0 m between rows. Each experimental unit consisted of one vase with holes in the bottom, containing a 1 cm layer of crushed stone, covered with geotextile to facilitate drainage; then layered with about 20 kg of soil.

The forage cactus cultivars used in the experiment were Orelha de Elefante - Palmepa - PB3 - *Opuntia Tuna* L. Mill; IPA - Sertânia (Baiana) or Palmepa - PB1 and Miúda or sweet or Palmepa - PB4 - *Napolea cochenillifera* Salm - Dyck. These were chosen because they are resistant cochineal carmine (*Dactylopius opuntiae* Cockerell).

Racquets outputs free from infestations of pests and diseases were selected for planting, these included cochineal-de-scales. These rackets passed through the shadow healing period where

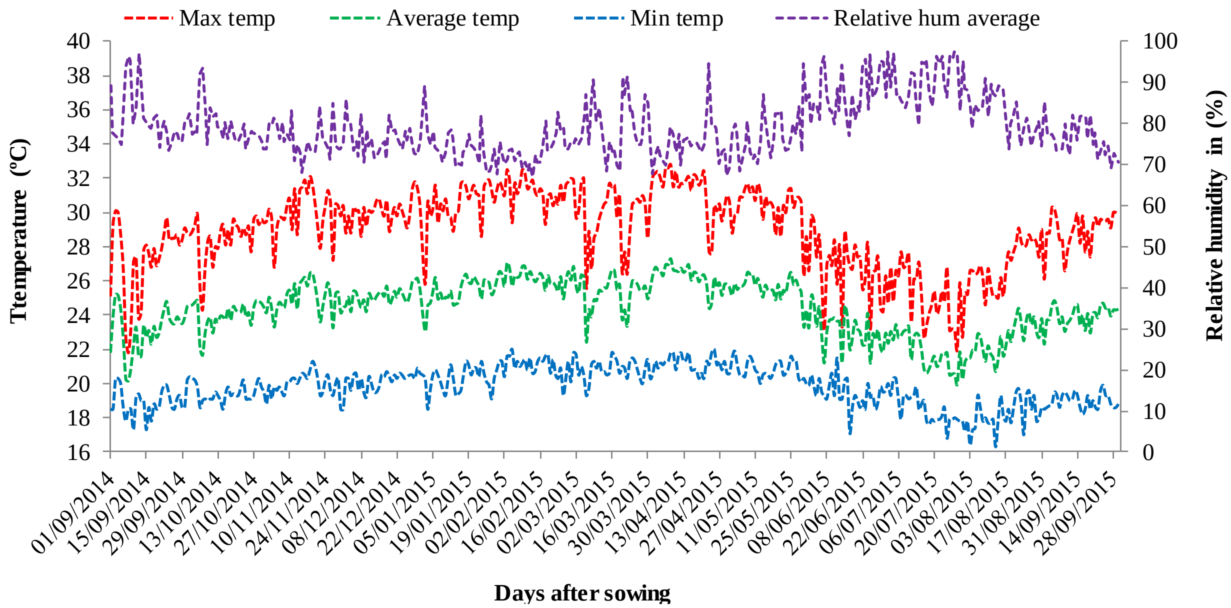

Figure 2. Maximum air temperature; average, minimum, and relative humidity during the experiment period.

Table 1. Physical and chemical characteristics of the soil used in the experiment.

pH	M.O	P	K	Na	Ca	Mg	Al	CTC	V
	g kg^{-1}	Mg dm^{-3}				cmol$_c$ dm^{-3}			%
6.8	222.55	486.36	2124	0.07	6.85	10.35	0.0	38.23	63.54

the cladodes lost some moisture caused by the cutting operation in the field. The rackets were arranged in front of the pits to the sun and buried 50% with an inclination of 45º.

Irrigation was carried out before planting to raise the soil capacity. This was done following the gravimetric method (standard) of Greenhouse, and a humidity capacity of U = 26.32% was obtained.

The irrigation was performed weekly, always at the end of the afternoon. The irrigation management was fixed and water slide was obtained by crop evapotranspiration (ETc) from the drain reading in the lysimeters (vases), which indicated the average balance of water inlet and outlet to keep moisture in values close to field capacity throughout the crop cycle, as Equation 1.

$$ETc = I - D \quad (1)$$

Where: ETc = crop evapotranspiration in mm day $^{-1}$; I = blade applied by irrigation in mm day $^{-1}$;
D = drainage blade in lysimeter in mm day $^{-1}$.

Fertilizer was applied in the foundation according to the result of soil analysis. In M2, urea was used as a source of nitrogen, single superphosphate as source of phosphorus and potassium chloride as source of potassium in doses of 200 kg ha^{-1} N, 150 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_2O according Novais et al. (1991)..

At 360 days after planting, variables were examined. Width and height of plants were measured, length and width of cladodes perimeter was measured using tape measure; cladodes were counted; thickness of cladodes was measured using digital caliper and finally, weight of green matter of cladodes and yield were taken.

The estimated production of green biomass of the cultivars was determined according to the methodology proposed by Menezes et al. (2005) and the average mass of cladodes estimated based on the Equation 2.

$$BMVC = C * L * E * 0.535 \quad (2)$$

Where: BMVC = biomass of green cladodes matter, in g; C = average length of cladodes in cm;
L = average width of the cladodes, in cm; E = average thickness of the cladodes, in cm.
The results of the study variables were subjected to analysis of variance by F test analyses where the interaction was significant, the mean comparison was calculated based on the 5% Tukey test, with the aid of Statistical Software SISVAR (Ferreira, 2008).

RESULTS AND DISCUSSION

The summary of the analysis of variance for plant height (AP), plant width (LP), number of cladodes (NC), length of cladodes (CC) and width of cladodes (LC) of forage cactus under mineral fertilization at 360 days after planting are shown in Table 2.

It should be noted that the variables were significant at 1% probability to cultivars, except for width of plants. For the fertilizer factor, there was a significant effect for the variables plant height, width and number of plant

Table 2. Analysis of variance of plant height (AP), plant width (LP), number of cladodes (NC), length of cladodes (CC) and width of cladodes (LC) of forage cactus in mineral fertilization at 360 days after planting.

Variation sources	G.L	AP (cm)	LP (cm)	NC	CC (cm)	LC (cm)
Cultivar (C)	2	476.16**	76.63^{ns}	151.54**	28.67**	139.40**
Fertilization (A)	1	450.66**	1247.04*	198.37**	0.63^{ns}	6.12^{ns}
Interaction (C x A)	2	27.16^{ns}	99.29^{ns}	27.12^{ns}	10.59^{ns}	10.37^{ns}
Residue	15	28.73	175.33	11.11	4.33	7.03
General average	-	57.33	74.13	11.71	23.52	14.13
Coefficient of variation (%)	-	9.35	17.86	28.48	8.85	18.77

^{Ns}Not significant at the 0.05 level of probability by F test; *,** Significant at 0.05 level of probability and 0.01, respectively, by F test.

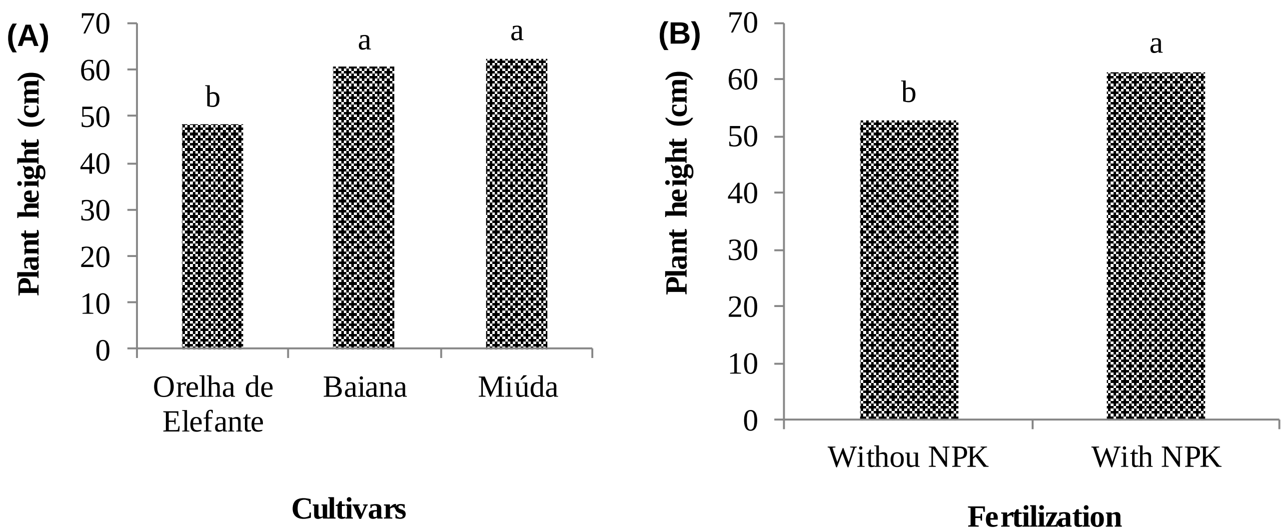

Figure 3. Height of spineless cactus plants depending on cultivars (A) and with and without NPK in the foundation (B) at 360 days after planting.

cladodes at 5 and 1%, respectively, with no significant effect for the length and width of cladodes. However, no significant interaction between cultivar (C) and fertilization (A) of the forage palm was found at 360 days after planting (Table 2).

Silva et al. (2015b) in their study of the growth and production of forage cactus in mineral fertilization observed that there was no difference in the evaluations of morphological characteristics at 60 days after planting. This result differs from those obtained in the present study; this fact is associated with the slow growth of the species with acid metabolism of CAM crassulacean in the initial phase (Silva et al., 2010).

Plant height in function of cultivar presented better performance in cultivars Baiana and Miúda when compared to elephant ear, at 360 days after planting (Figure 3A). This is probably related to genetic and morphological characteristics of the cactus pear, that is, species *Opuntia tuna* (L.) Mill (Elephant Ear) has horizontal growth and *Nopalea cochenillifera* (Bahia and Miúda) has vertical growth (Embrapa, 2002).

Silva et al. (2015a) reported that the cultivar IPA-Sertânia (Baiana) and Miúda are those with higher plant height compared to other cultivars, a result consistent with that obtained in the present study.

On the factor, fertilization, it is noted that plant height was higher in the treatment with NPK; this fact is possibly associated with an increased supply of nutrients culture as compared to the treatment without NPK (Figure 3B).

According to Silva et al. (2012) nitrogen is the nutrient with the most influence on the plant height, particularly under adequate phosphorus supply. Ramos et al. (2011) when studying the vegetative growth of *Opuntia ficus-indica* at different planting spacing found that plant height differed statistically at 5% probability, the highest average being 74.6 cm at 455 days after planting for cultivar Italiana. These results are similar to those obtained in the present study where the average height observed was 60 cm at 360 days after sowing.

There were significant differences observed in the

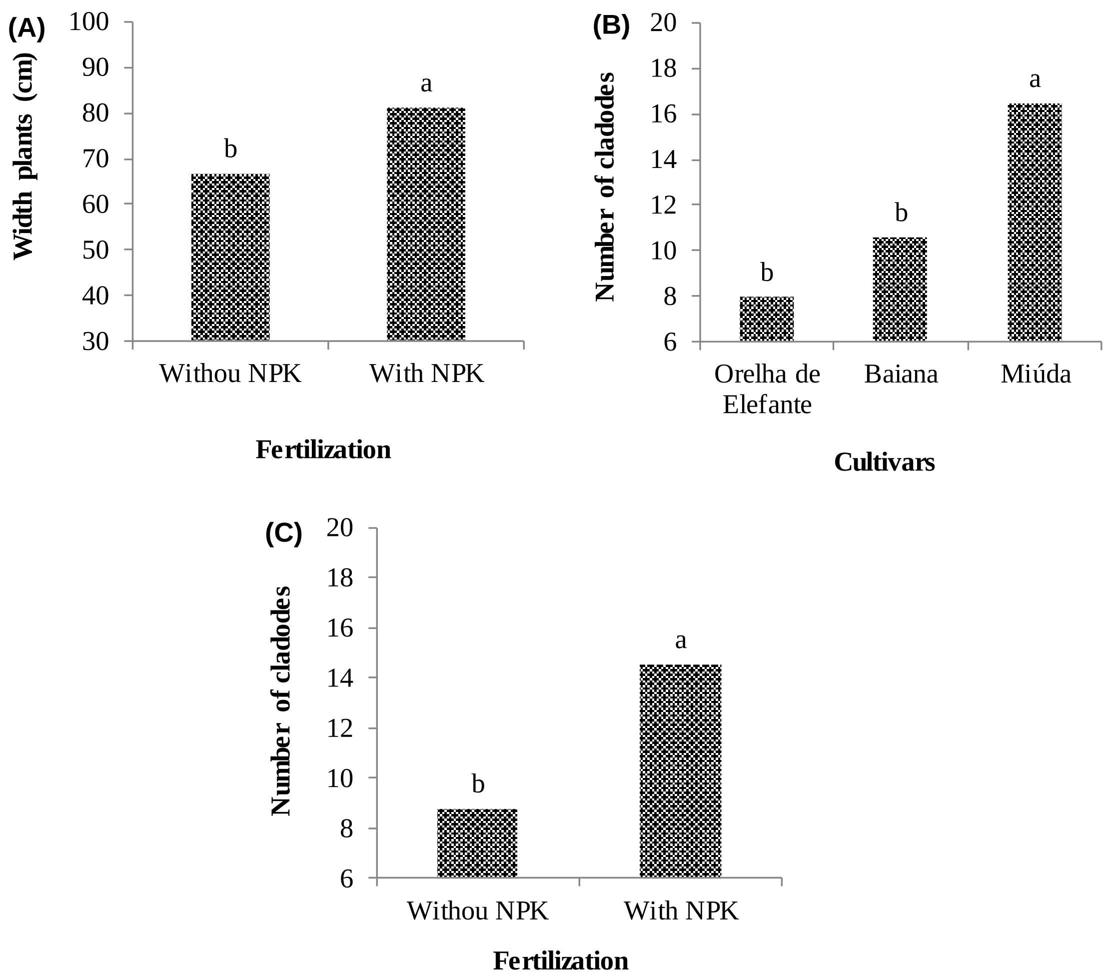

Figure 4. Plant width spineless cactus with and without NPK application in foundation (A), cladodes number depending on the cultivar (B) and with and without NPK foundation fertilization (C) at 360 days after planting.

Tukey test at 5% probability for plant width of cactus forage depending on fertilizer (Figure 4A), and treatment with NPK application was resulting in maximum increments LP. This fact associated with the availability of nutrients and consequently the absorption by the plant aiding in their growth.

Nascimento et al. (2011) studied the morphometric characteristics of forage cactus submitted to organic fertilizer, mineral and cutting. The study report shows that there was no significant difference between the fertilization used on the cactus forage in the experiment to the variable-width of plants.

The numbers of cladodes (depending on the cultivars) differed statistically in the Tukey test at 5% probability, and cultivar Miúda showed higher cladodes emission per plant (Figure 4B). This fact is closely related to the growth habit of the species gender Nopalea spp., since they have open growth habit favoring the formation of areolas, or axillary buds, allowing the emergence of new cladodes, not only in cladodes apex mother as well as at their side edges (Amorim et al., 2015).

Despite that the cultivar Miúda presents more cladodes, the result is lower in green biomass production. However according to Silva et al. (2015b), cultivar Miúda showed superiority of over 200% in the number of cladodes per plant compared to the cultivars IPA - Sertânia (Baiana) and Orelha Elefante.

Cunha et al. (2012) reports that lower cladodes are observed in cultivar Miúda. This function is needed to distribute the nutrients to a larger number of cladodes, but their superiority as the number is effectively associated with the morphological characteristics of the genre.

To study the influence of the fertilization on number cladodes, it was observed that the treatment with the NPK application which showed maximum emission of cladodes in cultivars (Figure 4C). Probably this fact is related to the existing synergistic interaction that makes nutrients available in the soil, making it readily available to plants (Araújo and Machado, 2006).

According to Silva et al. (2014) when soils are fertilized with phosphorus, there is an increase in the availability of

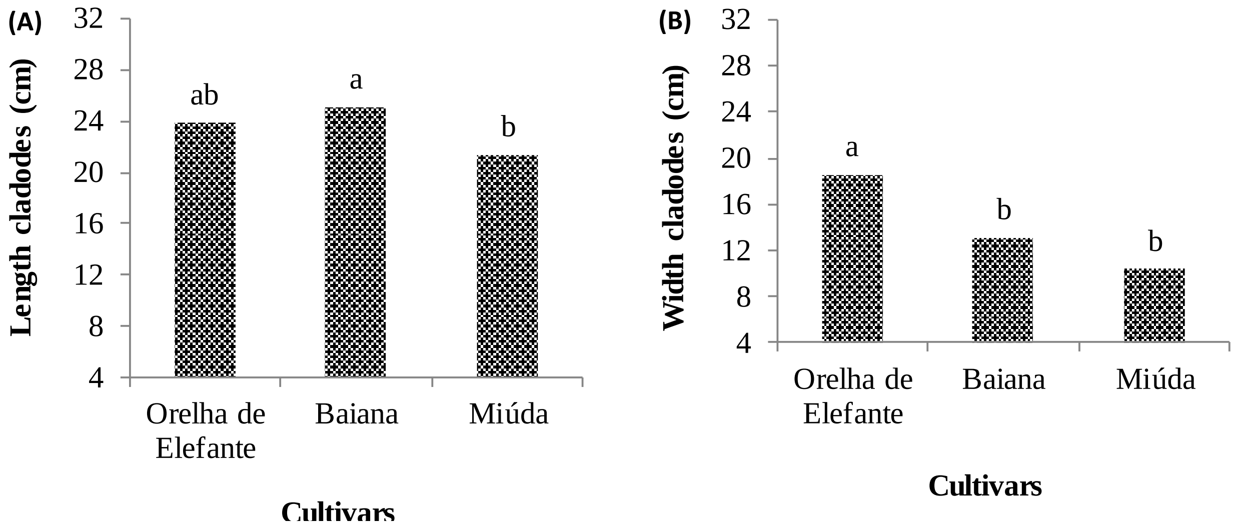

Figure 5. Cladodes length (A) and cladodes width (B) of forage palm at 360 days after planting.

Table 3. Analysis of variance for perimeter cladodes (PC), thickness of cladodes (EC), weight green cladodes matter (PMVC) and yield (R) of forage cactus in mineral fertilization at 360 days after planting.

Variation sources	G.L	PC (cm)	EC (mm)	PMVC (g)	R (kg vaso^{-1})
Cultivar (C)	2	369.77**	131.67**	4201639.62**	323.69*
Fertilization (A)	1	2.77^{ns}	3.98^{ns}	8123.89^{ns}	1218.29**
Interaction (C x A)	2	33.39^{ns}	7.71^{ns}	2094676.10^{ns}	258.31*
Residue	15	28.89	3.08	584643.41	60.28
General average	-	55.47	18.38	3201.32	34.52
Coefficient of variation (%)	-	9.69	9.55	23.88	22.49

^{ns}Non- significance at the 0.05 level of probability by F test; *,** Significant at 0.05 level of probability and 0.01, respectively, by F test.

adenosine triphosphate (ATP) which encourages the absorption of nitrogen as amino acids and promotes protein synthesis.

The length of the cladodes depending on the cultivars differed statistically at 5% according to Tukey test (Figure 5A). It should be noted that cultivar Baiana was statistically similar to Orelha and Elefante but different from cultivar Miúda. However, the highest length of cladodes was observed in cultivar Baiana.

According to Silva et al. (2015b) in the study of the productivity of forage cactus cultivars observed at different times, the cultivar showed better length of cladodes (21 cm) in the Orelha Elefante, at 150 days after planting, corroborating the findings of the present study where the cultivar of Orelha Elefante did not differ from that of Baiana and the observed mean was 24 cm.

Regarding the width of cladodes, it was observed that the growth rate of Orelha Elefante had higher value at 360 days after planting (Figure 5B). Note that the Baiana and Miúda cultivars did not differ by Tukey test, leaving only cultivar Orelha Elefante. The low value of cladodes width of Baiana and Miúda cultivars is associated with features of the cultivars that belong to *Nopalea* genre.

According to Dubeux Junior et al. (2010) the differences observed in the studied cultivars can be associated to their peculiar morphological structure which is seen in the width and thickness of each cladode.

The summary of the analysis of variance for the variables perimeter of cladodes (PC), thickness of cladodes (EC), weight of green cladodes matter (PMVC) and yield (Y) of forage cactus in mineral fertilization at 360 days after planting are shown in Table 3.

It is observed that the variables were significant at 1 and 5% probability factor to cultivar. For the fertilization factor, there was a significant effect for the yield variable at the level of 1%. Significant effect at 1% probability for interaction between cultivar (C) x fertilization (A) of the forage cactus was observed at 360 days after planting only for yield variable (Table 3).

There was a significant difference at 5% probability observed by Tukey test for perimeter of cladodes depending on the cultivars at 360 days after planting

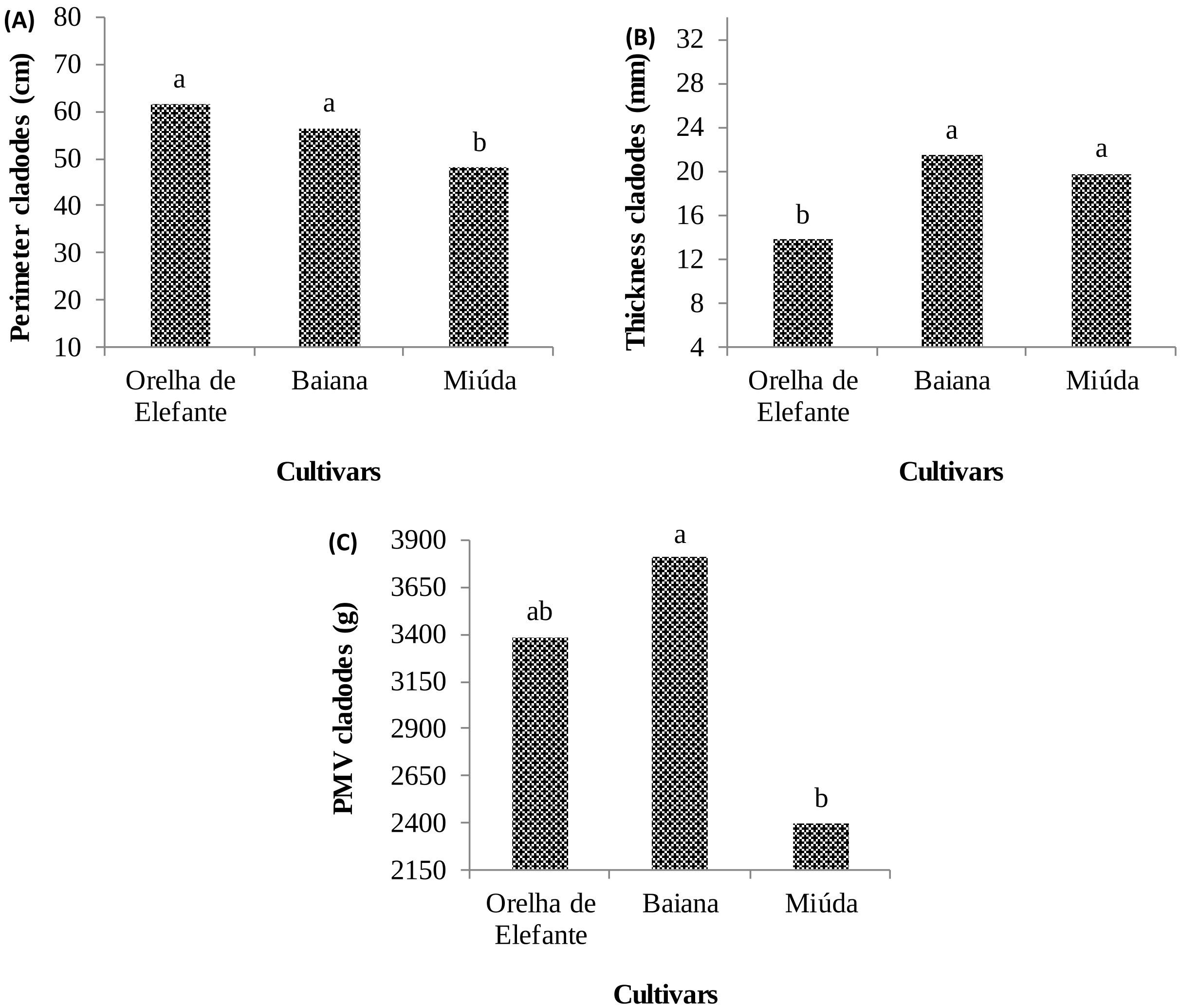

Figure 6. Perimeter of cladodes (A), thickness of cladodes (B) and green mass weight cladodes (C) depending on the cultivars at 360 days after planting.

(Figure 6A). It should be noted that the cultivars Orelha Elefante and Baiana did not differ; difference was only noticed in cultivar Miúda. It is also observed that the maximum perimeter was found in cultivar Orelha Elefante corresponding to approximately 66 cm. The largest perimeter observed in cultivar Orelha Elefante is associated with its anatomy which shows wider perimeter compared to other cultivars.

This result is consistent with those obtained by Leal et al. (2008) and Oliveira Júnior et al. (2009), using the same cultivars, they found averages for the perimeter of 60.42 and 60.50 cm to 270 days after planting, respectively.

The thickness of cladodes differ statistically by Tukey test at 5% probability, and the Baiana and Miúda cultivars presented the greatest thickness of cladodes corresponding to 21.5 and 19.8 mm, respectively (Figure 6B).

This fact probably is related to the genre cladodes format Nopalea be of oblong type that influences a greater thickness for the cladodes, as well as increasing the same volume resulting in increased biomass production by plants fact of great importance since cultivars of this kind generally have lower fresh mass production, when compared to the genre Opuntia (Dubeux Junior et al., 2010).

Regarding the green mass weight of cladodes, depending on the cultivars at 360 days after planting, it is noted that this differed statistically at 5% by Tukey test. It appears that the highest average value of green mass weight was obtained in cultivar Baiana, corresponding to 3800 g (Figure 6C). The greater the thickness of the cladodes, the higher their weight will be.

As a feature of simple visualization and measurement, green mass production in the semiarid region aids the calculation of the size and number of animal flow on the

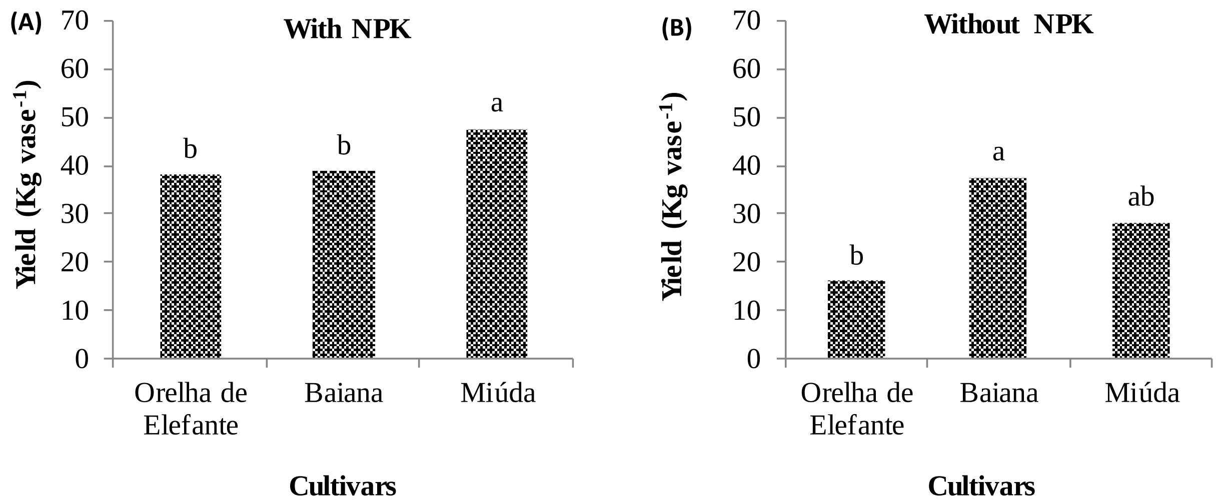

Figure 7. Yield per kg vase $^{-1}$ depending on cultivar x fertilization with NPK (A) and cultivars x fertilization without NPK (B) of forage palm 360 days after planting.

farm.

Silva et al. (2015) obtained similar results to those reported in this study, at 150 days after planting; cladodes green mass production reached approximately 1000 g in cultivar Baiana.

The interaction between Cultivar x Fertilization was significant at 5% by Tukey test for yield of forage palm at 360 days after planting (Figure 7). Note that the yield of treatments with NPK application in kg vase^{-1} was higher in 'Miúda, with a maximum yield of 47.0 kg vase^{-1} (Figure 7A).

Lopes et al. (2007) state that for spineless cactus to have good yields, it needs to be grown in well-structured soil with good aeration and excellent fertility, given that the plant requires large amount of nutrient due to its high number of cladodes. Considering all the above, it is preferable to cultivar Miúda, since it produces more cladodes than cultivars Orelha Elefante because of its morphological structure.

Almeida et al. (2012) reported that fertilization, either mineral or organic is indispensable when one wants to have high yields, since it provides the supply of macro and micronutrients necessary for the full development of the cactus forage.

Cultivar Miúda compared to Orelha Elefante is more nutritious and appreciated by cattle (palatable), but has a lower resistance to drought (Silva and Sampaio, 2015).

Regarding cultivars within the treatment without NPK application there was a significant difference at 5% probability by Tukey test (Figure 7B); it is observed that the Baiana, Orelha Elefante and Miúda cultivars did not differ. This is possibly due to the genetic characteristics of each gender and how it responds to conditions of low nutrient availability.

Silva et al. (2015) reports that when NPK was applied to the Baiana and Miúda cultivars, they showed higher yield in kg vase^{-1} at 150 days after planting, these results are consistent with that observed in this present study.

Conclusions

1. The isolated treatments significantly influenced the morphology and yield of the biomass of forage cactus.
2. Treatment with NPK application improved the morphology and yield of the biomass of cultivars cactus forage.
3. The length, width, thickness and green mass weight of cladodes had maximum yield for the cultivars Orelha Elefante and Baiana.
4. The interaction between the factors was significant only for the yield of cactus forage in kg vase^{-1} with the NPK fertilization that resulted in maximum yield for cultivar Miúda.

Conflict of Interests

The authors have not declared any conflict of interests.

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Quali-Quantitative study of biogas production from bio-digestion of cutting poultry

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The purpose of this study was to evaluate the biogas production and the potential production obtained with the anaerobic digestion of cutting poultry carcasses. The carcasses were used to supply batch digesters with 8 liters of capacity of fermentation material. To perform this process, analysis content of total solids (TS) and volatile solids (VS) were performed. We used a system of three treatments, TRA (Treatment A) - 8% of ground chicken and 92% of water; TRB (Treatment B) - 8% of ground chicken, 20% of swine inoculum and 72% of water; TRC (Treatment C) - 13% of ground chicken, 20% of swine inoculum and 67% of water. The results were evaluated statistically showing that TRB treatment has shown better performance compared to the other two treatments, resulting in obtaining biogas at 0,3854 m³/kg TS and 0,4158 m³/kg VS, with an average production of methane (CH4) of 75.63%, thereby producing 0,2748 m³ of methane per kg of TS added and 0.2964 m³ of methan/kg VS^{-1} (???), with a hydraulic retention time (HRT) of 31 days.

Key words: Anaerobic, methane, environment.

INTRODUCTION

The poultry industry has grown significantly in recent years, with its growth, there is also a need to produce increasingly higher quality. New management technologies, increased population density of poultry and the short period between batches, are factors that directly influence the quality of the meat produced and on the resulting increase of waste (poultry litter, carcasses, waste water, etc.). According to UBABEF (2014), the poultry activity began in Brazil in 1950 and started to show rapid development. The Brazilian chicken meat production increased 48.62% in 13 years, reaching a production of 12.30 million tons in 2014. Currently, the country is the largest exporter of this product and also one of its largest consumers, with a 41.80 kg per capita consumption. Among the producing states, Paraná is the largest one, with a share of 31.12%, followed by Santa Catarina with 16.66% of total production. Still in the 50's, Western Paraná has gone through massive changes since its colonization, due to modernization of national agriculture, replacing subsistence agriculture by the grain production. Brazil had several agricultural frontiers with expansion of grain production, in the Midwest, Northeast

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and North regions, taking also into account, places in the west mesoregion of Paraná State, which turned into a suitable region for the industries of slaughter and cattle, and poultry and swine meat processing (Dalmás et al., 2007).

In the West mesoregion of Parana, there are eight chicken slaughter houses, five cooperatives and three private companies, all of them exporting to other countries and distributing to the Brazilian domestic market (Belusso, 2010). According to IBGE (2015), Brazil slaughtered about 5.5 billion chicken carcasses in 2014. Abreu et al. (2009) mention that the poultry industry estimates approximately 3 to 5% mortality of birds during the process, causing a large environmental liability, together with the waste they had produced. The incorrect way to dispose of such carcasses, without prior treatment, can result in chemical and biological contamination of soil and water. Therefore, an alternative to mitigate environmental impasses generated by the improper disposal of poultry waste, would be the use of composting; however, many poultry farmers do not perform this procedure, thereby resulting in poor handling. Therefore, it is important to adopt a system of treatment of this waste in order to avoid possible environmental contamination (Güngör-Demirci and Demirer, 2004).

Bonturi and Dijk (2012) report that the biodigester is a biomass treatment system, to where the animal waste are conducted through pipes. This is where, through the anaerobic process, the bacteria that grow in total absence of oxygen causes the material to suffer a natural fermentation process and, at the end of the process, gases, pasty wastes and liquid effluents are produced. These by-products have economic value. The gas (methane) can be used in electric power generation, space heating, aviaries heating in winter and even in domestic stoves. The solid material turns into biofertilizer for farming, while the liquid effluents may be used for feeding algae in tanks that are then used as food for fish raised in ponds. Furthermore, according to Sánchez et al. (2001), the process of anaerobic digestion provides not only biogas, but also biofertilizer, which can replace conventional fertilizers when applied to various crops. This waste treatment in biodigesters also reduce pathogens and parasites, faecal coliforms and unpleasant odor, improving the environment of rural properties.

Without the presence of gaseous oxygen, anaerobic biodigestion is a decomposition process of organic matter by bacteria, divided into phases that together perform the decomposition of organic matter (Demirer and Chen, 2005). Initially, the liquefaction stage takes place, the organic matter is processed into dissolved compounds (volatile organic matter). In the following stage, the process is divided into two parts: acid fermentation and acetogenic fermentation. Even at this stage, the products of the previous subphase are transformed into acetate, hydrogen and carbon monoxide. In the third and final phase (methanogenesis), the acetogenesis products are transformed into methane in a greater proportion (Costa, 2006). According to Silva (2009), the anaerobic process takes places as follows: bacteria use the chemical components, such as carbon dioxide (CO_2), nitrate (NO_3), and sulfate ($SO_4$2-) to give the final product of oxidation of organic matter. This process consists of two stages. In the first one, the anaerobic and facultative bacteria process organic compounds of carbohydrates, lipids and proteins convert into volatile acids. In the second one, strictly anaerobic bacteria convert the volatile acids into gases such as methane and carbon dioxide, thus, resulting in biogas and in addition to contributing with the environment, it also generates income to producers.

China is one of the countries that mostly use biogas. They use it for cooking and home lighting in rural areas, including having their own biodigester model, with multiple working units installed (Nogueira and Zürn, 2005). In 1996, Brazil started studying the electric energy production using biogas and also in a more intensive way. The results achieved in Brazil are enough to demonstrate a good mastery of the biogas production technology, with competence and qualification to develop programs at the national level, both in rural and agro-industrial areas, as well as in urban and industrial areas (Seixas et al., 1980). At present, anaerobic digestion is the most widely used procedure in Brazil for the swine manure treatment, resulting in a large amount of gas, mainly composed of methane (CH4) (Oliveira et al., 2003). Salminen and Rintala (2002), performed a study on the potential for methane generation from the waste of poultry slaughter houses and demonstrated the importance of the use of this waste with potential: 0.20 to 0.25 m³ of CH4 kg^{-1} of poultry carcasses; 0.10 to 0.15 m³ of CH4 kg^{-1} poultry litter; 0.05 m³ of CH4 kg^{-1} of feathers; 0.10 m³ of CH4 kg^{-1} of blood, and 0.30 m³ of CH4 $kg^{-1}$of viscera, feet and head. The objective of this experiment was to verify the technical feasibility of using anaerobic biodigestion to treat cutting poultry carcasses in order to take advantage of this by-product of poultry and adding value to it.

MATERIALS AND METHODS

The experiment was conducted on a farm in São Miguel do Iguaçu - PR for a period of 102 days. The substrate for supplying the biodigesters was obtained in one of the aviaries of the farm where the experiment took place. A total of 22 chickens aged 21 days that were processed in an industrial crusher Siemsen brand, LSD-25 model was used. The grinding time was 30 s per bird, so that the resulting mass could stay on the desired consistency and be mixed for the experiment. After grinding, the whole mass was again mixed in a container, forming the substrate as shown in Figure 1. For the conduct and development of the research, 9 units of batch biodigesters were used, each with a total capacity of 8 L. This model was chosen because it is a most recommended system for treating waste which is removed less frequently, or in systems where the daily cleaning is not practiced.The batch biodigesters are composed of three distinct pairs, one of them being the container

Figure 1. Crushed and mixed dead chicken mass.

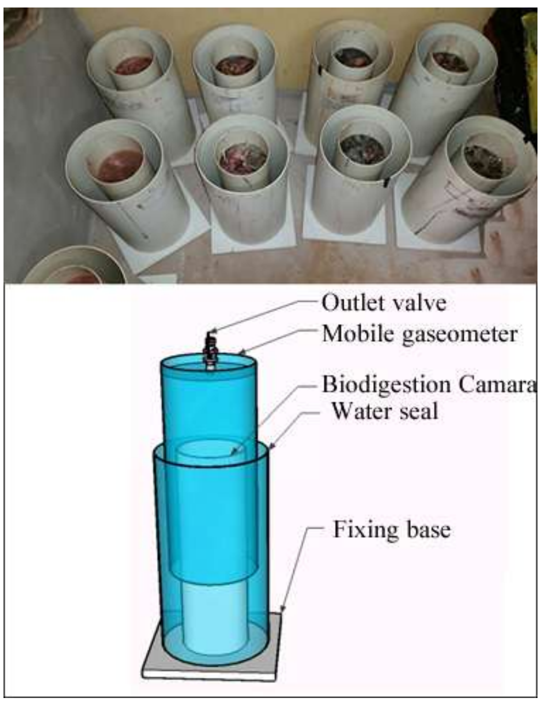

Figure 2. Batch biodigesters.

with the material in fermentation, the gasometer and the compartment is to form the water-seal. The container with material in fermentation was composed of a Polyvinyl Chloride (PVC) straight cylinder, measuring 150 mm in diameter and 480 mm in height. The gasometer consisted of a PVC straight cylinder with 480 mm diameter by 200 mm height, having one end closed by a valve that either allows or not the passage of gas for analysis and burning. The water-seal compartment was formed by a PVC straight cylinder with 250 mm diameter and 480 mm height. The biodigesters were located at room temperature in a masonry room with an area of 4 square meters, as shown in Figure 2.

The treatments were performed in triplicate and formed as follows: TRA - 8% ground chicken, and 92% water; TRB - 8% ground chicken, pig inoculum 20% and 72% of water; TRC - 13% ground chicken, pork 20% inoculum and 67% water. All treatments were performed in triplicate. According to Assis (2004), in his study with some models of digesters, the fact that the gasometer be willing or on the substrate or on the water seal reduces losses during the biogas production process, the author had reported that the substrate used for feeding these models digesters must present a concentration inoculum equal to 20% and a total solids (TS) of not more than 8% to improve the circulation of substrate through the chamber from fermentation thus, avoiding blockages in their inlet and outlet pipes. After preparing the substrate, the characterization was conducted, in order to know the VS and TS levels for the chickens and inoculum samples, according to the methodology described by (APHA, 2012). For the determination of TS, the samples were placed in previously tared and weighed porcelain crucibles, in order to obtain the wet material and, after that, they were placed in an oven with air forced circulation at a temperature of 105°C, until reaching constant weight; then, they were cooled and reweighed, as a means to know the dry material weight. In order to determine the VS, the resulting dry material was placed in the muffle furnace and kept at a temperature of 575°C for 30 min. After cooling, the resulting material was weighed, for the purpose of having the mineral matter weight. The standardization values of the treatments are presented in Table 1. In order to obtain the substrate weight which should be added to represent the desired percentage of TS, the following equations were used:

$$Pst = Mt.Sub \quad (1)$$

$$Psa = \frac{Pst.1000}{Ps} \quad (2)$$

Where:

Pst is Total Solid Weight (kg), Mt is the mixture total mass of the biodigester (kg), Sub is the added substrate percentage (%), Psa is the added substrate weight (kg) and Ps is the solid weight of wet substrate 1 kg (kg).

After calculating the quantities in Table 1, the mixture was mixed, according to the composition of each treatment, resulting in a total weight of 6.400 g for each treatment, which equals 80% of the biodigester capacity. The water used was collected in an artesian well at the site of the experiment. H is Ph value measured at 7, with a Schott Gerate CG818 model equipment. The inoculum was collected at the outlet of a biodigester in a swine farm in São Miguel do Iguaçu - PR. The substrate, inoculum and water were properly weighed, added in the biodigesters and mixed thoroughly. After this process, the biodigesters were closed with the gasometer, and water was added to form the seal. The biodigesters were subjected to daily readings gauge pressure, gasometer displacement and room temperature. The gauge pressure in cm H_2O was measured by a water column gauge, constructed by the authors. The gasometer displacement readings in cm was measured by a ruler attached to the gasometer itself. The temperature was measured by a TagTemp model Novus thermometer. After performing the readings, the generated gas was burned.

Weekly, the gas was collected and taken to the laboratory of the Federal University of Technology - Parana, Medianeira campus, to perform chromatography, in order to know the generated gas percentage. The chromatograph used was Clarus 680 model Perkin Elmer with Elite-Plot Q column, 30 m in length and internal diameter of 0.32 mm. To perform the sample collection, it was used with glass syringes of 60 ml capacity with metallic needles. A rubber hose was attached to the outlet valve of the gas meter, the valve was opened for the passage of gas and allow the exit of possible impurities contained in the hose. The end of the hose was closed with a catch and the needle was inserted to the suction gas which was made. After removing the needle, the same was immediately closed with a protective cover to prevent leaks and the air intake. For each case, treatment was collecting up 3 samples, they were

Table 1. Total solids (TS) and volatile solids (VS) of the chickens and the inoculum.

Treatment	Chicken (kg)	Inoculum (kg)	Water(kg)	Chicken(TS%)	Inoculum(TS%)	Chicken(TSkg)	Inoculum (TSkg)
TRA	1.613 (8%)	--	4.786	31.73	--	0.512	--
TRB	1.613 (8%)	1.600	3.186	31.73	4.28	0.512	0.068
TRC	2.622 (13%)	1.600	2.177	31.73	4.28	0.832	0.068

stored in a cooler and transported to the laboratory. The collection time until arrival at the laboratory was 30 min. In the 102nd day, having noticed that the daily production was already very low, the biodigesters were opened and the waste was discarded in the composting home of the experiment aviary. The correction of the biogas volume, for the conditions of 1 atm and 20°C, was made based on the Caetano (1985) work as equation 3. For correction of biogas volume, the average gauge pressure considered in this period was 9810 mm.

$$\frac{Po.Vo}{To} = \frac{P1.V1}{T1} \qquad (3)$$

Where: Po = Pressure at STP (760 mmHg), Vo = Volume at STP, To = Temperature at STP (293K), P1 = Local pressure in São Miguel do Iguaçu (981.03 mmHg), V1 = Measured volume (L), T1 = Measured temperature (K).

RESULTS AND DISCUSSION

After the characterization to get the moisture, TS and VS percentage, the sample had shown moisture 68.27%, with TS of 31.73% and, from this last percentage, VS of 29.41%. Under Brazilian table of food composition TACO (2011), the whole raw chicken moisture, with skin, reaches 66.5%. Guimarães and Adell (1995) and Ordóñez (2005) report that the chicken muscle contains the average of about 75% water of its weight. The muscle is the main component of the meat. As the animal organism, it contains water, proteins, fat, carbohydrates and inorganic constituents. The amounts of substrates added in the biodigesters were calculated from equation 1 and equation 2. Considering 8% substrate, we have Pst = 0.512 kg and Psa = 1.613 kg. Considering 13% of substrate, we have Pst = 0.832 kg and Psa = 2.622 kg. The experiment results showed biogas production data in the form of weekly and total accumulations as shown in Figure 3. We can see that TRB presented a higher production level than the other treatments, reaching 0.197 m³ of biogas accumulated in 15 weeks, against 0.069 m³ of TRC and 0.022 m³ of TRA. It is remarkable that TRA and TRB began to accumulate less at the end of the 12th week, while TRA had stabilized in the 7th week.

According to Steil (2001), in the anaerobic digestion system, a number of factors can have some influences, depending on the favor (or not favor) of the process starting. The substrate degradation, the growth and decline of the microorganisms involved, biogas production, anyone can determine the success or failure of the treatment of a particular waste. Among such factors, we can mention the pH, the presence of nutrients in the substrate composition, the total solids content and temperature, and as a consequence thereof, the interaction among the microorganisms involved in the process. The effect of temperature is one of the most important factors in the anaerobic digestion, since it acts on the speed of the bacterial metabolism, in the ionic equilibrium of substrates and their solubility (Foresti et al., 1999). The substrate pH and alkalinity were not observed either before or after the process, but due to the premature reduction of biogas production and to the characteristics of the added organic matter, it appears that the material suffered acidification, thereby inhibiting the TRA and TRB treatments production.

Hobson and Wheatley (1993) stated that one of the main reasons of a biodigester failure can occur at the beginning of the process, because the methanogenic flora which generally takes more time to be reduced than the acidogenic one, can not metabolize all produced organic acid, causing pH reduction. Low pH values inhibit methanogenesis and its production tends to get worse. Thus, input organic loads prevent the formation of large amounts of acids. Table 2 represents the weekly accumulated biogas production for three treatments. By statistically analyzing the three treatments production by F test, and comparing the samples two by two, all of them were significantly different from each other, with a 5% significance. Table 3 represents the biogas average production accumulated and weekly due to the reference treatments. When comparing TRB with TRC, we can verify that, at the end of the 15th week, TRB and TRC accumulated 0.1973 m³ and 0.0662 m³, respectively, thereby having TRB a greater biogas production, that is, 0.1311 m³. This difference is probably due to the fact that TRC contains more 5% of substrate. According to Mazzucchi (1980), it is necessary that the concentration of dry matter ranges from 7 to 9%, to be considered a satisfatory methane production. Figure 4 shows

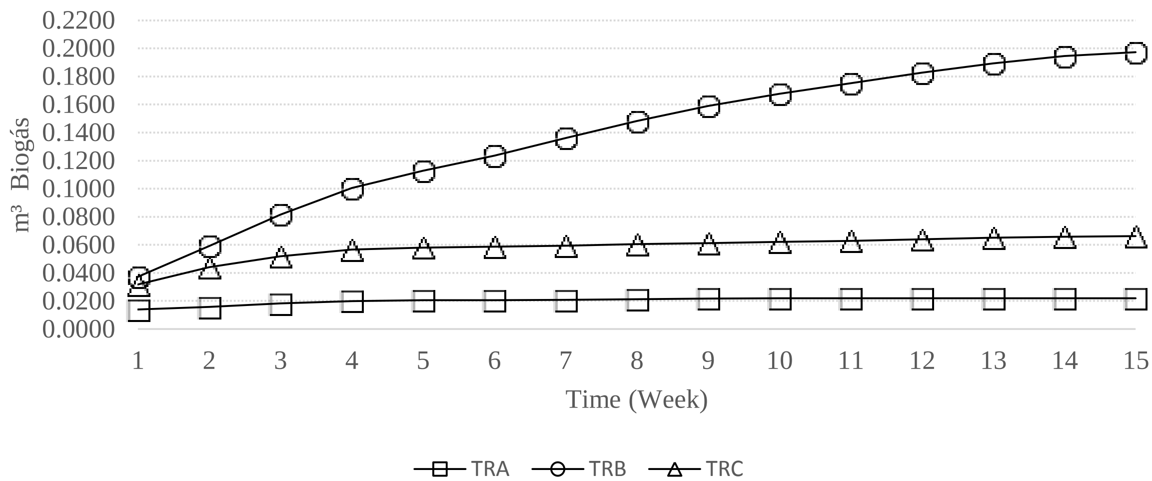

Figure 3. Average Biogas production, weekly accumulated due to the three treatments.

Table 2. F Test between TRA, TRB and TRC treatments, compared two by two.

Statistical parameters	*TRA*	*TRB*	TRC	TRA/TRB	TRA/TRC	TRB/TRC
Average	19.68775	130.9798	55.96832	-	-	-
Variance	15.51745	2766.677	155.6845	-	-	-
Observations	103	103	103	-	-	-
gl	102	102	102	-	-	-
F	-	-	-	0.00561	0.09967	17.771
P (F <= f) one-tail	-	-	-	0	0	1.50E-37
F critical one-tail	-	-	-	0.7209	0.7209	1.38715

Table 3. Biogas average production (m³) - Accumulated (A) and Weekly (S), due to the reference treatments.

Treatment	Production	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
TRA	S	0.0181	0.0028	0.0032	0.0024	0.0008	0.0002	0.0002	0.0008	0.0005	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000
	A	0.0181	0.0209	0.0241	0.0265	0.0273	0.0275	0.0277	0.0285	0.0290	0.0294	0.0294	0.0294	0.0294	0.0294	0.0294
TRB	S	0.0373	0.0219	0.0224	0.0188	0.0124	0.0108	0.0126	0.0119	0.0109	0.0087	0.0075	0.0075	0.0067	0.0051	0.0028
	A	0.0373	0.0592	0.0816	0.1004	0.1129	01236	0.1362	0.1481	0.1590	0.1677	0.1752	0.1827	0.1894	0.1945	0.1973
TRC	S	0.0317	0.0123	0.0078	0.0048	0.0015	0.0006	0.0007	0.0010	0.0008	0.0010	0.0006	0.0011	0.0012	0.0007	0.0004
	A	0.0317	0.0440	0.0518	0.0566	0.0581	0.0587	0.0595	0.0605	0.0613	0.0622	0.0622	0.0639	0.0651	0.0658	0.0662

the cumulative biogas production monthly. It is observed, that the TRB treatment showed higher production compared to the other treatments.

In figure 4, the biogas methane content data are weekly measured during the development of the experiment. Among the data, we can see that, in the 3rd week, the gas produced had methane contents above 50%, with the sole exception of that one produced by TRA treatment that had not yet recorded considerable rates of methane. From the 11th week, all substrates produced biogas with methane content above 70%, and the TRB treatment had methane content above 65% in the 3rd week. According to Seixas et al. (1980), since the microorganisms specifications and quality of life are met, the biogas generated will compose a gas mixture, with methane contents of about 60 or 65% of the total volume. The remaing content is made up of 35 or 40%, mainly carbon dioxide, and also smaller amounts of other gases. This biogas composition may vary, according to the type and quantity of the used biomass, as well as to climatic factors and to the biodigester dimensions, among others. Figure 5 represents the average of methane levels found among triplicates for each treatment, showing that the TRB treatments and TRC contained methane in its

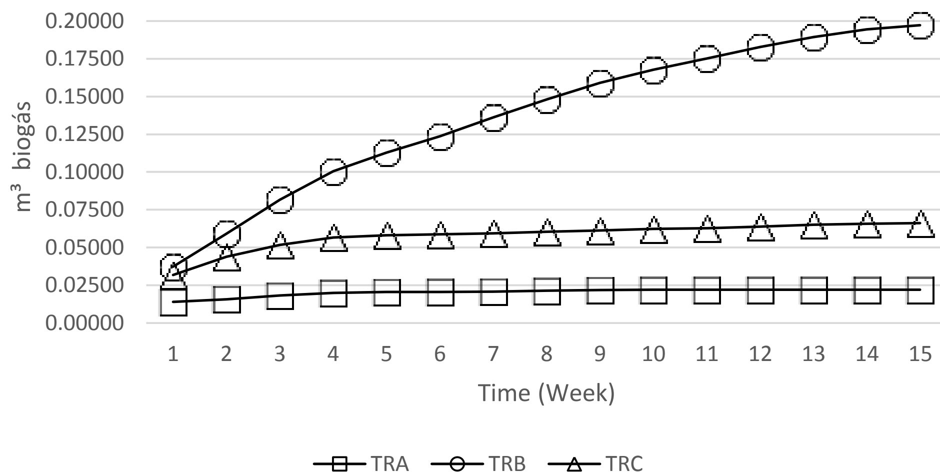

Figure 4. Weekly Accumulated Production - TRA.

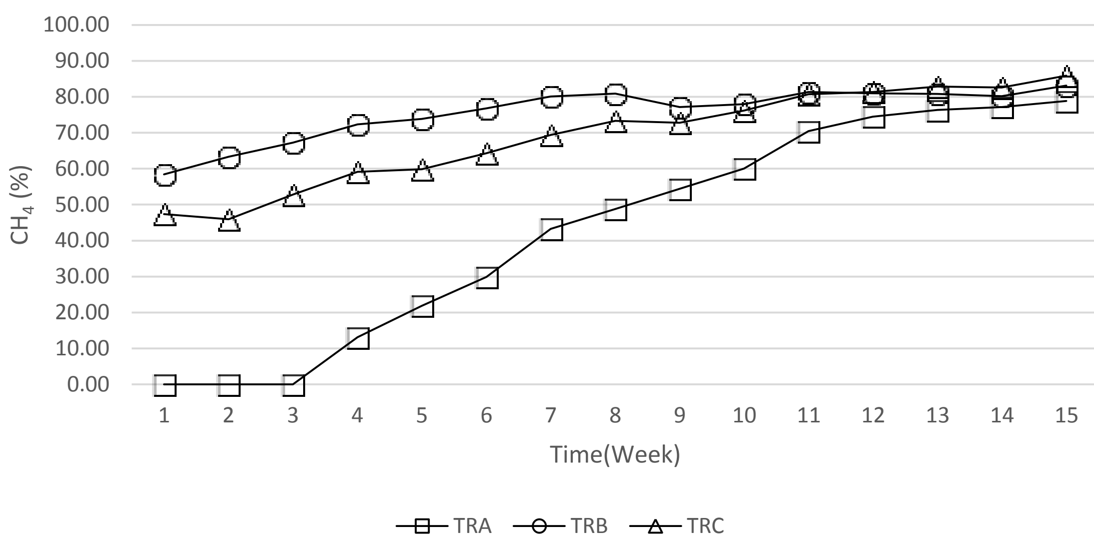

Figure 5. Weekly methane content.

composition at the beginning of the 2nd week as the TRA treatment presented behavior similar to the other treatments, in the middle of the 9th week.

When analyzing the TRA treatment with the TRB and TRC ones, we can see that the TRA methane production began at week 4 and had a low methane percentage, however, in the other treatments, the production began in the 1st week and had a high percentage of methane. This occurred due to the fact that TRB and TRC treatments have had inoculum in its composition, which accelerated the process. The purpose of analyzing non-inoculum TRA was precisely to know the substrate characteristics in natural situations. The obtained HRT (Hydraulic Retention Time) for each treatment was based on the percentage of the daily volume of biogas produced, according to the accumulated total volume. A production that showed a daily amount greater than 1%, in relation to the total accumulated volume, was considered acceptable. According to VDI (2006), when the daily production is less than 1% of the total accumulated biogas produced, we can consider it as the end of the production, since the production volume is no longer satisfactory. According to figure 6, the TRA treatment presented HRT of 27 days, TRB presented HRT of 31 days and TRC, 26 days.

The result of the biogas production is presented in a weekly and cumulative format, as shown in Table 3. From these data, we can see that the TRB and TRC treatments had cumulative production greater than TRA; we can also

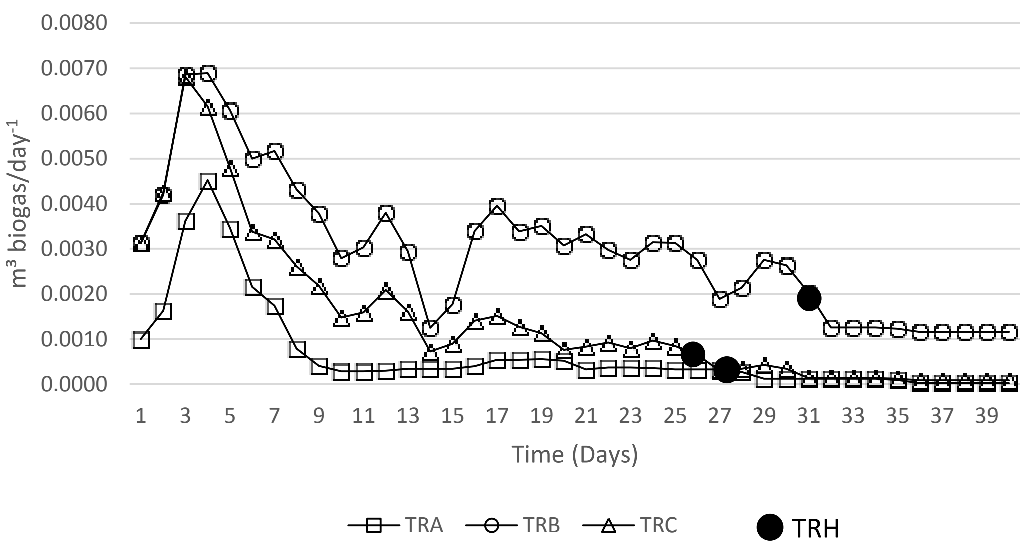

Figure 6. Hydraulic retention time.

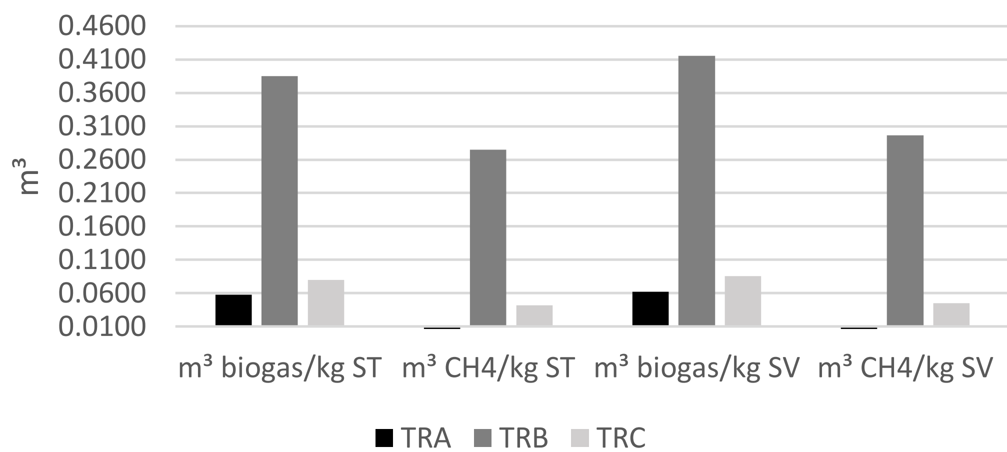

Figure 7. Biogas and methane yields, by ST and SV.

highlight that, at the end of the studied period, TRB had a much higher production than the others. In a general way, the period of maximum production of both treatments occurred in the first week, while the treatment that remained stable further until the 9th week was the TRB one, as TRC has started to decline at week 3. Moller et al. (2004) studied the ways of separating solid fractions of swine effluents and found production potentials of 0.210 m³ CH_4/kg VS to solid fraction of the waste resulting from centrifugation, 0.247 m³ CH_4/kg VS to solid fraction of the waste resulting from chemical precipitation and 0.506 m³ CH_4/kg VS for the liquid fraction of the waste resulting from centrifugation. In the Figure 7, we have the biogas and methane yields, by ST and SV. We can verify that TRB was superior in all results, reaching an average production of 0.4158 m³ biogas/kg SV and 0.3144 m³ methane/kg SV. According to Baldin (2013), the cutting poultry waste, in solid state, have a biogas production of about 0.36 m3/kg, in dry matter; secondly, we have the swine waste, in liquid state, presenting 0,35 m3/kg, and thirdly, we have livestock waste, with 0.30 m^3/kg.

Conclusion

We can observe that the TRB treatment was the one that presented the best results, both in the biogas production and in the amount of methane produced. The inoculum showed a good result for the production acceleration, showing that it can be used in future researches. The TRA treatment produced a good amount of biogas in the early stage of this study, but had its process practically reduced in the third week. The TRC treatment, with 5% more substrate than TRB, did not present an adequate result, thus, proving that 8% of substrate produces biogas in a satisfactory manner. Thus, it could be concluded that biogas production is feasible, when using cutting poultry by batch process, using 8% substrate, 20% swine inoculum and water. TRB showed that the ground cutting poultry waste gave 0.385 m³/kg TS, showing that this waste can present a great potential for various forms of energy. Testing the biodigestion with 6 and 4% of substrate concentration, in two treatments, is a suggestion for further researches, as well as using 30% inoculum in other treatment, in order to know whether the method used in this study, was actually efficient.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Corn hybrid seed damage as a function of metering device in corn planting

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Seed quality control is becoming increasingly important, since the market is increasingly competitive. The planting operation process should occur in order to avoid mechanical damage, which may affect the seed quality. This study aimed to evaluate the effect of mechanical damage caused by meter mechanisms (mechanical and pneumatic) on physical and physiological quality of corn seeds. The experiment was carried out in a Rhodic Hapludox, Southeast Brazil. A randomized block design with 2 × 3 factorial scheme (metering mechanism x seed shape) was used. The corn variety used was a corn hybrid SYN5R27. The seed samples were collected before and after the planter run, and the seed performance was evaluated by germination, accelerated aging, cold test, emergency in the soil and determination of mechanical damages with iodine. The metering mechanisms (mechanical and pneumatic) did not affect the seed quality, except for the saturated cold test that estimates the germination potential of the seeds under adverse conditions. Due to a better adaptation of the disks, the flat seeds presented less damage.

Key words: Mechanical meter, pneumatic planter, physiological quality, planting performance.

INTRODUCTION

The corn planters available in the Brazilian market have metering devices that do not attend completely to the prerequisites of uniform spatial distribution of seeds. Like some work done, pneumatic meters show superior results compared to the seeder with mechanical meter principle, in lower densities (Tourino et al., 2007). With increasing density, seeding the performance of two machines tend to equalize statistically. This is in accordance with claims Bragachini et al. (2003). According to the authors, the high seeding rate is one factor that reduces the advantage of pneumatic distributors in relation to mechanics. Another problem of uniform spatial distribution is that the passage of the seed through the metering mechanism decreases the percentage of germination of the corn seed and decrease the plants uniformity (Garcia et al., 2011).

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According to Silva et al. (1985), the ability to provide low level of damage to seeds during the seeding process is one of the most important features of a planter, seed size and shape are important factors that influence the occurrence of mechanical injuries in seeds. Seed shapes are classified into rounded, flat and size in sieves of different meshes (Von Pinho et al., 1995). However, little attention has been given to the meter mechanism as damaging agent, even knowing that when passing through this mechanism, the seeds are under pressure making them susceptible to mechanical damage that reduce germination and vigor (Carvalho and Nakagawa, 2000).

Mechanical damages can destroy essential seed structures, increase susceptibility to microorganisms and sensitivity to fungicides, besides reducing germination, vigor, storage potential and field performance. (Carvalho and Nakagawa, 2000). Quality control programs have always been important, since the seed market is more and more competitive. Identifying the intensity and possible consequences of deteriorative processes related to the loss of physiological capacity of the seeds by combining the results of germination and vigor, tests can be an alternative for the evaluation of such issues (Marcos Filho, 2005). This study aimed to evaluate the effect of mechanical damage caused by meter mechanisms on physical and physiological quality of corn seeds.

MATERIALS AND METHODS

The experiment was carried out in Rhodic Hapludox, next to the geodetic coordinates 21°14' S and 48°17' W, with an average elevation of 595 m. The experimental design was the 2 × 3 factorial scheme, with four replications. The treatments were two metering mechanisms (mechanical and pneumatic), common in precision planters, and three corn seed shapes, classified into a flat sieve (20F), which the seed passing through the sieve presented 5.16 mm diameter, round (20R), which seeds were retained on the 6.35 mm sieve; and thick (20T), which seeds were retained on 5.16 mm sieve. The corn variety used was a corn hybrid SYN5R27. For the planting operation, we used two precision planters: a pneumatic planter, COP Suprema 7/4 model, and a mechanical planter, PST² model, both with double discs for fertilizer and seed distribution. The characteristics of each metering mechanism to seed distribution are present in the Table 1.

The planter was equipped with 4 planting rows and in each of them we placed plastic bags in the conductor to collect the seeds. The corn evaluations were:

Moisture degree

This was determined before the installation of the tests, by the oven method at 105°C (± 3°C) for 24 h in two samples. The results were expressed as average percentage (wet basis) per sample.

Germination test

This was conducted with four replications of 50 seeds, in moistened paper towel rolls with an amount of water equivalent to 2.5 times the paper mass at 25°C for seven days.

The readings were conducted in accordance with the rules for seed analysis (Brasil, 2009) and the results were expressed as a mean percentage of normal seedlings for each sample.

Accelerated aging

The method used was the mini-chambers, and then the seeds were distributed in a single layer on a suspended screen inside a plastic box (11 × 11 × 3 cm), containing 40 ml of water. The seeds remained incubated for 96 h at 41°C (AOSA, 1983). After this period, we conducted the germination test and were considered the percentage of normal seedlings on the fifth day after planting.

Saturated cold test

Four samples of 50 seeds were sown in substrate composed of sand and soil mixture in 3:1 ratio in trays, placing the embryo upside down, pressing gently to make it parallel to the soil surface, so that the substrate involves the entire embryo, and preventing contact with the oxygen. The trays were placed in a cold chamber at 10°C for 4 days, and then, in the incubator for 3 days at 25°C, the aerial part that determined the percentage of normal seedlings was analyzed (Caseiro and Marcos Filho, 2000). Emergency in the soil: the seeds were exposed to conditions of temperature and substrate similar to those expected at the time of sowing in the greenhouse. A layer about 5 cm thick of soil was put in plastic boxes, where the seeds were placed and also covered with a layer of 1 to 2 cm of soil. It was evaluated after 7 days, and then we analyzed the total of normal seedlings emerged according to the rules for seed Analysis (Brasil, 2009).

Test to determine mechanical damage with iodine

The seeds were placed in boxes, added 150 ml of water and 10 ml of 2% iodine. Within 5 min the seeds as slight damage were evaluated (endosperm) and deep damage (embryo) (Marcos Filho et al., 1987).

Statistical analysis

The statistical programs used were the Sisvar (Ferreira, 2011) and Assistat, resulting in analysis of variance by F test of Snedecor and, when significant, we applied the Tukey test at 5% of probability ($p < 0.05$).

RESULTS AND DISCUSSION

The water content in the corn seeds (Table 2) showed very close behavior, being 0.4% the biggest difference, so it did not affect the study. The pneumatic and mechanical meter mechanisms did not affect significantly the physiological quality of the seeds (Table 3), when these were evaluated by the germination and accelerated aging tests. However, when these mechanisms were compared by the cold test, the vigor decreased when using the mechanical meter mechanism. These results agree with those obtained by Wortman and Rinke (1951), which noted efficiency of the cold test to stratify seed lots

Table 1. Characteristics of the metering mechanism for seed distribution.

Metering characteristics	Pneumatic	Mechanical
Disc type	Vertical	Horizontal
Holes to seed distribution	32	28
Hole diameter (mm)	5	12 15

Table 2. Water content of the corn seeds.

Meter Mechanisms	Seed shape	Water content (%)
Pneumatic	Thick	10.2
	Flat	10.4
	Round	10.2
Mechanical	Thick	10.0
	Flat	10.2
	Round	10.0

Table 3. Germination accelerated aging and saturated cold tests of different seed shapes after passing through the meter mechanisms.

Treatments	Germination	Cold Test	Accelerated aging
	---------------------------- (%)	--------------------------------	
Meter mechanisms (M)			
Pneumatic	98.60	94.92^{a}	98.42
Mechanical	98.60	93.08^{b}	98.50
Seeds (SE)			
Thick	98.40	95.25^{a}	98.50^{ab}
Flat	99.12	94.00^{ab}	98.50^{a}
Round	98.25	92.75^{b}	97.50^{b}
F Test			
M	0.00^{NS}	6.368*	0.027^{NS}
SE	1.99^{NS}	3.947*	4.503*
MxSE	1.80^{NS}	1.474^{NS}	1.785^{NS}
CV (%)	0.96	1.89	1.27

Means followed by different letters in the column differ by Tukey test at 5% probability. CV: coefficient of variation.

with different levels of mechanical damage, Borba et al. (1994) noted in the seeds of maize hybrid BR 201, a reduction in vigor, in an immediate and significant way with the increase in mechanical damage. The germination test was conducted under conditions considered as optimal environment and therefore must provide the theoretically maximum germination that may be expected in certain sample. This information is very important because it sets limits to the performance of the seed lot after planting (Marcos Filho, 2005). For the three seed sizes (thick, flat and round), after passing through the pneumatic and mechanical meter mechanisms (Table 4), there was a lower performance for round seed. In studies developed with corn seeds, there was a trend of

Table 4. Emergence test in soil and mechanical damage (slight and deep) according to the meter mechanisms and seed shapes.

Treatments	Emergence in the soil	Slight damage	Deep damage
	------------------------------ (%)	----------------------------------	
Meter mechanisms (M)			
Pneumatic	98.50	8.29	1.80
Mechanical	98.08	10.00	2.70
Seeds (SE)			
Thick	98.62	9.38^{b}	1.63^{ab}
Flat	98.08	1.69^{a}	0.75^{a}
Round	97.25	16.38^{c}	4.37^{b}
F test			
S	2.19^{NS}	1.53^{NS}	0.87^{NS}
SE	14.28**	37.80*	4.98*
MxSE	5.35*	1.03*	0.66^{NS}
CV (%)	0.70	36.95	106.55

Means followed by different letters in the column differ by Tukey test at 5% probability. CV: coefficient of variation.

Table 5. Interaction between treatments for Emergence in the soil.

Emergency in the soil (%)		Seed characteristics		
		Thick	Flat	Round
Meter mechanisms	Pneumatic	98.25^{Ab}	99.25^{Aa}	98.00^{Aa}
	Mechanical	99.00^{Aa}	98.75^{Ab}	96.50^{Ba}

Means followed by different lowercase letters in columns and uppercase letter in the rows differ by Tukey test at 1% probability.

Table 6. Interaction between treatments for slight mechanical damages.

Slight mechanical damages (%)		Seed characteristics		
		Thick	Flat	Round
Meter mechanisms	Pneumatic	9.12^{Ba}	1.62^{Aa}	14.12^{Ba}
	Mechanical	9.62^{Ba}	1.75^{Aa}	18.62^{Ca}

Means followed by different lowercase letters in columns and uppercase letter in the rows differ by Tukey test at 1% probability.

round seeds presenting higher incidence of mechanical damage than the flat seeds. In the round seeds, the embryonic axis occupy a very exposed position facilitating the damage (Menezes et al., 2002).

For the planter with pneumatic mechanism, the seed size did not influence the emergence(Table 5), however, for the mechanical meter the round seed obtained the worst result, probably due to the largest damages presented (Table 6). This type of seed did not present difference for the two distribution mechanisms tested. The seed emergence was better in the mechanical meter in relation to the pneumatic in thick seed, but with the flat seed, the opposite occurred. It can be explained by the greatest requirement for air suction to hold the thick seed. The flat seeds presented better results when compared to others, lower than 2%. For the pneumatic meter mechanism there was no difference between thick and the round shape, however, in the mechanical meter the round seed had higher amount of slight damages in relation to the thick and flat seed. George et al. (2003) also observed a higher percentage of injuries in the pericarp observed to round seeds when compared to flat seeds.

The deep damages were not affected by the mechanical and pneumatic meter (less than 3%). However, the flat seed shape presented less deep damage than the round shape. The thick seed was in the middle position.

Conclusion

The meter mechanisms (mechanical and pneumatic) did not affect the seed quality, except for the saturated cold test that estimates the germination potential of the seeds under adverse conditions. Due to a better adaptation of the disks, the flat seed presented less damage.

Conflict of interest

The authors have not declares any conflict of interest

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Full Length Research Paper

Genetic divergence between soybean cultivars grown in the Cerrado in Southwestern Piauí, Brazil

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Knowing plant traits and their contribution to genetic diversity is key to identify favorable genotypes and breed new cultivars. This article aims to describe and quantify the genetic divergence between soybean cultivars grown in the Cerrado in southwestern Piauí, Brazil, and identify favorable genotypes for hybridization. To do this, 13 soybean cultivars were grown in the region and assessed concerning vegetative and reproductive performance traits. Randomized block design was adopted, with four replications. Genetic divergence was determined by using the generalized Mahalanobis distance procedure, the Tocher's optimization method, and the unweighted pair-group method with arithmetic mean (UPGMA). Under the conditions analyzed, the Tocher's method and the UPGMA grouped the cultivars in a similar way. Genetic variability enabled the identification of dissimilar genotypes, and leaf area, thousand-seed weight, and pod length were the traits providing greater contribution. Hybridization between the cultivars Pampeana 10 RR x FT Campo Novo RR, BRS Sambaíba, M9350, M9144 RR, P99R03, or P99R09; between M8766 RR x Pampeana 20 RR, Pampeana 30 RR or P99R09; and between P99R09 x Pampeana 20 RR or Pampeana 30 RR showed to be favorable in order to obtain segregating populations with higher variability.

Key words: *Glycine max* (L.) Merril, agronomic performance, cluster analysis, parental selection.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) stands out in the global agribusiness, due to the relevance of its products and the possibility of cultivation in various environments (Rocha et al., 2012). The significant growth of soybean production in Brazil is linked to advances related to the creation of cultivars adapted to different regions and the availability of new production technologies (Freitas, 2011) which enabled increasing the productivity and cultivation area, especially in low-latitude regions. On the world scene, Brazil stands out as the second largest soybean producer, with 99 million tons (t) for the 2015/16 harvest, behind only the United States (106.9 million tons) and

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Table 1. Chemical characteristics of the experimental area after correction (0-20 cm) before the test facility.

pH	P	K^+	H+AL^{3+}	Al^{3+}	Ca^{2+}	Mg^{2+}	SB	CEC	V	M	O.M
H_2O	mg dm^{-3}				cmolcdm^{-3}				%		g kg^{-1}
6	6.9	40	1.73	0	2.8	1	3.9	5.6	69.0	0	1.6

SB, Sum of bases; CEC, cation exchange capacity; V, base saturation; O.M, organic matter.

ahead of countries such as Argentina (56.5 million tons) and China (11.8 million tons), its main competitors (USDA, 2016). This is a plant species of great economic interest, because it occupies a prominent place in the food industry, due to its high content of protein (around 40%), for animal feed, and oil (around 20%), for human consumption (Paiva et al., 2006; Sá, 2006; Klahold et al., 2006), and it is also the main source of raw materials to produce biodiesel (Goldemberg et al., 2008).

According to Behling et al. (2009), improved cultivars with genes capable of expressing higher productivity, better adaptation, and great resistance or tolerance to adverse factors represent the most significant contribution to efficiency in the productive sector. Plant improvement is associated with the existence of genetic variability, genotype selection and adjustment to the environment, and progress depends on the ability to determine selection criteria to promote the desired changes (Reis et al., 2004). Genetic variability in soybean breeding programs, in order to enable the emergence of new combinations by broadening the genetic basis of this crop, is crucial to meet new demands, such as increasing yield and quality (Costa et al., 2004). Much of the genetic variability in this crop is preserved and kept in germplasm banks in many eastern and western countries. In Brazil, several studies have been conducted to verify the existence of genetic variability in this crop (Priolli et al., 2004; Almeida et al., 2011; Rigon et al., 2012; Val et al., 2014). To determine the genetic divergence of a population, a number of multivariate methods may be applied (Clemente; Cahoon, 2009), such as the Tocher's optimization method, the hierarchical unweighted pair-group method with arithmetic mean (UPGMA), and dendrograms.

Knowing the genetic divergence between potential parents is important for hybridization purposes, as this enables the identification of genotypes that, when crossbred, provide greater heterotic effect on progeny, increasing chances to obtain better genotypes in yield and yield stability in segregating generations (Nunes et al., 2011). Addressing this, at a population level, involves agronomic and morphological variables and multivariate biometric techniques, gathering pieces of information from a set of traits. When this is undertaken through cluster analysis, potential parents are grouped according to classification criteria, so that there is homogeneity within the same group and heterogeneity between the various groups (Cruz et al., 2014). Knowing the interrelationship between plant traits has proven to be crucial, as it provides significant information for the selection process (Nogueira et al., 2012). The Cerrado in southwestern Piauí, Brazil, is a part of the last agricultural frontier in the country, named as "MAPITOBA" (on the border of the states of Maranhão, Piauí, Tocantins, and Bahia), and it has favorable conditions for producing grains (Freitas, 2011). However, there are rare scientific information and technical data on agronomic performance and adaptation of soybean cultivars in this region, as well as those concerning the identification of favorable genotypes to breed new cultivars adapted to local conditions. Thus, this article aims to describe and quantify the genetic divergence between soybean cultivars grown in the Cerrado in southwestern Piauí and identify favorable genotypes for hybridization.

MATERIALS AND METHODS

Experimental location and climatic conditions

The experiment was conducted in 2014 and 2015 at São João Farm, in Serra do Pirajá, Currais, Piauí, Brazil. The municipality is located in the center-southern portion of the state, in the mesoregion "SudoestePiauiense" and the geographic microregion "Alto MédioGurgueia," between the geographical coordinates 44° 18' W and 45°05' W longitude and 8' 26' S' and 9' 02" S latitude. It has an average annual temperature of 29°C and rainfall of 944.4 mm (IBGE, 2001) with a frequent occurrence of prolonged Indian summer weather (Andrade Júnior et al., 2009). The soil in the experimental area is classified as sandy. Its chemical features after correction is shown in Table 1.

Experimental establishment and management

The experiment was carried out using randomized block design, with 13 treatments and 4 replications. The treatments were represented by the cultivars (Table 2). The experimental plots consisted of nine 5-m-long rows, spaced at 0.50 m. For data collection, the 2 peripheral rows and 0.50 m in the ends were disregarded, they were seen as borders. The total experimental area was 1.300 m^2. Sowing was done manually on December 13, 2014. Fifteen days after emergence; plant thinning was carried out. Management and cultural practices during the cycle were uniformly conducted in all treatments, according to the guidelines for a soybean crop in Cerrado regions (Arantes and Souza, 1993).

Variables determined

In the phenological stage R_6 (full grain) (Fehr and Caviness, 1977),

Table 2. Relationship of soybean cultivars in the Cerrado of Piauí southwestern Currais-PI.

Cultivars	Company	Group maturation	Type growth
Pampeana 30 RR	Pampeana	10.0	Undetermined
Pampeana 20 RR	Pampeana	9.6	Undetermined
BRS Carnaúba	Embrapa	9.6	Determined
Pampeana 10 RR	Pampeana	9.4	Undetermined
BRS 333 RR	Embrapa	9.4	Determined
FT Paragominas RR	FT Sementes	9.3	Determined
M9350	Monsoy	9.3	Determined
BRS Sambaíba	Embrapa	9.1	Determined
M 9144RR	Monsoy	9.1	Determined
P99R 03	Pioneer	9.0	Determined
P99R 09	Pioneer	9.0	Determined
FT Campo Novo RR	FT Sementes	8.7	Determined
M 8766 RR	Monsoy	8.7	Determined

Table 3. Variance analysis (F values) for plant height (HP), plant branching (PB), leaf area (LA), shoot dry weight (SDW), number of pods per plant (NPP), average number of grains per pod (ANGP), pod length (PL), thousand-seed weight (TSW); and productivity (PROD) among soybean cultivars in the Cerrado of Piauí southwestern Currais-PI.

Source/ variation	GL	Mean square								
		HP	PB	LA	SDW	NPP	ANGP	PL	TSW	PROD
Blocos	3	41.39	158.41	115429.09	35.86	53.79	0.11	1.70	62.78	122094.20
Cultivars	12	314.95**	72.19**	1368287.92**	293.74**	194.09**	0.21**	21.33**	683.72**	241461.65**
Resíduo	36	105.70	10.35	204691.04	53.65	54.39	0.04	2.05	46.36	50275.37

**Significant at 1%.

there was an assessment, with 5 replications per plot, concerning these traits: plant height (PH) - shoot length from the ground level to the main stem apex; plant branching (PB) - quantifying the number of branches on the main stem; leaf area (LA) - determined with the aid of the equipment LI-3100 Area Meter (LI-COR, Inc. Lincoln, NE, USA); and shoot dry weight (SDW) - weighing the dry weight after drying in an oven, at 65°C, until reaching constant weight. After harvesting, done by hand in May, there was an assessment of: number of pods per plant (NPP) - quantifying the number of pods formed in the whole plant; average number of grains per pod (ANGP) - by counting; pod length (PL) - average length of 30 pods per plant; thousand seed weight (TSW) - following the methodology proposed by Brasil (2009); and productivity (PROD) – calculated through the average production in the plot converted into kg ha^{-1} (corrected for humidity of 13% L.H.).

Statistical analysis

Data underwent the normality test, as well as homogeneity and analysis of variance concerning all traits and subsequent grouping of average values according to the Scott-Knott test (Bhering et al., 2008); then, multivariate analysis of variance was conducted. Through the matrix of phenotypic mean values of the traits for each lineage and the matrix of residual variance-covariance, generalized Mahalanobis' distances (D^2_{ij}) were calculated, as described by Cruz et al. (2014). The cluster analysis was performed by using the D^2_{ij} matrix between cultivars, resorting to the Tocher's optimization method and the hierarchical UPGMA, and the identification of the relative contribution of each trait to genetic divergence was based on Singh's criteria (Cruz et al., 2014). All analyses were processed through the software *GENES* (Cruz, 2008).

RESULTS AND DISCUSSION

The summary of the analysis of variance for the nine traits assessed is shown in Table 3. The results demonstrate the existence of significant differences between the mean values of cultivars for all traits assessed through the F-test, suggesting the existence of genetic variability between populations. Regarding plant height, the cultivars were gathered into two groups, according to the Scott-Knott test ($p \leq 0.05$) (Table 4). The highest average heights were observed in the cultivars Pampeana 10 RR (84.7 cm), Pampeana 30 RR (76.29 cm), and Pampeana 20 RR (72.12 cm), the others showed average values ranging from 54.10 cm (M9144 RR) to 63.65 cm (M8766 RR), not differing from each other. This result may be assigned to the type of crop growth, since the greatest height was observed in the Pampeana cultivar group that had indeterminate growth, while the others had determinate growth although, this may be influenced by crop management and weather

Table 4. Average values of 9 traits out of 13 soybean cultivars in the Cerrado in southwestern Piauí.

Cultivars	Character								
	HP	PB	LA	SDW	NPP	ANGP	PL	TSW	PROD
Pampeana 10 RR	84.70[a]	14.05[b]	2510.42[a]	42.44[a]	49.80[a]	2.52[a]	42.72[a]	109.63[c]	888.1[c]
Pampeana 20 RR	72.12[a]	18.01[a]	2467.63[a]	41.22[a]	49.80[a]	1.65[c]	39.11[b]	108.19[c]	920.01[c]
Pampeana 30 RR	76.29[a]	12.00[b]	1611.16[b]	40.66[a]	41.80[a]	2.05[b]	38.08[c]	109.15[c]	1138.79[b]
BRS Carnaúba	62.22[b]	9.10[c]	1175.10[b]	32.72[b]	33.35[b]	2.02[b]	36.67[c]	115.68[b]	1630.67[a]
BRS Sambaíba	62.20[b]	4.60[c]	1027.02[b]	19.61[c]	28.25[b]	1.85[c]	37.46[c]	129.11[b]	1608.25[a]
BRS 333 RR	57.85[b]	5.50[c]	1000.91[b]	20.94[c]	35.85[b]	2.13[b]	41.24[a]	102.01[d]	1206.97[b]
FT Campo Novo RR	56.90[b]	4.60[c]	929.02[b]	21.91[c]	38.60[b]	2.09[b]	36.40[c]	97.50[d]	1297.03[b]
FT Paragominas RR	60.69[b]	9.00[c]	1585.77[b]	32.50[b]	32.65[b]	1.96[c]	39.36[b]	114.28[c]	1353.89[a]
P99R03	57.99[b]	5.60[c]	849.21[b]	19.54[c]	41.70[a]	1.81[c]	38.58[c]	124.29[b]	1397.86[a]
P99R09	59.05[b]	4.50[c]	1125.84[b]	29.91[b]	37.45[b]	1.95[c]	41.42[a]	143.66[a]	1569.31[a]
M9350	57.85[b]	7.40[c]	776.03[b]	26.27[c]	33.10[b]	1.70[c]	34.31[d]	108.82[c]	1591.25[a]
M9144RR	54.10[b]	4.85[c]	782.55[b]	23.94[c]	32.50[b]	1.85[c]	37.71[c]	120.76[b]	1306.22[b]
M8766RR	63.65[b]	6.00[c]	1422.47[b]	21.00[c]	46.60[a]	1.70[c]	40.06[b]	97.58d	1224.73[b]
General average	63.51	8.09	1327.93	28.66	38.57	1.94	38.70	113.90	1317.93
Fc	2.97*	6.97**	6.68**	5.47**	3.56*	4.89**	10.37**	14.74**	4.80*
CV(%)	16.18	39.75	34.06	25.54	19.11	10.75	3.70	5.97	17.01

HP, Plant height; PB, plant branching; LA, leaf area (cm^2); SDW, shoot dry weight (g); NPP, number of pods per plant; ANGP, Average number of grains per pod; PL, pod length (mm); TSW, thousand-seed weight (g); PROD, productivity (kg ha^{-1}); FC, estimate F Snedecor. **Significant at 1%; *significant at 5%. CV (%), Coefficient of variation. Means followed by the same letter in column belong to the same group by Scott-Knott test at 5% significance.

factors.

According to Rocha et al. (2012), plant height is a critical trait in determining the cultivar variety to be introduced in a region, since it is related to grain yield, weed control and losses during mechanical harvesting. This trait is related with productivity and the operational efficiency of harvesters, where soybean is expected to achieve heights between 60.0 cm and 110.0 cm (Shigihara and Hamawaki, 2005). Thus, the three Pampeana cultivars, the BRS Carnauba and Sambaíba, FT Paragominas RR, and M8766 RR fit the desired height range. Taller plants may favor lodging, as they have thinner stems (Guimarães et al., 2008), which is a fact that was not observed in this experiment.

Observing the distribution of mean values for traits and their respective significances, a correlation structure was suggested between plant height and the group of variables shoot dry weight, leaf area and plant branches, since the cultivars Pampeana 10 RR, 20 RR, and 30 RR stood out, showing the highest overall average values when compared to the other cultivars concerning these traits (Table 4). Thus, it is assumed that they have the highest potential for growing plant shoots, a significant trait for the growth of the photosynthetic apparatus and vegetative structures. However, larger shoots are not always turned into higher productivity, thus attention and preference must be devoted to crops more efficient in converting photoassimilates to the reproductive organs (Gubiani, 2005). So, according to Sediyama et al. (1985), combining these cultivars with those showing higher contrast, the trait concerned might be convenient, in order to obtain segregating populations with the heterotic effect and enabling the selection of better genotypes.

As for the average number of pods per plant, the cultivars were also grouped into two contrasting groups through the Scott-Knott test, where the cultivars Pampeana 10 RR, 20 RR, and 30 RR, as well as the P99R03 and M8766 RR, showed the highest levels, ranging from 41.7 to 49.8 pods per plant. The other 8 cultivars were grouped to the number of pods, ranging from 28.25 (BRS Sambaíba) to 38.6 (FT Campo Novo RR) per plant. This trait is regarded as the main variable contributing to grain yield in legumes, as it is positively correlated with the production (Santos et al., 2015) the number of grains per pod may influence seed size, its weight, and could be of consequence to the crop yield. Four groups were formed by pod length, with the largest ranging from 41.24 mm (BRS 333 RR) to 42.72 mm (Pampeana 10 RR), and the smallest ones have 34.31 mm, observed in the M9350. The cultivar Pampeana 10 RR had the highest average number of grains per pod (2.52), followed by the group formed by Pampeana 30 RR, BRS Carnaúba, BRS 333 RR, and FT Campo Novo, which ranged from 2.02 to 2.13 grains per pod, and the group of the others, ranged from only 1.65 to 1.96 grains per pod.

Except for Pampeana 10 RR, all others showed grain yield below the average value reported by Santos et al. (2011), when studying the divergence between soybean genotypes grown in irrigated lowland (2.14), by Almeida

Table 5. Estimates of the Mahalanobis distances (D^2_{ij}), maximum and minimum, of soybean cultivars assessed in the Cerrado in southwestern Piauí.

Cultivars	Maximum		Minimum	
	Distances	Cultivars	Distances	Cultivars
Pampeana 10 RR	173.37	M9350	28.87	Pampeana 20 RR
BRS 333 RR	98.84	Pampeana 10 RR	8.71	M 8766 RR
FT Campo Novo RR	134.72	Pampeana 10 RR	10.70	M9350
Pampeana 20 RR	118.02	BRS Sambaíba	20.88	Pampeana 30 RR
Pampeana 30 RR	71.10	P99R09	13.14	FT Paragominas RR
P99R03	143.38	Pampeana 10 RR	3.84	M 9144RR
M 9144RR	149.21	Pampeana 10 RR	3.84	P99R03
FT Paragominas RR	66.91	Pampeana 10 RR	12.82	BRS Carnaúba
BRS Sambaíba	157.85	Pampeana 10 RR	6.18	M 9144RR
M 8766 RR	103.76	Pampeana 10 RR	8.71	BRS 333 RR
M9350	173.37	Pampeana 10 RR	10.70	FT Campo Novo RR
BRS Carnaúba	105.02	Pampeana 10 RR	12.82	FT Paragominas RR
P99R09	130.86	Pampeana 10 RR	20.25	P99R03

et al. (2011) in soybean cultivars grown in the 2005 offseason, in southern Tocantins, Brazil (2.13). It is observed that only the cultivar Pampeana 10 RR showed consistency among the reproductive traits number of pods per plant, pod length, and average number of grains per pod, remaining in the group with the highest average values, something which may be due to the higher vegetative growth observed. Even so, it was not the cultivar showing the highest productivity. Four average value groups were established for thousand-seed weight. The cultivar P99R09 was better, with 143.66 g. The second group consisted of P99R03; M 9144 RR; BRS Sambaíba; and BRS Carnaúba. Group three consisted of the cultivars Pampeana 10 RR, 20 RR, and 30 RR; FT Paragominas RR; and M9350. And the cultivars BRS 333 RR; FT Campo Novo RR; and M8766 RR had the lowest average values for the trait, from 97.50 to 102.01 g. This trait is a determining factor for productivity, since it is related to grain yield and may be used to estimate whether there was good efficiency during the grain filling process, and it also expresses, indirectly, the size of these seeds and their physiological quality.

Regarding productivity, the formation of three contrasting groups is observed. The most productive consisted of P99R03; FT Paragominas RR; BRS Sambaíba; M9350; BRS Carnaúba; and P99R09, with productivity ranging from 1,353.89 to 1,630.67 kg ha^{-1}. The second group had an average productivity from 1,138.79 to 1,306.22 kg ha^{-1} and the least productive group consisted of Pampeana 10 RR and Pampeana 20 RR, with 888.15 and 920.01 kg ha^{-1}, respectively. The overall average productivity was 1,317.93 kg ha^{-1}, it is below that observed by Rocha et al. (2012), that is, 2,670.30 kg ha^{-1}, in 32 soybean genotypes under low latitude conditions, in Teresina, Piauí, Brazil. Productivity is a complex trait and its expression depends on other traits (Nogueira et al., 2012), including climatic factors. When studying populations, it provides a basis to select the most divergent and those with high productivity; it is among the traits prioritized for improvement (Rotili et al., 2012). Considering the multivariate effect, based on the maximum distance between cultivars, it was observed that the cultivar Pampeana 10 RR was the most divergent when compared to 10 cultivars, followed by the contrast between M9350 and Pampeana 10 RR, between BRS Sambaíba and Pampeana 20 RR, and between P99R09 and Pampeana 30 RR. Regarding the least divergent, the most similar pair was P99R03 and M9144 RR (Table 5). The relative contribution of each trait to genetic dissimilarity (Table 6), according to Singh's criteria, shows that the leaf area (17.12%), thousand-seed weight (16.92%), and pod length (16.19%) were the traits that contributed most to genetic divergence, and they must be prioritized for parental selection in improvement programs. The smallest contribution was observed in the number of pods per plant, with 3.82% of the proportion in estimated variation.

Productivity, in turn, contributed little to divergence (7.25%), suggesting genetic similarity among the genotypes for this trait and the need to obtain segregating populations to maintain improvement. The small relative contribution of productivity to genetic dissimilarity, in a study on genetic divergence, was also found by Dotto et al. (2010) with corn, Cabral et al. (2011) with beans, and Santos et al. (2011) with soybean. Genetic diversity between cultivars was found through the formation of contrasting groups by using the UPGMA, grouping in these types of graphs is performed subjectively, by selecting a threshold point on the distance scale (Fuzatto et al., 2002). In this study, the threshold level for grouping genotypes was defined as 70% similarity (Figure 1). The cultivars P99R03 and

Table 6. Contribution of each trait to genetic divergence, through Singh's criteria, among soybean cultivars under analysis in the Cerrado in southwestern Piauí.

Feature	Contribution to divergence (%)
Number of pods/plant	3.82
Pod length (cm)	16.19
Productivity (kgha^{-1})	7.25
Thousand-seed weight (g)	16.92
Plant height (cm)	7.59
Average number of grains per pod	6.89
Plant branching	12.74
Shoot dry weight (g)	11.45
Leaf area (cm^2)	17.12

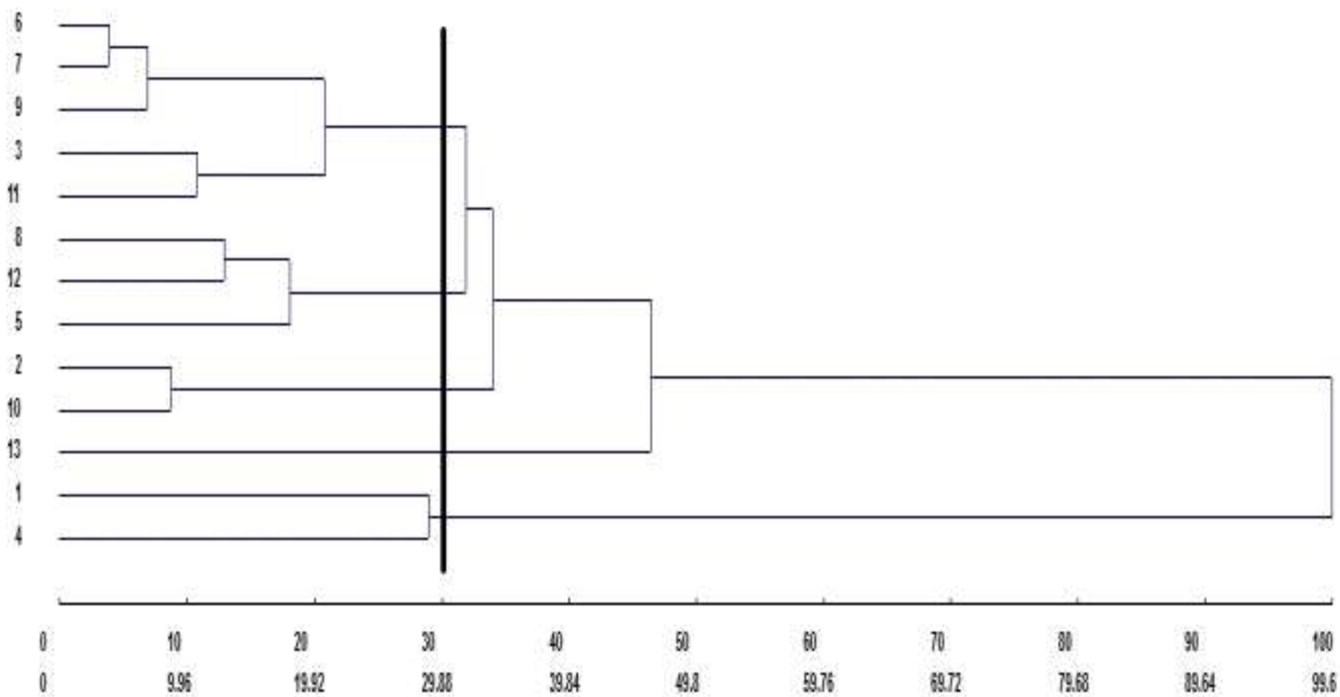

Figure 1. Dendrogram based on the UPGMA, calculated from the cophenetic correlation coefficient, or be, obtained through generalized Mahalanobis distances in 13 soybean cultivars assessed in the Cerrado in southwestern Piauí.
Cultivars: 1 – Pampeana 10 RR; 2 – BRS 333 RR; 3 – FT Campo Novo RR; 4 – Pampeana 20 RR; 5 – Pampeana 30 RR; 6 – P99R03; 7 – M9144RR; 8 – FT Paragominas RR; 9 – BRS Sambaíba; 10 – M8766RR; 11 – M9350; 12 – BRS Carnaúba e 13 – P99R09.

M9144RR were those with the shortest distance (D^2 = 3.85), in turn, the longest distance from the other cultivars was assigned to Pampeana 10 RR and BRS 333 RR (D^2 = 99.6), and 100% dissimilarity is considered for establishing the dendrogram.

These results may be used for hybridization purposes in breeding programs, because such information on these cultivars enable them to be used as sources to achieve better genetic bases.

Through this method, it was possible to observe the formation of two different groups. The first group consisted of the cultivars Pampeana 10 RR and 20 RR, a result which may have been influenced by the fact that these cultivars showed the highest values for the leaf area (2,510.42 cm^2 and 2,467.63 cm^2, respectively) (Table 4), which was the most significant trait to distinguish cultivars (Table 6); the second group consisted of the cultivar P99R09, which had the highest average value for the trait thousand-seed weight; the third group was formed by the cultivars BRS 333 RR and M8766 RR; the fourth group consisted of the cultivars Pampeana 30 RR, BRS Carnaúba, and FT Paragominas

Table 7. Cluster of 13 soybean cultivars assessed in the Cerrado in southwestern Piauí.

Groups	Genotypes	Average distance	
		Intragroup	Intergroup
I	P99R03, M 9144 RR, BRS Sambaíba, M9350, BRS Carnaúba, FT Campo Novo RR, FT Paragominas RR, BRS 333 RR	20.09	DI. II = 64.91; DI. III = 128.66; DI. IV = 35.78; DI. V = 40. 90
II	Pampeana 20 RR, Pampeana 30 RR	20. 88	DII. III = 33.22; DII. IV = 50.98; DII. V = 89.81
III	Pampeana 10 RR	-	DIII. IV = 103.77; DIII. V = 130.87
IV	M 8766 RR	-	DIV. V = 64.61
V	P99R09	-	

RR; and the fifth group was formed by the other cultivars. The Tocher's optimization technique distinguished the cultivars into 5 groups contrasting with each other (Table 7). The first group consisted of the cultivars P99R03, M 9144 RR, BRS Sambaíba, M9350, BRS Carnaúba, FT Campo Novo RR, FT Paragominas RR, and BRS 333 RR, representing around 61% of the cultivars under analysis. The second group consisted of 2 cultivars (Pampeana 20 RR and Pampeana 30 RR). Groups III, IV, and V consisted of only 1 cultivar each: Pampeana 10 RR, M 8766 RR, and P99R09, respectively.

According to Peluzio et al. (2014), the formation of groups is of great importance for parental selection, because the new hybrid combinations to be formed must be based on the magnitude of their dissimilarity and parental potential. By relating the Tocher's optimization method and the UPGMA, there was a concordance between them, and 5 groups show a similar constitution. The most dissimilar soybean cultivars are combined in rather distant groups; they may be regarded as favorable in artificial crossbreeding (Oliveira et al., 2005). However, in addition to dissimilarity, it is necessary that the parents correlate high average values for the other desired agronomic traits (Nunes et al., 2011; Peluzio et al., 2014). Thus, the distance between the cultivar Pampeana 10 RR and the others suggests that it may have a high heterotic effect after crossbreeding with another plant variety. As reported by Asmus (2008), the best hybrid combinations to be tested in an improvement program must involve divergent parents with a high performance. Thus, considering the group of cultivars under analysis, in principle, the first intersection recommended is between the cultivars Pampeana 10 RR x M9350, since the greatest Mahalanobis distance was found between them, also we also observe its allocation in various groups by using the Tocher's method (Table 7) and the UPGMA (Figure 1).

The cultivar Pampeana 10 RR may be crossbred with the cultivar M8766 RR, belonging to group IV, due to the magnitude of the Mahalanobis distance (Table 5). Given the magnitude of the Mahalanobis distances (Table 5), the cultivar Pampeana 10 RR may also be crossbred with the cultivars M9350, BRS Sambaíba, M9144 RR, and P99R03, which, in turn, belong to group I, formed by the Tocher's method. These cultivars have lower pod number and length, plant height, average number of grains per pod and branches, dry weight of shoots and leaf area when compared to the cultivar Pampeana 10 RR, but they are more productive, especially the cultivars BRS Sambaíba and P99R03 (Table 4). According to Cruz et al. (2014), the establishment of groups with homogeneous genotypes within and heterogeneous between the groups is the starting point for a careful evaluation of them, aiming at their use in improvement programs. Indeed, the identification of better genotypes was effective, for each trait under analysis, as well as the identification and recommendation of the favorable crossbreeding between cultivars. In this way, the following hybridizations are predicted as favorable: Pampeana 10 RR x FT Campo Novo RR; Pampeana 10 RR x P99R03; Pampeana 10 RR x M9144 RR; Pampeana 10 RR x BRS Sambaíba; Pampeana 10 RR x M9350; Pampeana 10 RR x P99R09; M8766 RR x Pampeana 20 RR; M8766 RR x Pampeana 30 RR; M8766 RR x P99R09; P99R09 x Pampeana 20 RR; and P99R09 x Pampeana 30 RR.

Conclusion

1. There was a partial concordance regarding the formation of groups with similarity in their constitution through the parental optimization method provided by Tocher and the UPGMA.
2. The traits that most contributed to the divergence were leaf area (17.12%), thousand-seed weight (16.92%), and pod length (16.19%).
3. Genetic variability enabled the identification of dissimilar cultivars and those with high average values for the traits under analysis.
4. The hybridizations Pampeana 10 RR x FT Campo Novo RR; Pampeana 10 RR x P99R03; Pampeana 10 RR x M9144 RR; Pampeana 10 RR x BRS Sambaíba; Pampeana 10 RR x M9350; Pampeana 10 RR x P99R09;

M8766 RR x Pampeana 20 RR; M8766 RR x Pampeana 30 RR; M8766 RR x P99R09; P99R09 x Pampeana 20 RR; and P99R09 x Pampeana 30 RR are favorable to obtain segregating populations with higher variability.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Comparison of means of agricultural experimentation data through different tests using the software Assistat

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In the analysis of variance, the comparison of means is essential when the calculated F is significant at 0.05 or 0.01 probability level and there are more than two treatments, because the significant F only rejects the null hypothesis, according to which the treatments or samples do not differ statistically. This study was aimed at to evaluate the similarities and differences between the classifications of means of Tukey, SNK, Scott-Knott and Duncan tests, as well as to demonstrate the performance of the software Assistat in the analysis of experimental data of the agricultural research. Data of agricultural experiments were analyzed using the models of the analysis of variance (ANOVA), as completely randomized and randomized block experiments. It was concluded that the Tukey test provides more-detailed results in comparison to the tests of Duncan, Scott-Knott and SNK, but not very different, and it is the most used test. The tests of Duncan, Scott-Knott and SNK tend to show similar results, except for the fact that, in the Scott-Knott test, no mean can belong to more than one group. The tests of Duncan and SNK, for being similar, except for the utilized distribution, almost always show the same results.

Key words: Assistat, Tukey test, Duncan test, Scott-Knott test, SNK test.

INTRODUCTION

For the comparison of means, according to Zimmermann (2004), currently there are more than ten tests, but the most common are: t or LSD, Tukey or HSD, Duncan, Student-Newman-Keuls or SNK, Scheffé and Dunnett. Comparatively, each one of them has advantages and disadvantages and can be used in the comparisons between all pairs of treatments (Tukey, Duncan, SNK and LSD), or between groups of treatments (Scheffé, LSD and Scott-Knott), or between each treatment against one of them (control), which is the case of the Dunnett test. The selection of the test to be used depends solely on the researcher, according to the type of hypothesis formulated. Two of these tests are different; Dunnett's, which compares each one of the means with the control (Zimmermann, 2004), and Scott-Knott's, which is actually a grouping technique that separates the means in different groups; its advantage is that no mean can belong to more than one group. The other tests compare the means and classify them with letters.

Vieira (2006) claims that, in order to answer questions

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Table 1. Data of grain production of irrigated rice, in kilograms/hectare.

Replicates	Treatments			
	1	2	3	4
1	6276	7199	6457	7202
2	6035	6890	6174	7173
3	6086	6586	6612	7169
4	5594	7149	6087	6590
5	6321	6657	5797	6444
6	6746	6210	5865	6740
7	5751	6128	6498	6370
8	6191	6393	6486	7270

Source: Zimmermann (2004), page 54.

comparison of means, but there is no test better than the of the researchers on which is/are the best mean(s) and which is/are different, it is necessary to apply a test of others; all of them have advantages and disadvantages, and it is also worth remembering that the tests of comparison of means must be seen more as indicators of the reality than as exact solutions.

For Santos et al. (2008), the knowledge on the power of the tests is extremely limited and variable, to the point of allowing the selection of procedures with very discrepant characteristics (error rate through experiments or through comparisons). This causes these procedures to lose credibility, since the conclusions can be different according to the procedure employed. In the field of biology, there are also restrictions, because often it is more adequate an estimation procedure than a test of hypothesis, since a difference statistically significant could be depreciable from the biological point of view.

According to Gomes (2009), the application of the Duncan test is more laborious than that of Tukey’s, but more-detailed results are obtained, that is, Duncan’s indicates significant results in cases in which the Tukey test does not allow to obtain statistical significance. As the Tukey test, Duncan’s, for being exact, requires that all treatments have the same number of replicates. Still according to Gomes (2008), the Scheffé test is of more general use compared with Tukey’s and Duncan’s and the Bonferroni test is an improvement over the t-test, which is very good for a small number of contrasts. This study aimed at to evaluate the similarities and differences between the classification of means of the Tukey, SNK, Scott-Knott and Duncan tests, as well as to show the performance of the software Assistat in the analysis of experimental data of the agricultural research.

MATERIALS AND METHODS

The evaluations used data of agricultural experiments found in the literature, for completely randomized and randomized block designs. For the completely randomized design, data of one experiment (Zimmermann, 2004) were used. This experiment tested four forms of application of nitrogen fertilization in irrigated rice, and the response variable was the production, whose data are shown in Table 1. The treatments were the following amounts of fertilizer in kilograms/hectare:

1= 80 at planting;
2 = 40 at planting and 40 at 40 days after emergence (DAE);
3 = 13.2 at planting and 66.8 at 40 DAE; and,
4 = 13.2 at planting and 33.4 at 40 and 60 DAE.

For the randomized block design, data of two experiments were used. In the first one (Campos, 1984), the response variable was the content of copper (in ppm) in sugarcane leaves (Table 2), with eight blocks, testing the following treatments:

A = Leaves with no cleaning;
B = Leaves cleaned with only the passing of an attached brush and vacuum cleaner;
C = Leaves washed with running water and rinsed off in distilled and demineralized water;
D = Leaves washed in diluted detergent solution (at 0.1%), then distilled water, 0.1% N HCL and finally demineralized water; and,
E = Leaves washed in diluted detergent solution (at 0.1%), rinsed off with distilled water to remove the detergent and finally with demineralized water.

The second experiment in randomized blocks (Gomes, 2009) evaluated the competition between potato varieties and the response variable was the production, with eight treatments and four blocks, as shown in Table 3.

The software Assistat (Silva and Azevedo, 2006) was used to evaluate the data. The comparison of means was performed through the tests of Tukey, SNK (Student, Newman and Keuls), Scott-Knott and Duncan, which are according to Gomes (2009), Scott and Knott (1974) and Zimmermann (2004). The software Assistat is available at http://www.assistat.com. The software Assistat was developed by Professor Francisco de A. S. e Silva of the Federal University of Campina Grande, Brazil. This software is distributed free of charge. Figure 1 shows the steps of an analysis, in the results screen you can go back and choose another test of comparison of means.

RESULTS AND DISCUSSION

Table 4 shows the result of the analysis of variance for the three experiments, whose data were shown in Tables

Table 2. Data of the content of copper (ppm) in sugarcane leaves.

Blocks	Treatments				
	A	B	C	D	E
1	11.5	7.7	9.8	10.7	12.0
2	12.7	9.0	8.0	10.8	10.9
3	12.6	9.1	7.4	10.2	10.3
4	12.2	8.6	9.5	9.6	9.8
5	10.4	8.8	8.3	9.8	9.4
6	12.0	8.6	8.9	10.1	9.5
7	12.2	8.4	10.5	11.1	9.7
8	8.5	8.4	10.4	11.5	10.1

Source: Campos (1984), page 66.

Table 3. Data of production in ton/hectare.

Treatments	Block1	Block2	Block3	Block4
Kennebec	9.2	13.4	11.0	9.2
Huinkul	21.1	27.0	26.4	25.7
S. Rafaela	22.6	29.9	24.2	25.1
Buena Vista	15.4	11.9	10.1	12.3
B 25-50 E	12.7	18.0	18.2	17.1
B 1-52	20.0	21.1	20.0	28.0
B 116-51	23.1	24.2	26.4	16.3
B 72-53 A	18.0	24.6	24.0	24.6

Source: Gomes (2009), page 76.

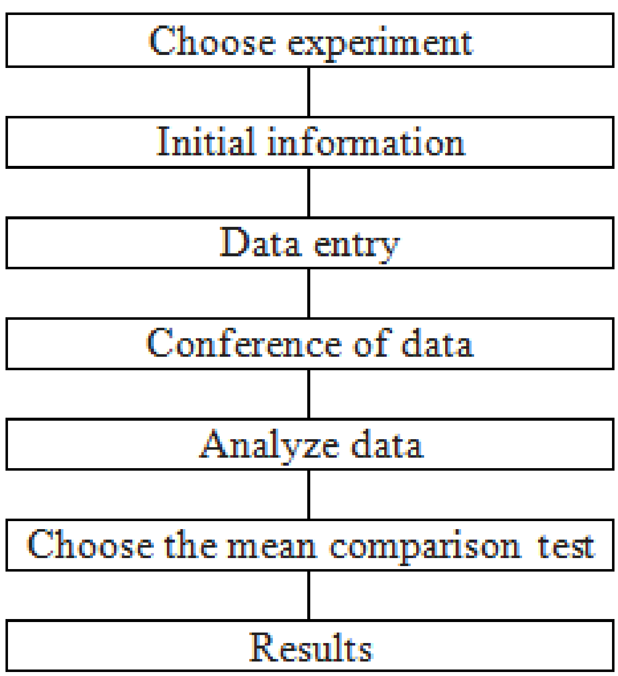

Figure 1. Steps of analysis of variance in the software Assistat.

Table 4. Results of the analysis of variance for the data of the three experiments.

Experiment	Source of variation	Degrees of freedom	Mean square
1 - Zimmermann (2004)	Treatments	3	963873.1250 **
	Residual	28	131497.9821
2 -Campos (1984)	Blocks	5	0.6141 ns
	Treatments	7	10.7919 **
	Residual	28	1.0189
3 - Gomes (2009)	Blocks	3	16.8433 ns
	Treatments	7	131.3886 **
	Residual	21	8.5460

** Significant at 0.01 probability level ($p < 0.01$), * Significant at 0.05 probability level($0.01 \leq p < 0.05$), ns Not significant ($p \geq 0.05$).

Table 5. Comparison of means through four tests for the data of Table 1 at 0.05 probability level.

Treatments	Mean	Tests			
		Tukey	SNK	Scott-Knott	Duncan
1	6125.0000	c	b	b	b
2	6651.5000	ab	a	a	a
3	6247.0000	bc	b	b	b
4	6869.7500	a	a	a	a

Means followed by the same letter in the column do not differ statistically.

Table 6. Comparison of means through four tests for the data of Table 2 at 0.05 probability level.

Treatments	Mean	Tests			
		Tukey	SNK	Scott-Knott	Duncan
1	11.5125	a	a	a	a
2	8.5750	c	c	c	c
3	9.1000	bc	c	c	c
4	10.4750	ab	b	b	b
5	10.2125	ab	b	b	b

Means followed by the same letter in the column do not differ statistically.

1, 2 and 3. The effect of treatment was significant in the three cases, which means that there is a difference between the treatments and that it is necessary to apply a test of comparison of means. For better evaluation of the differences between treatments, the means of the data in Table 1 were compared using four tests of comparison of means, as shown in Table 5. This allows to evaluate the most concordant and discordant ones. It is noted that the tests of SNK, Scott-Knott and Duncan showed identical classifications, but the Tukey test showed a more detailed classification; however, for the means 2, 3 and 4, their classification was the same.

In addition, it is observed that the four tests agreed with respect to the difference existing between the means 1 and 4.The comparison of means for the treatments of the data in Table 2 was also performed through four tests, as shown in Table 6. As in the previous analysis, the classification of means by the tests of SNK, Scott-Knott and Duncan was the same and, again, the Tukey test showed a more detailed classification, demonstrating a pronounced sensitivity to small differences between means, which always occurs with this test. In regard to the means 1 and 2, the four tests agreed on their classification.

Table 7 shows the comparison of means for the data of Table 3. It is observed that the four tests showed the same results for the treatments 1, 2 and 3 and, considering the first letters, they were concordant in the treatments 5, 6, 7 and 8. For the treatment 4, the tests of Tukey and Scott-Knott showed a result different from

Table 7. Comparison of means through four tests for the data of Table 3 at 0.05 probability level.

Treatments	Mean	Tests			
		Tukey	SNK	Scott-Knott	Duncan
1	10.70000	c	c	c	c
2	25.05000	a	a	a	a
3	25.45000	a	a	a	a
4	12.42500	c	bc	c	bc
5	16.50000	bc	b	b	b
6	22.27500	ab	a	a	a
7	22.50000	ab	a	a	a
8	22.80000	ab	a	a	a

Means followed by the same letter in the column do not differ statistically.

those of SNK and Duncan, which were in agreement, as the first two. Once more, the Tukey test was sensitive to the smallest differences, showing a more detailed result.

The classifications of the means of Tables 5, 6 and 7 indicate that, for number of treatments lower than or equal to 8 and well defined differences between them, the tests of SNK, Scott-Knott and Duncan tend to show the same results, and that the Tukey test tends to show results that partially agree with those of the other three, but with a more detailed classification. This more detailed classification of the Tukey test may be due to a somewhat excessive rigor of this test, according to Gomes (2009), and to a higher control of Type I error, reported by Sousa et al. (2012) and Girardi et al. (2009), who observed lower percent rates of this error in comparison with the tests of Duncan and SNK.

In the three tables, it is also noted that the tests of Duncan and SNK have precisely the same results, which was expected, since the only difference between them is basically that the SNK test uses the q distribution of Tukey, whereas Duncan's uses the z distribution (Zimmermann, 2004). Although there is no reason for the Tukey test to be the most used, because definitely there is not a test better than the others, all of them have advantages and disadvantages (Vieira, 2006). In spite of that, it is by far the most used. Twenty articles that used the software Assistat were reviewed and, in sixteen of them, the Tukey test was applied. Therefore, sixteen out of twenty, i.e., 80% used the Tukey test and this is consistent with Caeirão (2006), who observed that, in 103 tests with barley, the Tukey test was used in 79.9% of them.

Conclusions

The Tukey test showed more-detailed results compared with Duncan, Scott-Knott and SNK, but not very different, and it is the most used test. The tests of Duncan, Scott-Knott and SNK tend to show similar results, except for the fact that, in the Scott-Knott test, no mean can belong to more than one group. The tests of Duncan and SNK, for being similar, except for the utilized distribution, almost show the same results.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Soybean yield and productivity components according to spacing and population density

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Soybean has great importance in world economy. Their productivity is influenced by numerous factors like nutrition, pests, diseases, genetic factors, and weather and plant density to name a few, and the spatial distribution of plants per area a resource to increase productivity the aim of this study was to evaluate main items of productivity and soybean yield with different arrangements of plants with spacing (0.25 and 0.50 m), and population density of plants per unit area (351,000, 310,000, 270,000, 229,500 plants per hectare). The experiment was conducted in agricultural year 2013/2014, with cultivar CD 2610 IPRO® with determined type of growth and CD2611 IPRO® with indeterminate type of growth. The experiment design was conducted in a randomized block design with split-plot, containing three repetitions and for statistical analysis, we used Assistat program in sub-divided form. The two varieties tested showed significant results in 0.25 m space between plants with a density of 351,000 plants per hectare.

Key words: Arrangement, *Glycine max,* densities, growth.

INTRODUCTION

Soybean (*Glycine max*) is a legume grown in almost all arable regions. Their large number of varieties adapted for macro and micro regions, leads crops to have diversity in climatic demands, allowing its exploitation in South and North America, Asia, Africa, Europe and Oceania. Its high nutritional value and its exploitation of various products from this legume have favored its exploitation around globe. Brazil is the second largest

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Table 1. Summary of the treatments which were used in the experiment with the cultivar CD 2610 IPRO with determined type of growth.

Treatments	Advised population (%)	Population (plants ha^{-1})	Spacing (cm)
T-1	75	229.500	25
T-2	100	270.000	25
T-3	115	310.500	25
T-4	130	351.000	25
T-5	75	229.500	50
T-6	100	270.000	50
T-7	115	310.500	50
T-8	130	351.000	50

T= CD 2610 IPRO.

producer, exporter and soybean consumer, and annually planted area were 12 million hectares (Conab,2016). The greatest combination with productive potential of crop depends on environmental conditions where plants will be install and how they will develop. Thus, changes related to plant population can reduce or increase productivity gain, because it depends into spacing and density between plants. The plant population is the factor that least affects productivity, since plants were distribute uniformly in the area (Endres, 1996; Luca and Hungria, 2014).The spacing between lines and density among plants can be manipulated in order to establish a more suitable arrangement to obtain higher productivity. According to Procópio et al. (2013), in arrangement, where row spacing is equal to plant spacing along lines, it observed increases in soybean yield. The best use of incident radiation can be main responsible factor for obtaining higher yields in smaller spacing (Reunião, 2012). Soybean plants can provide great flexibility in response to different spatial arrangements, and can adapt to large-scale environmental managements (Heiffig, 2002; Heiffig et al., 2006). The same author obtained for smaller spacing a better and faster lock between lines, providing better control of weeds.

According to Parcianello et al. (2004), reduction in spacing also increases radiation interception, leaf area index and grain yield, being highest number of pods per m^2, this parameter explains increase in grain yield. Ventimiglia et al. (1999), determines higher yield potential and actual yield, which justifies the increase in productivity. Plants distributed inappropriately prevent correct use of available resources such as light, water and nutrients. In soybean cultivation, high plant population at some points can cause development of higher plants, less branched, with less individual production, reduced stem diameter and therefore more prone to lodging (Endres, 1996). On other hand, blank spaces between plants and between lines can facilitate weeds development, not to mention the fact that they can lead to soybean plants establishment with reduced size.

According Pitelli (1985), factors that influence interference degree of a weed plant in crop may related to weed community (specific composition, density and distribution), culture (cultivar, spacing and density) and environment (management practices, soil and climate).The major advantages observed with reduced spacing, compared to commonly used (40 to 50 cm) are verified in delayed sowing using early cultivars (Johnson et al., 1982), and fertility soils at suitable levels (Ventimiglia, 1996).The aim of this study was evaluate main components, and soybean yield productivity, with different plants arrangements of two varieties: one with a determined type of growth (CD 2610 IPRO®) and second with indeterminate growth type (CD 2611 IPRO ®) with spacing (50 and 25 cm) and density of plants per unit area (351.000, 310.500, 270.000, 229.500 plants per hectare).

MATERIALS AND METHODS

The experiment was applied in experimental area of Coodetec (Agricultural Research Central Cooperative) company, Located in Cascavel city, in west Paraná (BR), Brazil, during October 2013 to March 2014, in an area with altitude of 781 m. The soil was classify as typical dystrophic, gentle relief, basalt substrate (Embrapa, 2006). The management of experiment was carried out in accordance with technical recommendations given to their cultivation. Sowing was applied on 26 October 2013. The cultivars used were CD 2610 IPRO® with determined habit of growth and CD 2611 IPRO® with indeterminate habit of growth. The seeds were treated with fungicide (Maxim®) and insecticide (Standak®), all measurements were in accordance with recommendation of manufacturers products. The fertilization was performed along with sowing, using the commercial formula 02-20-20NPK at a dose of 295 kg ha^{-1}. We used experimental design of randomized blocks with split-plots and three repetitions, totaling 48 parcels. Each plot with dimensions of five meters long by two meters wide, with a total area of 10 m^2 per plot.

For cultivar CD 2610 IPRO® with determined type of growth, used treatments were shown in Table 1. For cultivar CD 2611 IPRO® with indeterminate growth, used treatments were shown in Table 2. For treatments density accuracy, were sown with 5% more seeds per plot, and after seedlings emergence, they were thinned out, leaving only exact population for each treatment. For

Table 2. Summary of the treatments which were used in the experiment with the cultivar CD 2611 IPRO with indeterminate type of growth.

Treatments	Advised population (%)	Population (plants ha^{-1})	Spacing (cm)
T-1	75	229.500	25
T-2	100	270.000	25
T-3	115	310.500	25
T-4	130	351.000	25
T-5	75	229.500	50
T-6	100	270.000	50
T-7	115	310.500	50
T-8	130	351.000	50

T= CD 2611 IPRO.

Table 3. Comparative table of cultivar interaction analysis (A) versus spacing (B) versus density (C).

FV	F.Cal.
Interaction AXB	^{ns} Interaction
AXC	177.1105**
Interaction BXC	178.6477**
Interaction AXBXC	50.8978**

^{ns}Non-significant, ** Significant at 1% probability

chemical control of pests and diseases, two systemic insecticide applications from group of pyrethroids (Betacyflutrina and Lambda-cyhalothrin) were apply. A preventive fungicide (Opera®) it was apply for bug's control. Three more applications of systemic fungicide from chemical group Benzimidazole + triazole (Derosal® + Aproach Prima®) using a trailed sprayer with conical nozzles in the flow of 200 L ha^{-1} were applied. During cultivars cycle we assessed blooms days, being on average 42 days after emergence and material full cycle on average 115 days of maturation.

The harvest was held on 8 March, 2014 with a parcel harvester Wintersteiger (100% of the plot was harvested). Following were performed in the laboratory evaluations. The evaluations were: weighing of parcels were carried out, 1000 grain weight, moisture and productivity transformation of plots in sacks h^{-1}. When the f test was significant, the collected data were analyzed in the Assistat Software on Turkey's test program at 5% probability in sub-subdivided form of the harvested yields.

RESULTS AND DISCUSSION

During experiment period (from planting to harvest), rainfall were 727 mm not well distributed causing stress to crop in grain formation stage. Interaction analysis between data, shown no significant interaction between the variety with determined kind of growth and the variety with indeterminate kind of growth versus spacing 0.25 and 0.50 cm (AxB). On the other hand, when comparing the interaction test of the two varieties versus density (AxC) there was significant difference, and when comparing the spacing versus density (BxC), there was significant difference and also when we compared all against all (AxBxC) there was a significant interaction according to Table 3. In the different spatial arrangements, it was possible to see the inherent characteristics of each plant with regard to the architecture and management of the culture. Analyzing the spacing of 25 cm between rows of determined kind of growth, there was a greater regularity among plants in the experiment. In this spacing the plants were better distributed and will compete less for space, water and nutrients, so that they can express their full potential. As negative aspect, there was the formation of a favorable environment for the development of pests and diseases by promoting greater protection to the sun and spraying.

According to Almeida et al. (2005) the lower spacing can cause major problems with diseases such as White Mold (*Sclerotinia sclerotiorum*). According to Embrapa (2000), the cultural weed control consists of management techniques that foster the development of soybeans over the weed and in this case was obtained by canopy closure reached quickly due to the reduced spatial arrangement. Spacings smaller than 40 cm between rows are even better for weed control in the crop. By and large, the spacing of 50 cm had the highest irregularity in the development of the architecture of

Table 4. Interaction spacing between rows and population density of plants per hectare, in the two cultivars, on the productivity.

Density of plants (plants ha^{-1})	Spacing between rows (cm)							
	25				50			
	CD 2610 IPRO		CD 2611 IPRO		2610 IPRO		2611 IPRO	
	Productivity		Productivity		Productivity		Productivity	
	Sc ha^{-1}	*	Sc ha^{-1}	*	Sc ha^{-1}	*	Sc ha^{-1}	*
(100%) 270.000	94.77	C	89.60	B	91.60	B	81.07	C
(115%) 310.500	101.77	B	92.07	B	110.43	A	90.53	A
(130%) 351.000	106.53	A	110.70	A	83.90	C	93.63	A
(85%) 229.500	90.37	D	86.70	D	75.77	D	85.47	B

* The averages followed by the same letter are not statistically different from each other. Tukey test was applied at 5% of error probability. msd: minimum significant difference to lines = 2.8709, CV% Cultivars = 0.71, CV% Spacing = 0.92, CV% Density = 1.37.

plants, because the competition between them was greater. Another important aspect is that the tested cultivars are semi-early and have a smaller branch capacity, then the lines remained open throughout the cycle, favoring the development of weeds. The weeds damage the crop owing to the fact that they compete for sunlight, water and nutrients, hinder the harvesting operation and compromise the quality of grain, depending on the weed species and the infestation level (Embrapa, 2000). On the other hand, a positive aspect was the control of diseases and pests which became more efficient, probably because there was no formation of an ideal microclimate for the development of them. With the smallest gap was associated with the largest number of plants per plot, however, this also raises the level of productivity for the two varieties where statistically the two varieties in the same spacing and the same density (351,000 plants / ha^{-1}) produce equally among each other, according to Table 4.

When compared both cultivars within each spacing, it was observed that for the conventional line spacing (0.50 cm), the cultivar CD 2610 IPRO® was the one which obtained the lowest result of agricultural productivity, while in the reduced spacing (0, 25 cm), the same cultivar CD 2610 IPRO® was the one which most produced in the experiment reaching 110.7 ha^{-1} bags (Table 4).The cultivars CD 2610 IPRO® and CD 2611 IPRO® do not respond when seeded with lower densities than recommended in the case (85%), also comparing with different spacing, as reflected in the soybean plant physiology, this is because their ability to adjust their production components to maintain the level of agricultural productivity of farming in different situations spacing between plants and between rows (Table 4). Comparing the behavior of the cultivar CD 2611 IPRO® in two densities with 310,500 and 351,000 plants per hectare, they are statistically equal among each other in spacing of 0.50 cm between rows (Table 4). Therefore, we can affirm that this cultivar can withstand higher plant population per unit area with reduced spacing without the occurrence of layering, thus having major agricultural productivity.

Conclusion

For the two varieties tested, there was significant results in the spacing of 0.25 cm with 351,000 plants density per ha^{-1}, this spacing and this density provided less competition between plants, resulting in higher productivity. In low density with 85% rating (229,500 plants ha^{-1}) showed lower results in productivity when compared to the others.

Conflict of Interests

The authors have not declared any conflict of interests.

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Processing, storage methods and quality attributes of spices and aromatic herbs in the local merchandising chain in Benin

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This study investigated the processing, the commercialization and the consumption practices of spices and aromatic herbs in different districts of Benin. The investigation was done through a survey using a questionnaire. The questionnaire mainly focused on the socio-cultural profile of actors, the spices and aromatic herbs commercialized, the processing techniques, the quality attributes according to the actors, the forms of consumption and the storage methods. The data collected were analysed using descriptive statistics. The results revealed that spices and aromatic herbs processing and commercialization are mainly female activities (80% of surveyed). The spices and aromatic herbs used in Benin are under several forms: fresh ones, dried ones, and powder obtained from one or a mixture of several spices and aromatic herbs. The products are commercialized through local markets and sub-regional markets (Nigeria, Ghana, Togo, Guinea). The appreciation of the quality of spices and aromatic herbs is based on quality attributes such as the aroma that must be marked and the physical aspect of fresh aromatic herbs. The spoilage of spices and aromatic herbs resulting in pungent taste, the attack by mould, and maggots, and the loss of aroma and weight of aromatic herbs during storage are the major problems claimed by the actors interviewed. Regarding the consumption, the spices and aromatic herbs are mainly used as flavouring and taste enhancer agents in all types of dishes. The current field investigation work also provided guidelines for the improvement of processing and storage practices of spices and aromatic herbs.

Key words: Flavouring, taste enhancer, processing methods, consumption, quality, Benin.

INTRODUCTION

Spices and aromatic herbs (SAH) constitute an important group of plants of the biodiversity including the Zingiberaceae, the Myrtaceae, the Liliaceae and the Solanaceae. Different parts of these plants: the root, the leaves, the fruit, the flora bud, the peel, the seed, the flowers, the stems and the rhizomes (De Vienne, 2007)

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are used for their flavoring and savoring characteristics (Tchiegang and Mbougueng, 2005; Chassaing, 2006) as whole record, powder or mixture of numerous spices and aromatic herbs (Peter, 2006; Tchiegang and Mbougueng, 2005).They have the particularity to be used in food in small amounts all over the world (Betts, 2014). Spices and aromatic herbs are also used for medicinal, cosmetic and religious ritual purposes as reported by Thomas et al. (2012).The consumption of the spices and aromatic herbs depends in general on the socio-cultural groups, the food habits and their availability in the zones of dwelling (Sossa, 2013). In African context or even Beninese, the SAH in addition to their culinary role, conferred to foods in which they are incorporated, a nutritional role (contribution in lipids, carbohydrate, proteins and mineral), functional properties and pharmacological virtues (Tchiegang and Mbougueng, 2005). Indeed, the spices and aromatic herbs contained a remarkable quantity of vitamin A, C, B and mineral as calcium, phosphorus, sodium, potassium, and iron (Al-Jasass and Al-Jasser, 2012). They also have antibacterial properties against numerous gastroenteritis pathogenic bacteria as the *Listeria*, the *Staphylococci* and *Escherichia coli* (Tchiegang and Mbougueng, 2005; El Kalamouni, 2010). As reported by Mah et al. (2009), the mixture of garlic and other spices inhibit the production of biogenic amine in Myeolchi-jeot, a Korean salted and fermented anchovy product and limits the development of numerous microorganisms. In addition, the spices and aromatic plants constitute an important source of antioxidants and natural antibacterial substances for food processing factories (Abdou, 2009) by preventing the deterioration of the free radicals (Popovici et al., 2009) and by delaying the oxidization of the lipids permitting to prolong the preservation duration of food (Nyegue, 2006; Popovici et al., 2009). However, the SAH can be heavily contaminated during processing, storage or transportation (Qaher, 2005). In this respect, the SAH present numerous health risks that required to be clarified. Similar to other agricultural products, the spices and aromatic herbs can be exposed to a large range of biological and chemical contamination during the post-harvest period, including the stages of processing, storage, distribution, sale and use (Hackl et al., 2013). Traditional drying of spices and aromatic herbs on the floor associated with unhygienic conditions exposed them to microbial and chemical contamination by heavy metal (Prakash et al., 2011). Moreover, the spices can also be contaminated by other toxic substances, such as the aflatoxins that can be produced through the presence of *Aspergillus flavus* (Roige et al., 2009). The presence of other pathogenic microorganisms such as *Salmonella* has also been reported by Eke et al. (2013). However, very few investigations have been done on the sanitary status, the nutritional value and the potential antibacterial properties within the large diversity of SAH consumed in the country. Therefore, this survey aimed to identify the different spices and aromatic herbs (SAH) processed, commercialized and consumed in Benin and West Africa regions. In addition, the socio-cultural characteristics of actors, the processing and the preservation methods used and hygiene practices, the definition of quality attributes of SAH according to the actors, and the main constraints faced by the actors were investigated with the objective to improve the processing and the quality of SAH commercialized at national and regional levels.

METHODOLOGY

Investigation sites and sampling of actors

Study areas explored were Dangbo, Adja-ouèrè, Adjohoun, Kétou in the Oumé/Plateau District, Savalou in the Zou/Colline District and Malanville in the Borgou/Alibori District which constituted the main zones of spices and aromatic herbs production (Assogba et al., 2009) and some cosmopolitan cities (Cotonou, Porto-Novo and Parakou) where processing activities of SAH were mainly done. The sampling size was determined according to Chadare et al. (2008). A total of 814 respondents comprising 216 traders, 216 processors, and 432 consumers were randomly sampled and interviewed from different localities (Table 1).

Data collection

Field investigation was conducted on SAH processors, traders and consumers of the different zones of Table 1. An exploratory study was conducted in order to identify production sites and introduce the purpose of this study to potential stakeholders. The questionnaire elaborated was pretested for its validity and reliability. Apart from the administration of the questionnaire to respondents, participant observations were made to the processing of SAH. The interviews were conducted in French, Goun, Fon, Mahi, Yoruba and Nago. Data collected were related to the sociocultural characteristics of various respondents, the forms of consumption and use of SAH, the virtues associated with the identified SAH, the processing and storage methods of SAH, the problems associated with the processing and the preservation of the SAH, and the quality attributes of SAH according to the different respondents.

Data analysis

Data collected were analysed through descriptive statistics using R software (version 3.2.2). Principal component analysis (PCA) was also performed with SPSS (version 16) to establish the link between different SAH identified and related socio- cultural groups.

RESULTS AND DISCUSSION

Social-cultural characteristics of respondents

The majority of respondents (87.6%) interviewed were adults aged between 30 and 68 years while the young people aged less than 30 years representing 11.5% (Table 2). The survey also showed that the commer-cialization of spices and aromatic herbs were carried

Table 1. Distribution of actors surveyed per locality and categories of actors in the survey zones.

Municipalities	Number of actors			Total people interviewed
	Traders	Consumers	Processors	
Adja-Ouèrè	13	26	4	43
Adjohoun	8	18	4	30
Cotonou	80	154	53	287
Dangbo	11	21	4	36
Kétou	18	35	8	61
Malanville	19	37	19	75
Parakou	29	56	22	107
Porto-Novo	31	58	23	112
Savalou	17	33	13	63
Total	226	438	150	814

out both by illiterate women (76.5%) aged between 20 and 66 years and men (23.4%) while the majority of processors (80%) were female aged between 22 and 61 years, and 72% of them were illiterate. For the majority of processors and sellers (80%), the processing and the commercialization of SAH constitute their main source of income. The main socio-cultural groups involved in the commercialization of spices and aromatic herbs were: Fon (13.9%), Yoruba (13.7%), Wémènou (13.4%), Goun (12.3%), Nago (10.7%) and Mahi (9.3%) (Table 2). Other minor socio-cultural groups included: Bariba (3.5%), Malian (2.9%), Zerma (2.9%) and Mina (2.5%), and to a lesser extent Adja (1.7%), Aïzo (1.7), and Pila-Pila (1.3%). Socio-economic data collected showed three categories of traders: the wholesalers earn more than three millions franc CFA (1 US$ = 450 CFA) of income/ month, the intermediary traders earn one hundred thousand to one million franc CFA of income/month, and the small scale traders earn twenty to forty thousand franc CFA of income/month. In addition, the survey revealed that the wholesalers and the intermediary sellers serve domestic and region markets while the small scale sellers only serve domestic markets. Moreover, the wholesalers (76%) and the intermediary sellers (48%) also processed the SAH.

Regarding the processors, the majority of them (80%) were women. These results are consonant with those of Kindossi et al. (2012) about *Lanhouin*, a fermented fish condiment used as taste enhancer and flavouring agent in many dishes in Benin. Men were also involved in the processing of SAH but they represented only 20% of processors surveyed. Among the processors, the adults and the young represented 91.3 and 7.3% respectively, and 1.3% of adults were more than 60 years old. Three categories of processors were identified: the small scale processors (87%) processed less than 200 kg/month, the medium-scale processors (2%) processed between 200 and 400 kg/month and the large scale processors (11%) processed between 400 and 1000 kg/month (Table 3).

Many socio-cultural groups were also involved in the processing of SAH but the main ones were: Wémènou (15.6%), Fon (14.6%), Nago (11.3%) Goun (10.6%) and Mahi (10%) (Table 2). The study also showed that the consumers of SAH belonged to all socio-cultural groups and fairly distributed between men and women.

Spices and aromatic herbs identified and their frequency of consumption

The survey showed that many spices and herbs were used by people interviewed (Table 4). Similar list has been reported by Pouillart and Pouillart (2013) and Codex (2015). Some of these spices and aromatic herbs were used alone, but several of them were also mixed within the group of spices or with aromatic herbs for culinary uses (Table 5). The frequency of consumption of spices and aromatic herbs varied according to the respondents and from one spice to another. Thus, chili (claimed by 14.0%), ginger (13.6%), garlic (12.9%), pepper (11.8%), onion (10.4%), anise (10.3%), sweet pepper (8.5%), nutmeg (8.3%), clove (6.5%), and Guinea pepper (6.4%) were the more spices consumed by the respondents (Figure 1a), while laurel (43.8%) and basil (38.3%) were the most aromatic herbs used (Figure 1b). Data also showed that mint, celery, Gambia tea, and rosemary were the less aromatic herbs used, while thyme, dill, cumin, cinnamon and star anise were the less spices consumed.

Processing of spices and aromatic herbs and difficulties claimed by respondents

The processing of SAH varied according to the type of spices and the areas investigated. The processing steps are summarized through the Figure 2. The main steps

Table 2. Socio-cultural characteristics of actors interviewed.

Characteristics	Frequency (%)
Age (years)	
30-68	87.6
<30	12.4
Sex	
Female	78.2
Male	21.7
Educational level	
Illiterate	81.2
Primary level	12.2
Secondary level	6.5
Marital status	
Married	91.4
Unmarried	6.8
Divorced	1.6
Religion	
Animist	36.8
Christian	44.6
Islam	18.3
Main socio-cultural groups*	
Fon	14.3
Goun	13.9
Wémènou	13.5
Nago	11.4
Yoruba	9.3
Mahi	8.9
Dendi	6.1
Bariba	4.5
Mina	3.8
Aïzo	3.4

***** Average data of actors involving in the processing and the commercialization of SAH

Table 3. Amount of SAH processed per categories of processors.

Quantity (kg) of SAH processed per month	% of processors	Categories of processors
Less than200	87	Small-scale
200-400	2	Medium-scale
400-1000	11	Large-scale

included the following.

Sorting and heating

Sorting is the first step of the processing of SAH. During this step, the SAH are sorted to remove the undesirable parts (rotting or mouldy parts). Regarding the heating, which is mainly applied in the case of chili, 2 L of water is added to 25 kg of product and the mixture is heated at 80 to 90°C during an average time of 5 min before being sun dried. During the heating step, palm oil (1/2 L for 25 kg of

Table 4. Spices and aromatic herbs identified in the survey areas.

Common names	Local names	Scientific names
Spices		
Anise	Plèplè	*Pimpinella anisum*
Chili	Takin	*Capsicum sp*
Cinnamon	Cannelle	*Cinnamomum zeylanicum*
Clove	AtinkinKpadotà	*Eugenia caryophyllus*
Cumin	Hâ	*Cumynum cyminum*
Dill	Sokounnou	*Anethum gravealens*
False nutmeg	Sassagbakun	*Monodora myristica*
Garlic	Ayo	*Allium sativum*
Guinea pepper	Kpédjlélékun	*Xylopia aethiopica*
Ginger	Dotè	*Zingiber officinalis*
Nutmeg	Salikun	*Myristica fragrans*
Onion	Mansà	*Allium cepa*
Pepper	Linlinkun	*Piper nigrum*
Star anise	Etoile	*Illicium verum*
Sweet pepper	Poivlon	*Capsicum annuum*
Thyme	Tein	*Thymus vulgaricus*
Aromatic herbs		
African basil	Tchioyo, tchiayo	*Ocimum gratissimum*
Celery	Céléri	*Apium gravealens*
Citronela	Timan	*Cymbopogon citrates*
Laurel	Loriéman	*Laurus nobilis*
Mint	Manti	*Mentha aquatic*
Parsley	Persil	*Petroselinum crispum*
Rosemary	Romarin	*Rosmarinus officinalis*

chili) is added to the hot water (practiced by 18% of processors) in order to improve the organoleptic characteristics of the sun died product.

Sun drying of SAH

The sun drying is one of the most important steps of the processing of the SAH as reported by Dufresne (2008). According to Bonazzi and Dumoulin (2014), the sun drying technique reduces the water activity in the SAH, and consequently prevents the development of microorganisms, but it also reduces the weight of SAH and unfavourably output after drying (claimed by 98% of processors interviewed). Similar observations have been reported by Dufresne (2008). Unfortunately, the drying technique applied by the processors remained even traditional (98% of surveyed processors) because limited to the sun drying as reported by Tunde-Akintunde (2010) while only 1.2% of them use the electric dryer. The SAH are dried either on the ground (13.4% of processors) concerning the aromatic herbs such as Citronella (*Cymbopogon citratus)* and Laurel (*Laurus nobilus*), even on the floorbord (27.2% of processors), on jute bags (17.1% of processors), on the flat baskets (12.1%) of processors) and on the sidewalks (29.3% of processors).

In contrast to the sun drying practices of SAH in Africa, drying using modern driers in developed countries limits the risks of contamination. Another problem resulting from the sun drying is that it is not possible to determine the drying time as the sun intensity is variable. Consequently, the duration of sun drying can influence the active compounds, the colour or the burst of spices and aromatic herbs (Peter, 2006). In addition to this, the drying on the sidewalks and on the ground can lead both to chemical and microbiological contamination of foods making them inappropriate for consumption (Salari et al., 2012). In fact, this method of drying can also be a source of contamination by heavy metals, pathogenic micro-organisms or spore of pathogenic organisms, and physical agents such as twig or stone (Mediss, 2012). Similar observations were also reported by Peter (2006) who revealed various types of micro-organisms infestation during the sun drying of spices and medicinal plants.

Table 5. Mixtures of processed spices and aromatic herbs commercialized.

Names of products	Components
Two spices	Garlic, ginger Garlic, ginger, salt
Three spices	Garlic, ginger, pepper Garlic, chili pepper
Four spices	Garlic, ginger, pepper, laurel Garlic, ginger, chili cinnamon Garlic, ginger, pepper, chili
Five spices	Garlic, ginger, anise, cumin, rosemary ; Garlic, ginger, pepper, laurel, nutmeg
Six spices	Garlic, ginger, green anise, thyme, cumin, rosemary
Sept spices	Garlic, ginger, nutmeg, anise, clove, pepper, cinnamon Garlic, ginger, nutmeg, clove, pepper, laurel, Guinea
Twelve spices	Garlic, ginger, curry, thyme, pepper, laurel, nutmeg, clove Basil, nutmeg, garlic, clove, ginger, curry, thyme, pepper, laurel
Spice for tchachanga (a roasting meat)	Chili, cumin, anise, laurel, peanut or cake of peanut, maggi cube or glutamate, dried tomato, dried eggplant, corn flour.

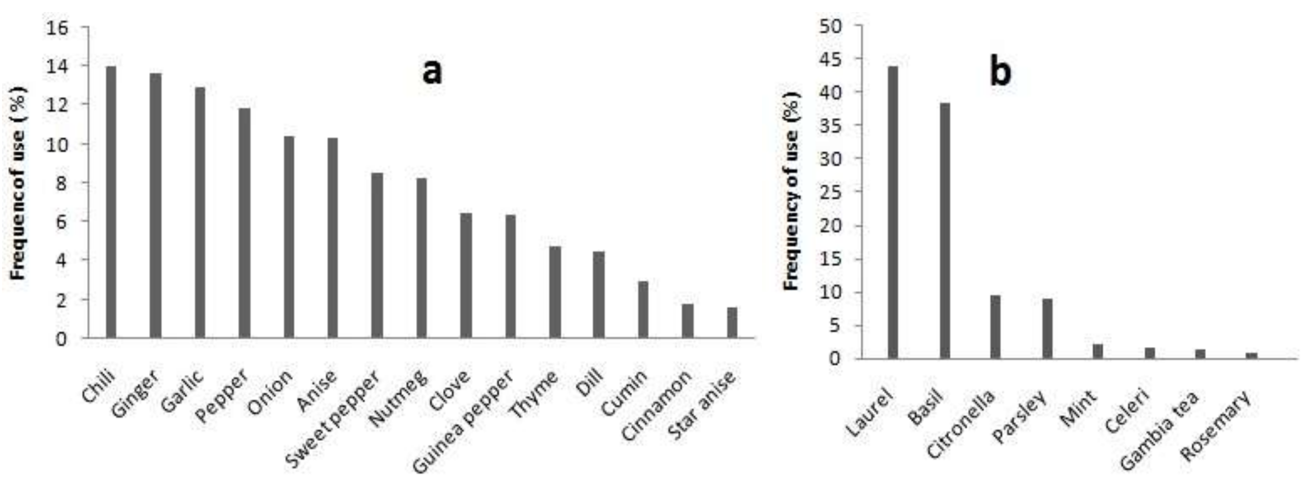

Figure 1. Frequency of use of individual spices (a) and aromatic herbs (b) identified.

Milling of SAH

The sun drying of SAH is followed by the milling step as reported by Hossain (2010). Various traditional milling methods were noticed during the survey. They are: use of cemented millstone (68.3%), built stone (11.4%) and mortars (2.5%). Different modern mills were also used by the processors interviewed: small mill (8.9%), plate disc mill for corn (58.2%) and plate disc mill for condiments (39.4%). The mills are often installed in unhygienic places (33%). According to Sung et al. (2012), the milling operation can lead to the loss of flavour of spices and

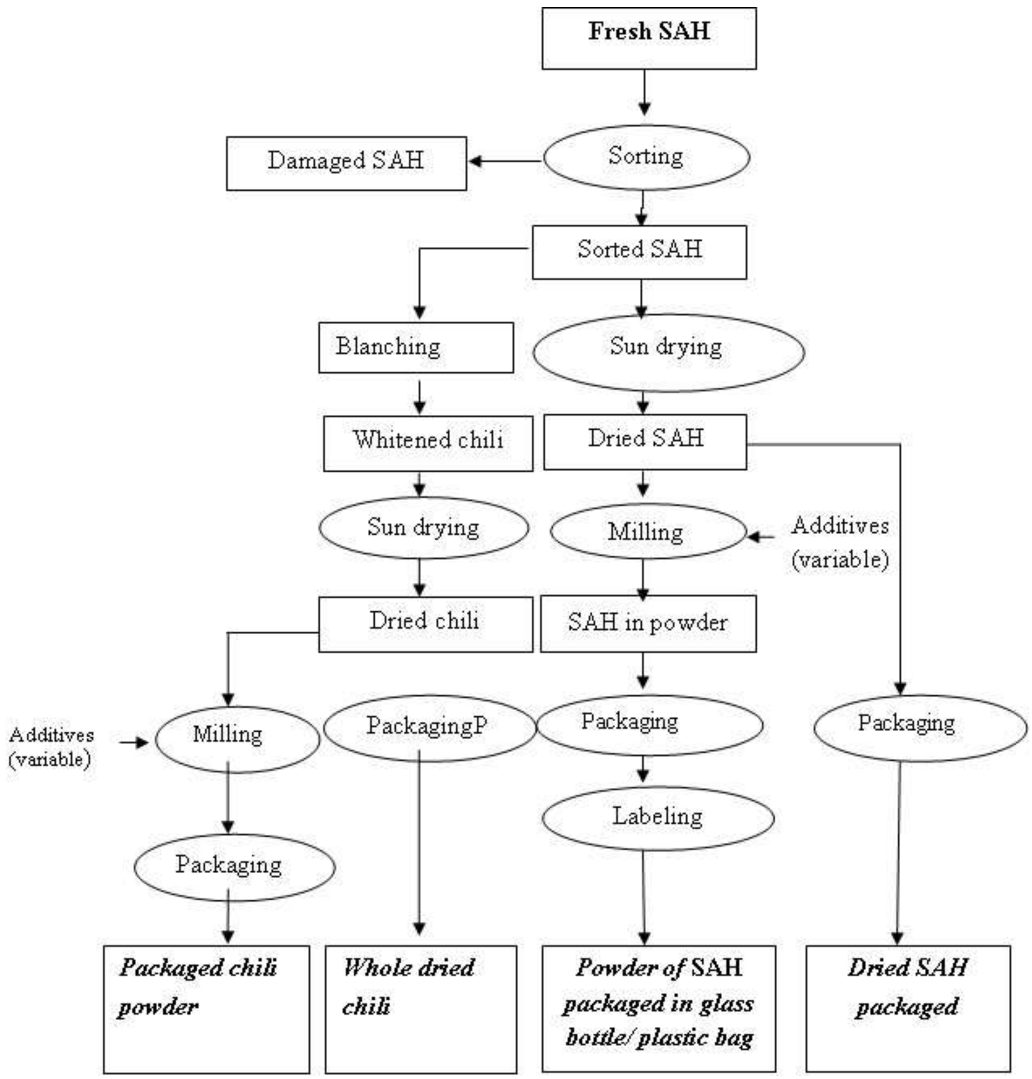

Figure 2. Flow diagram of processing of spices and aromatic herbs

aromatic herbs. This was mentioned by 14.55% of processors interviewed. Furthermore, the plate disc mills may be contaminated by other matter because the mill is used to grind other types of products such as leguminous, piece of dried yam, etc. Therefore, the type of milling can negatively impact the microbial status of ground spices and aromatic herbs and the colour as well.

Packaging of SAH

The ground spices obtained are packaged in different types of materials such as glass bottles (64.6%) or in small plastic bottles (6%) while jute bags (19.3%), plastic bag (6.7% and baskets or bowls (3.3%) are used as packaging materials for non-ground spices. From these results, it appeared that glass bottle is the packaging material mostly used by the processors of spices. According to the processors interviewed, the glass bottles allowed a good presentation of the product and long conservation period as well. These results are in accordance with the findings of various authors who reported the use of various package materials, sometimes conventional like paperboard boxes, multi-wall paper bags, plastic sacks that conferred to the spices and aromatic herbs a better conservation (Prashant, 2014).

According to the processors interviewed, various constraints were associated with the processing of spices and aromatic herbs. The most cited by these actors were: the sun drying practice, because of its duration and the climatic risks (80%), the milling (70%) because of the irritating characters of spices. Concerning the chili, the

Table 6. Quality attributes per category of respondents.

Respondents	Quality attributes (%)		
	Aroma	Absence of rotting part	Absence of foreign element
Processors	52.5	32.5	46.8
Traders	22.2	26.6	32.9
Consumers	61.8	86.2	92.4

heating applied before the sun drying was also mentioned as a constraint (18%) because they need to watch the fire to avoid the over cooking of the product.

Storage of spices and aromatic herbs and problems associated with the storage

After processing, the SHA were immediately sold or stored by using various techniques. For 75% of processors interviewed, the non-processed SAH are sun dried twice or three times a week for long period conservation (two years) or dried with electric drier (1.2%) to avoid the attack by mould. The storage of fresh SAH (chili, ginger, anion, and garlic) by the cool is practiced by 5% of the processors. The maximum storage duration of the SAH kept in cool conditions is 10 days according to 80% of the processors who practiced this technique. Thus, the moisture level for most spices and aromatic herbs should be reduced to below 11% in order to prevent the proliferation of microorganisms. According to the European Spice Association, the moisture content in spices after drying is very decisive for the microbial proliferation (Yogendrarajah, 2014; Tulu et al., 2014). For 98% of processors, the salt added to SAH during the milling allowed to keep them at least for one year. But, the SAH in powder already lost after 3 months some of their organoleptic characteristics such as the taste revealed by 11.4% of the respondents and 9.9% for the aroma.

In summary, the duration of the storage of spices is a function of the storage methods used (refrigeration, drying, packaging). From the survey data, it appeared that the products that did not last longer in storage were those stored with the cool (that is, at most 7 days for chili). In contrast, the dried products last longer and can be stored during two years as mentioned by 86% of the processors. The survey also showed that the major storage problems faced by the processors were the rotting of fresh SAH (cited by 65.3% of the respondents), the attack by mould related to the no ground dried products pointed out by 63.3% and powder products mentioned by 26% of the interviewees. The presence of dead maggots (38.2%) of processors, the loss of weight (18.1%) and aroma (9.98%) during the storage were also reported for dried products in general and the ground ones in particular. In addition, the attack by mould during the storage period can lead to the production of aflatoxins as reported by Set and Erkmen (2010). Other majors problems observed were the general unhygienic conditions of the processing sites and the processing material leading to the contamination of the product.

Quality attributes of SAH

All the respondents (processors, consumers, traders) interviewed based their choice on quality criteria including: the physical integrity of the SAH (absence of rotting or mouldy parts) (48.4%), the absence of foreign elements (57.3%) and the aroma, that is, the most important quality attribute (45.5%) (Table 6).

However, some of the processing practices mentioned earlier can affect the quality criteria. In fact, during the drying step, about 13.4% of the processors spread their products on the floor, while 29.3% spread them on the sidewalks contrary to hygiene related to dried spices and aromatic plants (Codex, 2015) that recommends a drying on platforms built with suitable materials. In the same time, the drying on the sidewalks or the floor does not guarantee the protection of the spices against contamination by house animals, rodents, birds, bugs, arthropods, and microorganisms as well. Similar results have been reported by Romagnoli et al. (2007) who noted the presence of *Aspergillus* species, *Penicillium* species and *Fusarium* species in various food products including spices and aromatic herbs. In this respect, the sun drying of SAH mainly practiced by the majority (98%) of processors and traders could increase the risks of attack of SAH by the mould during the rainy season.
Indeed, according to Nguyen et al. (2007), the rate of growth of *Aspergillus parasiticus* in SAH samples increased to 60% in the rainy season. These findings showed that there is a relationship between the moisture content in SAH after the drying and the proliferation of moulds or other microorganisms (Iqbal et al., 2011).

Consumption and forms of use of the SAH

In the markets, the consumers bought the SAH in fresh form, as powder obtained from one or a mixture of

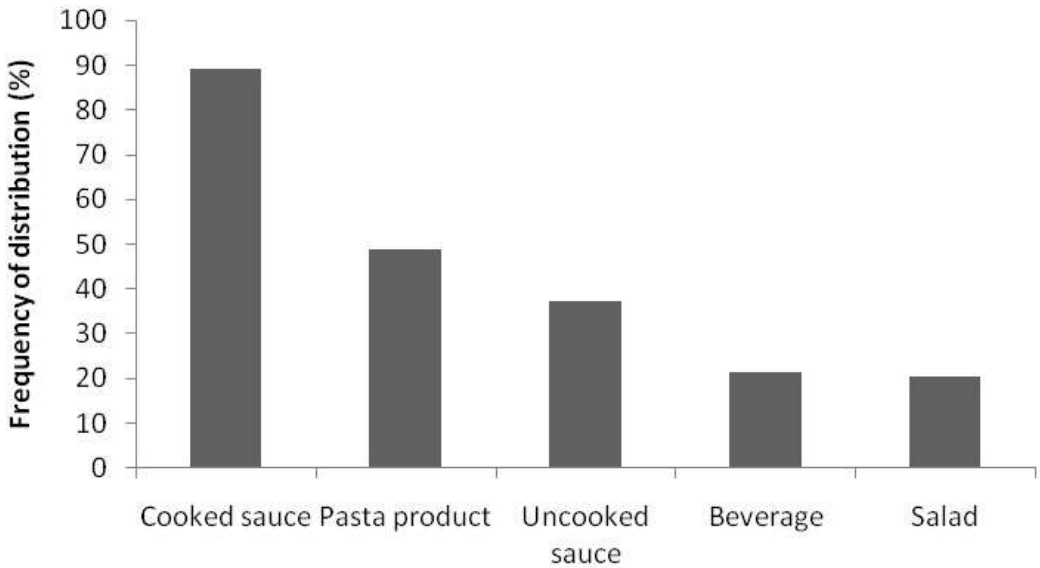

Figure 3. Frequency distribution of the main dishes containing SAH.

Table 7. Frequency of consumption of the main dishes containing SAH recorded.

Dishes containing SAH	Frequency of consumption (days/week)	Occasion of consumption	Place of consumption
Cooked sauce	7	Lunch, dinner	Home, restaurant, street food, funeral
Pasta products	3-5	Lunch, breakfast	Home, restaurant, street food, funeral
Uncooked sauce	3-4	Lunch, dinner	Home, restaurant, street food, funeral
Beverage	1-2	Breakfast	Home, restaurant, street food, funeral
Salad	Rarely	Lunch, dinner	Home, restaurant, street food, funeral

several SAH. Several reasons explain the use of the SAH by the consumers. They are mainly used as taste and aroma enhancer: chili, garlic, onion, ginger, clove, pepper, thyme, dill, cumin, anise, nutmeg, cinnamon and laurel (68.5% of the respondents), for medicinal reasons: basil, clove, onion, garlic and ginger (46.5%), against snakes: chili, guinea pepper (43.8%), for cosmetic use: mint, chili (41.1%), as preservative: chili for various cereals (maize, sorghum) and bean (39.5%), for spiritual reason: mint, clove, pepper of Guinea, false nutmeg, basil, tea of Gambia (39.2%) and as aphrodisiac product: ginger (2%). The SAH were usually incorporated in several types of dishes including: sauces (89%) (tomato sauce, vegetable sauce, groundnut or egoussi sauce, and sauce dja which is a fried tomato sauce), pasta products (49.%), uncooked tomato sauce called monyo (37.4%), beverage (21.5%) (syrup of ginger, mint and garlic) and salad (20.3%) (Figure 3). These dishes are consumed at breakfast, lunch and dinner, and at various places including home, street food, restaurant, and funeral ceremonies (Table 7).

Relationship between socio-cultural groups and types of SAH

The principal component analysis (PCA) performed on various spices and aromatic herbs, and social-cultural groups resulted in two axes accounting for 62.01% of the total variation, of which 32.71% was explained by the first axis (Axis 1) and 29.3% by the second (Axis 2) (Figure 4). Spices and aromatic herbs consumed by particular socio-cultural groups were grouped together with the groups. Considering Axis1, the Adja, Bariba, Dendi, Ditamari, Goun, Natimba, Yoruba, Mahi, and Nago groups mainly used African basil, anise, chilli, pepper, cumin, false nutmeg, garlic, ginger, laurel, guinea pepper, nutmeg, onion, rosemary and sweet pepper. With respect to Axis 2, celery, cinnamon, pepper, and thyme are more consumed by the Aizo, Fon, Haoussa, Djoula (Malian),

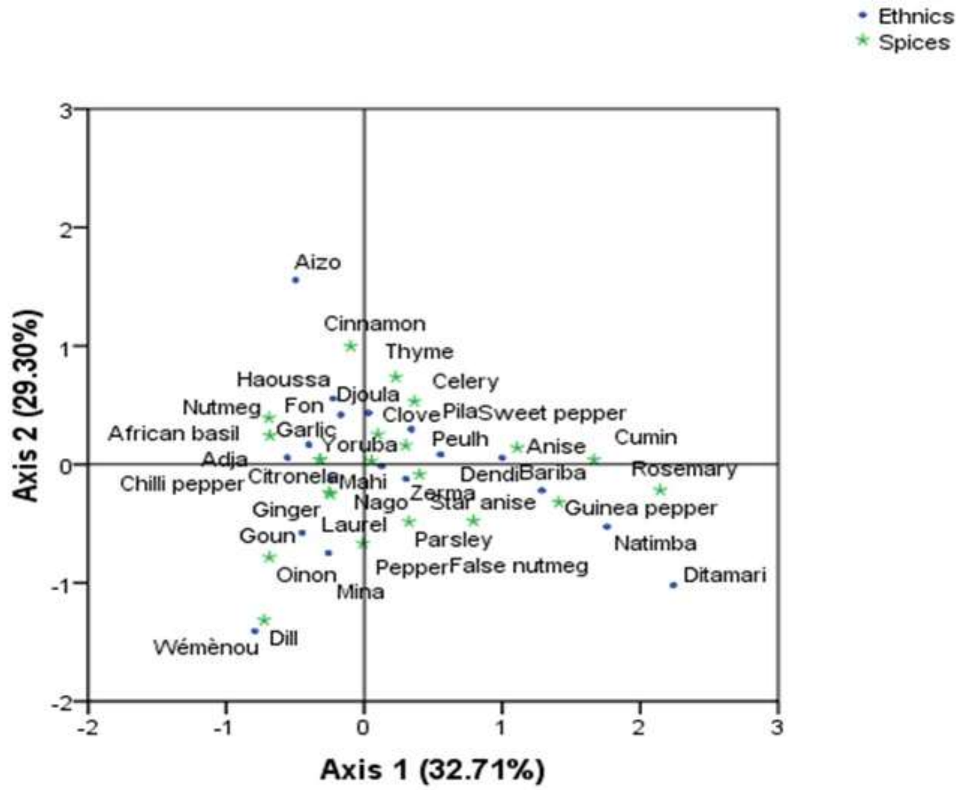

Figure 4. Correspondence Analysis to show linkages between socio- cultural groups and types of SAH mostly used.

Mina and Wémènou.

Conclusion

The survey enabled to identify the various SAH used in most localities of Benin. Most of them were imported and were consumed throughout the world. The survey also revealed that the processing and the commercialization of SAH were carried out both by women and men in contrast to other products such as *Lanhouin* and *Afitin* used to enhance taste and flavour in many traditional dishes in West African region. In addition, it appeared from this study that the processing of SAH is mainly done in very unhygienic conditions favourable to potential contamination at both microbiological and toxicological aspects. Packaging materials are mostly constituted of rudimentary containers that could also be a source of contamination of processed SAH. In addition, PCA analysis demonstrated that the use of SAH is associated with the socio-cultural groups identified during the survey. Based on hygiene practices observed, there is a need to characterise and improve the processing practices and the end-products, in order to establish the effect of processing practices on the quality of the end-products widely consumed in urban and rural areas in Benin and in the neighbouring countries of Nigeria, Ghana, Togo, etc. This will provide clear guidelines for the improvement of processing of SAH and consequently, the improvement of the microbiological, physicochemical and toxicological quality of SAH commercialized in the country.

Conflict of interest

There is no conflict of interest

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Full Length Research Paper

Temperature and pre-germinative treatments for overcoming *Acacia farnesiana* (L.) Willd. (Fabaceae) seeds dormancy

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***Acacia farnesiana* L. is a species known for its uses in recovery of degraded pastures, animal feeding, medical and fungicide properties. However, it is one of the most problematic invasive species in agriculture, due to the little known dormancy aspects of its seeds that results in the propagation and dispersion to distinct areas and the establishment of the invasive plant. The knowledge on ecophysiological characteristics of invasive species seeds aiming at the dormancy process is important for the comprehension of aggressive regeneration unities, and allows the development of strategies against infestation of new areas and reduction of soil seed banks. The objective of this research was to assess how *A. farnesiana* seeds overcome dormancy using different temperatures and pre-germinative treatments. The study was conducted in Federal University of Paraiba, using seeds obtained from fruits of ten matrix trees in Paraiba State, Brazil. The completely randomized design was adopted, with treatments arranged in a 3 x 15 factorial scheme representing temperatures and pre-germinative treatments with four replicates. Parameters related to germination percentage, germination and emergency index were assessed, with best results observed in seeds scarified with sandpaper 80 followed by imbibitions of water at environmental temperature (25-30°C) for 24 h.**

Key words: Germination, invasive species, ecophysiological characteristics, dormancy process.

INTRODUCTION

Acacia farnesiana L. Willd. is a bush plant which belongs to the Fabaceae family, characterized by its height that might reach 4 m, thorny aspect and continuous flowering and fruiting, that results in an annual yield of 13 thousand

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seeds per plant (Camacho et al., 2012), which germinate in river beds and wetter regions of lowlands (Dias et al., 2008). This species is an important option for recovery of degraded pastures, whilst its fruits can be used for animal feeding (Erkovan et al., 2013) and its leaves can be explored due to its antifungal properties and for phytotherapy medicines production (Kingsley et al., 2014).

Besides its beneficial properties, this plant is considered as one of the worst invasive plant species in the world (Camacho et al., 2012) because of its seeds dormancy break by animals (endozoochory), that influences the dispersion and propagation to different areas and contributes to the formation of new populations of this invasive species (Arévalo et al., 2010). The knowledge of ecophysiological characteristics related to the seed dormancy process of invasive species is important for the understanding of aggressive regeneration unities, and might be useful for the development of strategies to control invasive populations, reduce soil seed banks and the infestation of new areas (Martins et al., 2013a).

Tropical and subtropical forestry species frequently have viable seeds that do not germinate even in favorable environmental conditions. This is explained by the dormancy process, which evolved as a surveillance mechanism that allows physical and temporal distribution of germination in different environments (Camacho et al., 2012).

The overcoming of dormancy in seeds is regulated by several factors, but temperature is one of the most important by promoting the rupture of their coat, raising their permeability to water and gases, which are essential for germination metabolism (Martins et al., 2008). For germination tests, temperature is employed under certain limits, marked by an optimal singular value or temperature intervals, in which the germination rate occurs at max efficiency (Martins et al., 2013b).

Similarly, some seeds from *Acacia* spp. also have dormancy (Rodrigues et al., 2008), specially related to the impermeability of the coat (Martins et al., 2012). However, there are available treatments to overcome this kind of dormancy in *Acacia* spp., but the proper moment of application, the recommended temperature and the most efficient pre-germinative treatments depend on the species (Martins et al., 2008). Smiderle et al. (2005) found the best germination results for *Acacia longifolia* (Andrews) Willd through thermal scarification at 100°C for 1 min followed by germination at 25°C, while Rodrigues et al (2008) noticed that *Acacia mangium* Willd. seeds presented highest germination values after chemical scarification with H_2SO_4 for 90 min; Escobar et al. (2010) and Tapia et al. (2013) reported highest values for *Acacia caven* (Mol.) Mol. after abrasion with sandpaper and germination at 30°C.

Due to the diversity of the dormancy process related to the variability of germination, invasive forestry species is hard to predict infestations. For this reason, the knowledge of specific characteristics linked to the germination process is indispensable (Erkovan et al., 2013) and there is a lack of information on *Acacia farnesiana* (L.) Willd seeds. This research aimed to evaluate the effect of different temperatures and pre-germinative treatments on the dormancy break of *A. farnesiana* seeds.

MATERIALS AND METHODS

Seed acquisition

The present work was conducted at the Seed Analysis Lab (SAL) from the Crop Production and Environmental Sciences Department of the Federal University of Paraiba, located in Areia, Paraiba state, Brazil. Seeds from *Acacia farnesiana* were obtained from mature fruits harvested from ten matrix trees in Souza City, Paraiba State, Brazil. After the harvest, the fruits were kept in polyethylene bags and taken to the laboratory, pulped manually for the removal of seeds, and then homogenized.

Seed moisture content and pre-germinative treatments

The water content was determined using an oven at 105 ± 3°C, for 24 h (BRASIL, 2009), considering four replicates of 25 seeds per matrix tree.

A. farnesiana seeds with an initial moisture content of 10% was subjected to the following pre-germination treatments: Thermal scarification – immersion in water at 60°C (T_1: Immersion water 60°C), 70°C (T_2: Immersion water 70°C), 80°C (T_3: Immersion water 80°C), 90°C (T_4: Immersion water 90°C) and 100°C (T5: Immersion water 100°C) for 1 min.

Chemical scarification – seeds were immersed in absolute sulfuric acid (H_2SO_4) for 10 (T_6: Sca. sulfuric acid 10’) and 20 min (T_7: Sca. sulfuric acid 20’), with and without imbibition for 12 h (T_8: Sca. sulfuric acid 10’, imb. 12 h), (T_9: Sca. sulfuric acid 10’, imb. 12 h) or 24 h (T_{10}: Sca. sulfuric acid 10’, imb. 24 h), (T_{11}: Sca. sulfuric acid 10’, imb. 24 h) at environmental temperature (25 ± 1°C), after washing with running water for 5 min.

Mechanical scarification

Seeds were rubbed manually with sandpaper no. 80 at the opposite side of the micropile to avoid damaging the embryo, without (T_{12}: Sca. Sandp.) and with imbibition for 12 (T_{13}: Sca. Sandp., imb.12h) and 24 h (T_{14}: Sca. Sandp., imb. 24 h) at environmental temperature. In Check or control treatment (T_{15}: intact seeds), seeds were not exposed to any treatments.

Physiological treatments

Germination test was carried out in germination chambers adjusted to 20-30, 25 and 30°C with a 12 h photoperiod provided by fluorescent lamps (4 x 20 W), using four replicates of 25 seeds per treatment. Following the pre-germinative treatments, seeds were subjected to disinfection with the fungicide captan, then sown on rolls of humidified paper towel. The assessments were conducted through daily counts from the second to the eighth day after sowing, adopting the concept of normal seedlings determined by Brasil (2009).

Emergency test was performed in a greenhouse (average temperature of 30°C and relative humidity towards 80%, by the time of the evaluations), with four subsamples of 25 seeds, previously. Before sowing in plastic seed trays (49 x 33 x 7 cm), the seeds received chemical treatment with the fungicide captan, and then disposed in sand previously autoclaved. The reposition of substrate moisture was done regularly. From the third to the twelfth day after sowing, the number of emerged seedlings with the hypocotyl above the substrate (Martins et al., 2013b) was computed daily.

At the end of the experimental setup, the first count, the germination and emergency percentage were determined. The germination and emergency speed indexes were also calculated, following the methodology suggested by Martin et al. (2013).

Experimental design and statistical analysis

It was a completely randomized design, with the treatments arranged in a 3 x 15 factorial scheme (temperatures and pre-germinative treatments, respectively), with four replicates. Data were submitted to analysis of variance, using F test for comparison of mean squares, and the means were compared by Anova and Scott-Knott test, with a 95% confidence limit.

RESULTS AND DISCUSSION

The best results relative to germination percentage and first count (Table 1) were observed at the seeds scarified with sandpaper no. 80 (T_{12}) at 30°C and scarified with sandpaper no. 80 followed by imbibition for 24 h (T_{14}) at 25 and 30°C. However, considering percentage of germinated seedlings apart from the temperatures, these treatments did not present significant difference from the seeds immersed in sulfuric acid for 10 and 20 min with 24 h of imbibition of water (T_{10} and T_{11}, respectively) and from the seeds submitted to chemical scarification and 12 h of imbibition of water (T_9: Sca. sulfuric acid 10’, imb. 12h) at 30°C. It is also noticeable that thermal scarification treatments (T_1: immersion water 60°C and T_2: immersion water 70°C) implied in lower values of germination of *A. farnesiana* seeds.

The results (Table 1) prove that the treatment with sandpaper, despite being viable only for little amounts of seeds (Martins et al., 2008), promotes superior efficiency in the overcoming dormancy of *A. farnesiana* seeds as compared to the others, because it results in higher germination percentage and first count values. Similar results were found through scarification with sandpaper no. 80, with and without posterior imbibition of *Acacia mearnsii* Willd.(Roversi et al., 2002) and *Acacia caven* (Mol.) Mol. (Escobar et al., 2010; Tapia et al., 2011), with germination rates at 30°C. In the same way, *Apeiba tibourbou* Aubl. (Guedes et al., 2011) and *Cassia fistula* L. (Guedes et al., 2013) seeds submitted to mechanic scarification with sandpaper, with and without imbibition in water for 24 h and set to germinate at 30°C presented a larger number of normal seedlings at first count.

It was also noticeable that there was a significant interaction between the evaluated temperatures and the scarification with H_2SO_4 for 10 and 20 min followed by imbibition of water for 24 h. This combination was also effective to overcome *A. farnesiana* seeds dormancy probably by causing necessary ruptures at their coat, raising their permeability to water and gases, demanded factors for the germinative metabolism. These results corroborate with the ones reported for *Ormosia nitida* Vog. (Lopes et al., 2006), *Caesalpinia pyramidalis* Tul. (Alves et al., 2007), *Merremia aegyptia* L. (Pereira et al., 2007), with seeds scarified by H_2SO_4, as well observed in *Caesalpinia leiostachya* (Benth.) Ducke. (Biruel et al., 2007), *Adenanthera pavonina* L. (Rodrigues et al., 2009) and *Piptadenia moniliformis* Benth. (Azeredo et al, 2010) seeds treated for 20 min with sulfuric acid.

Analyzing the germination rates, it is possible to infer that *A. farnesiana* seeds are adapted to wide thermal amplitude, as germination succeeded both in constant and alternated temperatures. This statement is also confirmed by studies with other species of this gender, like *Acacia polyphylla* DC. (Araújo neto et al., 2003), *A. mangium* Willd. (Rodrigues et al. 2008) and *A. caven* (Mol.) Mol. (Escobar et al., 2010), where maximal germination results were reached at various temperatures (25, 30 and 20-30°C). According to Azeredo et al. (2011), these results illustrate the ability of adaptation and distribution of these species to a range of habitats, granting best chances to support adverse environmental conditions.

For germination speed index (GSI), the best results were found for the treatments with sandpaper, with and without imbibition of water (T12: Sca. Sandp. And T13: Sca. Sandp. Imb. 12h, respectively), at 25 and 30°C, not differing from the treatments with chemical scarification followed by imbibition of water for 12 (T_9: Sca. sulfuric acid 10’, imb. 12h) and 24 h (T_{11}: Sca. sulfuric acid 10’, imb), at 30^{O}C (Table 2). Chemical and mechanic scarification caused fissures on the seeds testa of this study, which allows the absorption of water and oxygen (Martin et al., 2008), enzymatic activation and energetic reserves hydrolysis, resulting in cell division and elongation and a faster and more constant germination (Albuquerque et al., 2009).

Such data were obtained with chemical and mechanic scarification, assessed at 25 and 30°C in *Caesalpinia ferrea* Mart. ex Tul (Medeiros filho et al., 2005), *A. mangium* Willd. (Rodrigues et al., 2008), *Adenanthera pavonina* L. (Costa et al., 2010) seeds and in *A. caven* (Mol.) Mol. propagules (Escobar et al., 2010).

The emergency results (Table 3) had the same tendency of the germination values. Higher percentages measured through the number of normal seedlings (82%) and first count (65%) were associated with mechanically scarified seeds (T_{14}: Sca. Sandp., imb. 24h). Nonetheless, the treatment with sandpaper with (T_{13}: Sca. Sandp., imb.12 h) and without (T_{12}: Sca. Sandp.) water imbibition were as well linked to greater emergency percentages in this study. T_{13} and T_{14} also contributed to the best values of emergency speed index (ESI), while the treatments with immersion in water at 60°C (T_1: immersion water 60°C)

Table 1. *A. farnesiana* seed germination percentage and first count due to different pre-germinative treatments.

Treatments	Temperatures (°C)					
	Germination (%)			First count (%)		
	20-30	25	30	20-30	25	30
T_1	0^{gA}	$0^{há}$	0^{fA}	0^{dA}	0^{dA}	0^{gA}
T_2	4^{gA}	$0^{há}$	4^{fA}	0^{dA}	0^{dA}	0^{gA}
T_3	2^{gA}	6^{gA}	0^{fA}	0^{dA}	0^{dA}	0^{gA}
T_4	5^{gA}	5^{gA}	4^{fA}	0^{dA}	0^{dA}	0^{gA}
T_5	10^{fC}	17^{eB}	24^{dA}	0^{dA}	0^{dA}	0^{gA}
T_6	9^{fC}	35^{cB}	45^{cA}	0^{dA}	0^{dA}	0^{gA}
T_7	41^{eA}	27^{dB}	17^{eC}	0^{dA}	0^{dA}	0^{gA}
T_8	83^{cA}	84^{bA}	81^{bA}	0^{dA}	0^{dA}	0^{gA}
T_9	91^{bA}	87^{bB}	95^{aA}	14^{cB}	4^{dC}	73^{cA}
T_{10}	98^{aA}	96^{aA}	96^{aA}	0^{dB}	0^{dB}	39^{eA}
T_{11}	95^{aA}	93^{aA}	95^{aA}	37^{bB}	20^{cC}	60^{dA}
T_{12}	61^{dB}	89^{bA}	93^{aA}	19^{cC}	76^{aB}	85^{aA}
T_{13}	13^{fA}	12^{fA}	15^{eA}	1^{dB}	1^{dB}	10^{fA}
T_{14}	78^{cB}	96^{aA}	97^{aA}	46^{aB}	79^{aA}	84^{aA}
T_{15}	83^{cA}	85^{bA}	80^{bA}	40^{bC}	69^{bB}	79^{bA}
CV (%)		8,04			10,16	

Means followed by the same lower case letter within a column and by the same upper case letter within a row are not significantly different by Scott-Knot test (P > 0.05). T_1: Immersion water 60°C; T_2: Immersion water 70°C; T_4: Immersion water 90°C; T5: Immersion water 100°C; T_6: Sca. Sulfuric Acid 10';T_7: Sca. Sulfuric Acid 20'; T_8: Sca. Sulfuric Acid 10', imb. 12h; T_9: Sca. Sulfuric Acid 10', imb. 12h; T_{10}: Sca. Sulfuric Acid 10', imb. 24h; T_{11}: Sca. Sulfuric Acid 10', imb. 24h; T_{12}: Sca. Sandp.; T_{13}: Sca. Sandp., imb.12h; T_{14}: Sca. Sandp., imb. 24h; T_{15}: intact seeds.

Table 2. Germination speed index of *A.farnesiana* seeds under different pre-germinative treatments.

Treatments	Temperatures (°C)		
	20-30	25	30
T_1	0.00^{fA}	0.00^{gA}	0.00^{fA}
T_2	0.22^{fA}	0.08^{gA}	0.33^{fA}
T_3	0.06^{fA}	0.38^{gA}	0.00^{fA}
T_4	0.18^{fA}	0.19^{gA}	0.33^{fA}
T_5	0.39^{fA}	0.53^{gA}	1.09^{eA}
T_6	0.33^{fB}	1.66^{fA}	1.90^{dA}
T_7	2.48^{eA}	1.70^{fB}	1.10^{eB}
T_8	6.06^{cA}	5.63^{eA}	5.21^{cA}
T_9	7.02^{bB}	6.88^{dB}	11.04^{aA}
T_{10}	5.92^{cC}	8.15^{cB}	8.87^{bA}
T_{11}	9.28^{aB}	7.84^{cC}	10.44^{aA}
T_{12}	4.43^{dB}	10.84^{aA}	11.33^{aA}
T_{13}	0.73^{fA}	0.84^{gA}	0.61^{fA}
T_{14}	7.26^{bB}	10.98^{aA}	10.87^{aA}
T_{15}	8.83^{aB}	9.91^{bA}	9.00^{bB}
CV (%) 10,91			

Means followed by the same lower case letter within a column and by the same upper case letter within a row are not significantly different by Scott-Knot test (P > 0.05). T_1: Immersion water 60°C; T_2: Immersion water 70°C; T_4: Immersion water 90°C; T5: Immersion water 100°C; T_6: Sca. Sulfuric Acid 10';T_7: Sca. Sulfuric Acid 20'; T_8: Sca. Sulfuric Acid 10', imb. 12h; T_9: Sca. Sulfuric Acid 10', imb. 12h; T_{10}: Sca. Sulfuric Acid 10', imb. 24h; T_{11}: Sca. Sulfuric Acid 10', imb. 24h; T_{12}: Sca. Sandp.; T_{13}: Sca. Sandp., imb.12h; T_{14}: Sca. Sandp., imb. 24h; T_{15}: intact seeds.

and 70°C (T_2: immersion water 70°C) for 1 min proved to be inefficient in breaking *A. farnesiana* seed dormancy. Although, the scarification with sand paper revoked the seed dormancy, it is perceptible that a longer imbibition time (24 h) was able to cause gradual increase of the emergency speed registered by the first count and ESI. This method results in small damages at the coat region contrary to the micropile of the seed, improving its permeability to oxygen and water and benefiting imbibition and metabolic reactivation with consequent growth of the embryo axis (Martins et al., 2008). These results are in agreement with the others reported for *Sterculia foetida* L. (Santos et al., 2004), *Rollinia mucosa* (Jacq.) Baill (Ferreira et al, 2009) and *Myracrodruon urundeuva* (Fr.All.) (Guedes et al., 2009) seeds scarified with sandpaper and then exposed to water imbibition for 24 h.

In nature, *A. farnesiana* seeds can be overcome by environmental factors. Scarification may occur through acidification, when seeds are eaten by seed disperser animals, like bovids, equids and some rodent species (Camacho et al., 2012), besides soil microorganisms. Additionally, the weakening of the coat of this species is common through the synergic action of fires and floodings in its native environment, caused by the high temperature amplitude (Erkovan et al., 2013).

This way, correlating the data presented in this paper with *A. farnesiana* attractiveness to animal feed, vast seed production (Camacho et al., 2012) along with the

Table 3. Emergency, first count and emergency speed index of *A. farnesiana* seedlings from seeds subjected to different pre-germinative treatments.

Treatments	Emergency(%)	Firstcount(%)	ESI
T_1	0^g	0^f	0f
T_2	5^g	0^f	0.33^f
T_3	12^f	1^f	0.53^f
T_4	45^d	0^f	1.88^e
T_5	49^d	0^f	1.25^e
T_6	32^e	1^f	1.56^e
T_7	79^b	17^d	4.80^c
T_8	15^f	0^f	0.61^f
T_9	9^f	0^f	0.50^f
T_{10}	65^c	0^f	4.14^d
T_{11}	76^b	7^e	4.78^c
T_{12}	84^a	36^c	5.88^b
T_{13}	84^a	59^b	7.00^a
T_{14}	82^a	65^a	6.95^a
T_{15}	4^g	0^f	0.22^f
CV (%)	10.84	20.58	15.08

Means followed by the same lower case letter within a column and by the same upper case letter within a row are not significantly different by Scott-Knot test ($P > 0.05$). T_1: Immersion water 60°C; T_2: Immersion water 70°C; T_4: Immersion water 90°C; T5: Immersion water 100°C; T_6: Sca. Sulfuric Acid 10';T_7: Sca. Sulfuric Acid 20'; T_8: Sca. Sulfuric Acid 10', imb. 12h; T_9: Sca. Sulfuric Acid 10', imb. 12h; T_{10}: Sca. Sulfuric Acid 10', imb. 24h; T_{11}: Sca. Sulfuric Acid 10', imb. 24h; T_{12}: Sca. Sandp.; T_{13}: Sca. Sandp., imb.12h; T_{14}: Sca. Sandp., imb. 24h; T_{15}: intact seeds.

ways to overcome seed dormancy, it is possible to conclude that *A. farnesiana* seeds have a strong capacity to germinate in several habitats, invading and changing the structure and composition of environments and native pastures.

This fact has been by researchers and is a serious problem in savannah areas in Brazil (Erkovan et al., 2013), North America (Arévalo et al., 2010) and in semiarid areas in Australia (Erkovan et al, 2013), based on studies on the probability of invasion and establishment, reproductive potential and dispersion and germination of soil seed banks, respectively. Still, more detailed researches on *A. farnesiana* dormancy and its aspects related to aggressive unities regeneration, like reproductive potential in the presence of disperser animals and long distance dispersion are required for a more precise conclusion.

Conclusion

Scarification with sandpaper 80 followed by imbibition of water at environmental temperature for 24 h, associated with temperatures of 25 and 30°C during germination, can be employed with high efficiency to overcome the mechanical resistance of the coat and to promote germination of *A. farnesiana* seeds and seedlings emergency in a lesser time.

Conflict of Interests

The authors have not declared any conflict of interests.

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Artificial form factor equations for *Tectona grandis* in different spacings

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The use of form factors is a practical alternative for estimating tree volume. However, only a few studies in this topic are relevant to *Tectona grandis*, and only few to those that evaluate the effect of spacing on it. The goal of this study was to generate form factor equations and evaluate spacing influence on it in teak plantations. Samples were taking in 3 × 2 m, 4 × 3 m, 5 × 2 m, and 6 × 2 m plots. The database consisted of rigorous cubage of 120 trees, 30 of which were utilised in spacing for the calculation of the artificial form factor models; these were compared using statistical indicators and graphic analyses. In order to assess possible differences among the equations, the identity test of models at the p < 0.05 significance level was utilised. The results of the identity test of models show that it is possible to utilise a single equation (the reduced model) to estimate the form factor for all spacings, indicating that the factor was not influenced by plantation spacing. It was found that with increasing diameter, teak tree trunks became more cylindrical. Stabilisation occurs starting at a diameter of 27 cm and a height of 25 m, with a form factor of 0.47.

Key words: Teak, volumetric, plantation density.

INTRODUCTION

Tectona grandis L.f. (teak), originally from Asia, produces wood of excellent quality and is considered unequalled for shipbuilding; it is also utilised in the production of fine furniture in luxurious ambients (Lamprecht, 1990). In addition, the use of this species is becoming widespread for building simple furniture, made from panels of young wood, at more accessible prices. To guarantee profits from the cultivation of this specie, it needs to be correctly managed based on information obtained using technical parameters (Drescher et al., 2010). The volumetric function, form function, and form factor are the standard parameters used to assess tree volume. The natural form factor is obtained by measuring the diameter at a relative height (10% of total height). However, in some cases diameters are not measured at the relative height. In such instances, the artificial form factor, based on the

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measurement of the diameter at the breast height (DBH at 1.30 m from the ground) is a more convenient calculation (Van and Akça, 2007; Péllico Netto, 2014). It is important to note that different countries adopt different measuring points (Morais et al., 2014). In most countries, the DBH is measured at 1.30 m above ground level, but 1.37 is used in the USA and 1.2 m is used in Japan and Korea (Van and Akça, 2007).

Because of the difficulty in defining the form of a stem, which may have a variety of forms at different heights, it is common to use the form factor for calculating volume (Campos and Leite, 2013). In such cases, the tree volume is obtained by multiplying the cylinder volume by the form factor, which may be understood as a percentage of the real volume constituted by the volume of a cylinder (Soares et al., 2006). It functions as a factor of reduction of the cylinder volume (Péllico Netto, 2014).

According to Figueiredo et al. (2009), the form factor is an option that should be utilised when greater speed of inventorying is required. Recent diagnoses of forest plantations in Mato Grosso conducted by Shimizu et al. (2007) and Famato (2013) also used this technique. In Karnataka, India, the use of this methodology is also common to volumetric estimates in teak plantations (Tewari et al., 2013). Figueiredo et al. (2009) stressed the importance of calculating volume by using the form factor and considering the diametric class, as well as the importance of other factors, to obtain further reliable estimates. One strategy for obtaining further precise results is to use statistical models to calculate the form factor (Drescher et. al, 2001).

Tree forms may be influenced by different factors, such as species, fertilisation, site quality, management, age, and stand density. Although, the form factor is not a description of the form of the tree, it is nonetheless influenced by it. According to Campos and Leite (2003), because the form factor is measured as a ratio of volumes (that is, the ratio of the volume obtained through rigorous cubage to the cylinder volume), it is possible that identical factor forms for different stems do not necessarily imply that stems have the same form, just as stems of the same form do not necessarily have the same form factor. Cardoso et al. (2014) found the form factor to be superior for pruned plantations of *Pinus taeda* L. and *Pinus elliottii* Engelm, compared to the non-pruned ones at the end of a rotation. In a study of form factor for teak at different ages, Figueiredo et al. (2005) found lower readings at higher ages. Drescher et al. (2010) also developed a form factor for young stands of teak and reported that the values decline and tend to remain constant with increasing tree diameter. Only few studies are available on the form factor for teak and just the research performed by Adegbeihn (1982) was found for this dendrometric attribute for different spacings. Based on this scenery, we aimed to evaluate the influence of spacing on the form factor and to develop artificial form factor equations, with bark, for non-thinning stands of teak in different spacings.

MATERIALS AND METHODS

Location and description of the experiment

The experiment was conducted in the Cáceres campus of the Federal Institute of Mato Grosso (IFMT). The geographic coordinates are 57°40′51″W and 16°11′42″S, and the altitude is 117 m. The climate according to the Köppen classification is Awi (tropical savannahs with humid summer and dry winter) characterised by a rainy tropical climate in which the mean annual temperature varies from 23 to 25°C. The annual pluviometric index is high (1.277 mm). The surface is flat, with soil classified as a red-yellow latosol (Oxisol) (Passos et al., 2006). Teak was planted in December 1998 on a total area of 4 ha. This procedure was carried out manually using stump seedlings with four spacings: 3 × 2 m, 4 × 2 m, 5 × 2 m, and 6 × 2 m. The experimental design involved randomised blocks and 3 blocks were utilised. The pits were fertilised using simple superphosphate and 10 g FTE BR-15. Disbranching occurred at 9, 14, and 22 months (Passos et al., 2006). Thunning was not conducted in any of the blocks. The trees were 15 years old at the time of evaluation.

Sampling and data gathering

In order to select the trees to be sampled, an inventory (or census) of the stand was conducted. The survey was done along the central lines of each parcel, thus, avoiding the edge effect. The circumference at breast height (CBH) of 1.30 m was measured using a measuring tape. The data was then converted into diameter at breast height (DBH). Next, 5 classes of DBH were defined with a breadth of 5 cm, and 10 trees per treatment were selected in each block, thus, 30 trees per spacing. The sample included all diametrical classes; the number of trees sampled was proportional to the frequency observed in the population of each spacing (Table 1). The trees were cut down and the diameter measured at heights of 0.15 m (stump height) and 1.30 m. Afterwards, the trunks were divided into 2 m long sections up to the first bifurcation. Then, the mensuration continued through the principal (thickest) bifurcation, using sections of 1 m, up to a minimum diameter of 5 cm. Volumes were obtained using the Smalian method. The descriptive statistics on the main dendrometric variables for the stands are summarised in Table 2.

Models of form factor

In order to generate the estimates, four models of artificial form factor (Models 1, 2, 3, and 4) were tested. The models were adjusted for spacing, utilising the R software (R Core Team, 2015), and the variables were selected by *stepwise*.

$$ff_{1.3} = \beta_0 + \beta_1\left(\frac{D_{0.3}}{DBH^2}\right) + \varepsilon \quad (1)$$

$$ff_{1.3} = \beta_0 + \beta_1\left(\frac{D_{0.3}}{DBH^2}\right) + \beta_2\left(\frac{1}{DBH^2}\right) + \beta_3 D_{0.5} + \varepsilon \quad (2)$$

$$ln(ff_{1.3}) = \beta_0 + \beta_1 ln\left(\frac{D_{0.3}}{DBH^2}\right) + \varepsilon \quad (3)$$

$$ln(ff_{1.3}) = \beta_0 + \beta_1 ln\left(\frac{D_{0.3}}{DBH^2}\right) + \beta_2 ln\left(\frac{1}{DBH^2}\right) + \beta_3 \ln(D_{0.5}) + \varepsilon \quad (4)$$

Where: ln = Napierian logarithm; $ff_{1.3}$ = artificial form factor; D_i, Hohenadl's relative diameter (cm); HT = total height (m); DBH = diameter at a height of 1.3 m (cm); β_i = parameters of the model; and ε = random error, where $\varepsilon \sim N(0, \sigma^2)$.

Table 1. Total number of sampled trees by diametrical class and spacing of *Tectona grandis*, in Cáceres-MT, Brazil.

Class of DBH (cm)	Spacing			
	3 × 2 m	4 × 2 m	5 × 2 m	6 × 2 m
5–10	2	2	1	0
10–15	9	5	5	2
15–20	16	15	15	11
20–25	3	7	7	14
25–30	0	1	2	3
Total	30	30	30	30

Table 2. Descriptive statistics on diameter at a height of 1.3 m (DBH, cm), total height (HT, m), and volume (V, m³) for stands of *T. grandis* in Cáceres-MT, Brazil.

Spac.	Variables	Mean	Standard deviation	Minimum	Maximum	CV (%)
	DBH	15.29	0.658023	7.5	22.25	23.57
3 × 2 m	HT	12.74	0.544886	5.3	17.03	23.43
	V	0.12964	0.012821	0.01909	0.31246	54.17
	DBH	17.27	0.712667	8.55	25.25	22.60
4 × 2 m	HT	13.98	0.584514	7.3	19.30	22.90
	V	0.17677	0.016854	0.03257	0.45726	52.22
	DBH	17.58	0.846492	6.5	26.05	26.37
5 × 2 m	HT	13.80	0.53121	3.3	19.30	21.08
	V	0.18638	0.017334	0.01094	0.40869	50.94
	DBH	20.42	0.709799	9.1	26.55	19.04
6 × 2 m	HT	14.83	0.454184	10.3	21.30	16.77
	V	0.25194	0.018248	0.05881	0.47337	39.67

Spac. = spacing; CV = coefficient of variation.

The criterion for choosing the best model obeyed initially the lowest relative standard error of the estimate-$S_{yx}\%$ (equation 1), the largest adjusted coefficient of determination-R^2_{ajus} (equation 2), the graphic distribution of the bias-free residuals-$E_{\%}$ (equation 3); and the normality of the residuals, by using the Shapiro–Wilk test, at $p < 0.05$ significance level.

$$S_{yx} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-p}} \therefore S_{yx}\% = \frac{S_{yx}}{\bar{y}}100 \quad (1)$$

$$R^2_{ajus} = 1 - \left(\frac{SQ_{res}}{SQ_{tot}}\right)\left(\frac{n-1}{n-p}\right) \quad (2)$$

$$E_{\%} = \left(\frac{y_i - \hat{y}_i}{y_i}\right)100 \quad (3)$$

Where y_i = observed value of the variable; $\hat{y}_i$ = estimated value of the variable; $\bar{y}$ = arithmetic mean of the observed variable; n = number of data observed; p = number of coefficients in the model; SQres = sum of squares of residuals; and SQtot = total sum of squares.

Identity test of models

For cases in which the same model was selected in different spacings, the equations were compared using the F test, through the Graybill test of identity, at the 5% significance level (Graybill, 2000). The tested hypotheses were as follows: H_0, where all the equations are equal and can be represented by one single common equation, and H_1, where the equations are different and cannot be represented by one common equation. Where the identity test was not significant, detailed graphic analysis were performed in order to evaluate the quality of the estimates. For that, it was used the dispersion of the observed values versus estimates through simple linear regression, and histograms of residues density.

RESULTS AND DISCUSSION

It is worth noting that the statistical indicators of adjustment (R^2_{ajus}), for the form factor equations for the different spacings, ranged from 0.177 to 0.870, and the $S_{yx}\%$, from 19.425 to 6.344%, considering the best and

Table 3. Parameters and statistics of adjustment of the regression models employed to estimate the artificial form factor for *T. grandis* in different spacings.

Spac.	Mod.	β_0	β_1	β_2	β_3	S_{yx}%	R^2_{ajus}
3 × 2 m	1	0.2802	4.6492	-	-	7.147	0.822
	2	**0.1282**	**5.8041**	**-0.6006**	**0.0107**	**6.344**	**0.870**
	3	0.7854	0.4826	-	-	7.354	0.811
	4	0.8057	0.5705	0.0608	0.2656	12.412	0.500
4 × 2 m	1	0.2655	5.3869	-	-	7.223	0.844
	2	**0.1402**	**6.8126**	**-3.8505**	**0.0074**	**6.932**	**0.866**
	3	0.8858	0.5013	-	-	7.476	0.832
	4	0.8388	0.6132	-0.0139	0.1384	13.446	0.496
5 × 2 m	1	0.2994	4.8003	-	-	10.589	0.761
	2	**-0.0691**	**8.1625**	**-6.0636**	**0.0214**	**9.058**	**0.837**
	3	0.6741	0.4280	-	-	11.974	0.694
	4	0.4754	1.1210	-0.4190	-0.0134	19.425	0.252
6 × 2 m	1	0.2958	5.3243	-	-	8.173	0.793
	2	**0.1647**	**4.3792**	**15.795**	**0.0099**	**8.173**	**0.838**
	3	0.7394	0.4374			9.333	0.731
	4	0.5403	0.6964	-0.1249	0.1184	16.924	0.177

Spac. = spacing; Mod. = model; βi = regression coefficient; Syx% = standard error of estimate; and R²ajus = adjusted coefficient of determination. Obs.: the values in boldface correspond to the selected equations. Obs.: numbers in boldface represent the selected model.

worst adjustments, respectively (Table 3). For the denser spacings (3 × 2 m and 4 × 2 m), it is important to note that the adjustments were greater than the ones of the broader spacings (5 × 2 m and 6 × 2 m). Following careful observation of the form factor models, the residual analysis were performed to determine whether a bias was present in the estimates or the assumptions of normality were met (Figure 1). All equations had normal distributions according to the Shapiro–Wilk test (*p-value* ≥ 0.05). Most of the distributions of residuals for Equations 3 and 4 in the larger spacings (5 × 2 m and 6 × 2 m) presented greater dispersion width all along the regression line. From Figure 1, it can be noted that Models 3 and 4, which utilise the dependent and independent variables in their transformed form (*ln*), were the ones that obtained the worst artificial form factor adjustments in comparison with the other studied models. In contrast, Model 2 stands out as the one with the best indicators of precision and adjustment in relation to the adjusted coefficient of determination and the standard error of estimate. This model showed R^2_{ajus} values similar to the best adjustments observed by Drescher et al. (2001) for form factor equations for *P. elliottii*, and inferior to the form factor adjustments for teak obtained by Drescher et al. (2010). Regarding S_{yx}%, the obtained values were similar. Throughout the stages of analysis for model selection, Equation 2 showed the best performance on artificial form factor for teak in the different evaluated spacings. Therefore, since the same equation was selected for the four treatments, the identity test of models was applied assuming that it is possible to reduce the number of employed equations. From the identity test of models, it was observed that the equation derived from the reduced model was not significant, showing an F value (1.023) lower than its tabled value (2.025), indicating that this model is flexible, because it enable to estimate the form factor using one single equation, for spacings 3 × 2 m, 4 × 2 m, 5 × 2 m and 6 × 2 m.

This procedure permitted a 75% reduction in the number of equations. The equation derived from the reduced model (Equation 4) showed values of S_{yx} = 7.535% and R^2_{ajus} = 0.836, as well as a trend-free distribution of residuals, an adequate dispersion of observed versus estimated values, and normality of errors, with the mean centred at zero (Figure 2).

$$ff_{1.3} = 0.0926 + 6.6766\left(\frac{D_{0.3}}{DBH^2}\right) - 3.0141\left(\frac{1}{DBH}\right) + 0.0120D_{0.5} + \varepsilon \quad (4)$$

Where ln = Napierian logarithm; $ff_{1.3}$ = artificial form factor; Di = Hohenadl's relative diameter (cm); HT = total height (m); DBH = diameter at a height of 1.3 m (cm) and ε = random error, with ε~N (0, σ²).

Hohenadl's relative diameters $D_{0.3}$ and $D_{0.5}$ were estimated indirectly, using equations 5 and 6.

$$ln(D_{0,3}) = -0,3554 + 0,3521ln(DBH^2) + 0,1603ln(DBH.HT) + \varepsilon$$
$$R^2_{ajus}=0,88;\ S_{yx}=8,7\% \quad (5)$$

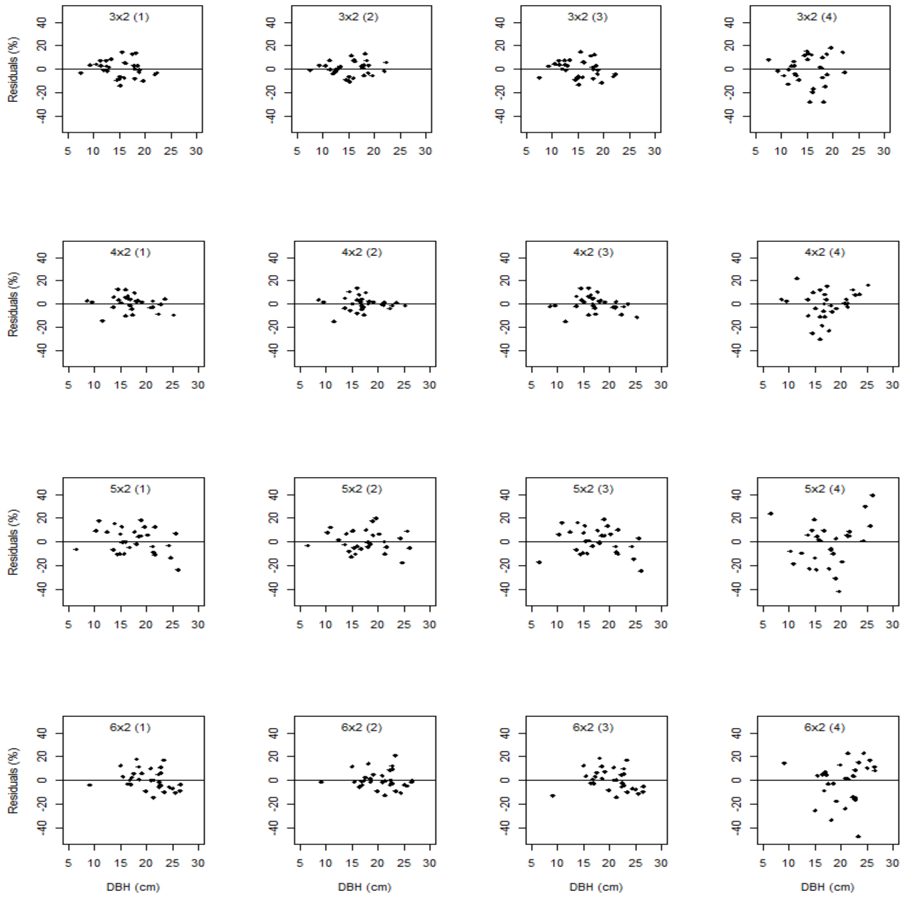

Figure 1. Distribution of residuals for the artificial form factor models tested.

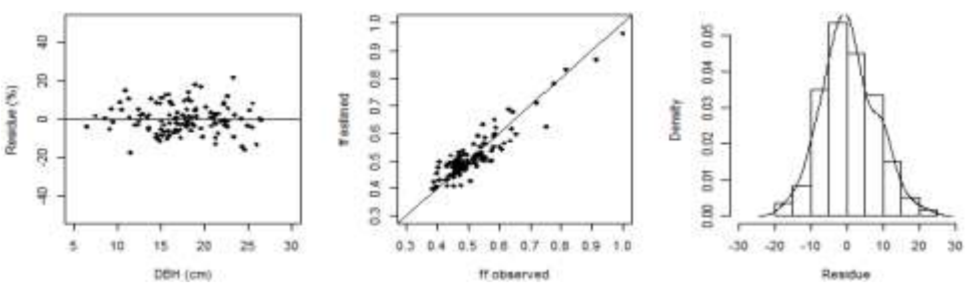

Figure 2. Distribution of residuals, observed versus estimated form factor, and classes of error for the model in its reduced form.

$$ln(D_{0,5}) = -0{,}6736 + 0{,}3089 ln(DBH^2) + 0{,}2221 ln(DBH.HT) + \varepsilon$$

R^2_{ajus}=0,81; S_{yx}=11,4% (6)

Where: ln = Napierian logarithm; Di = Hohenadl's relative diameter (cm); HT = total height (m); DBH = diameter at a height of 1.3 m (cm) and ε = random error, with ε~N (0, σ²). Spacing is one of the silvicultural factors that may affect the form of the trees, reflecting on the form factor. In spite of this, no spacing effect on the dendrometric variable in question was identified. This may be related to the age of the stands, together with fact that no thinning was performed. The different spacings seem to have attained similar growth conditions due to the obvious condition of competition in all stands and the absence of thinning. The first thinning in teak plantations could be carried out in the fifth year of age (Caldeira and Oliveira, 2008). Pérez and Kanninen (2005) observed that different intensities ofthinning had no effect on the form factor of teak. Nevertheless, intense thinning had a positive effect on the trunk form, producing trees with the

Table 4. Artificial form factor for *Tectona grandis* in Cáceres-MT, Brazil.

DBH	Height (m)																				
(cm)	5	6	7	8	9	10	11	12	12	13	14	15	16	17	18	19	20	21	22	23	24	25
6	0.840	0.865	0.887	0.906	0.924	0.940	0.954	0.968	0.968	0.981	0.992	1.004	1.014	1.024	1.034	1.043	1.051	1.060	1.068	1.076	1.083	1.090
7	0.741	0.762	0.781	0.798	0.813	0.826	0.839	0.850	0.850	0.861	0.871	0.881	0.890	0.899	0.907	0.915	0.922	0.929	0.936	0.943	0.949	0.956
8	0.667	0.686	0.702	0.717	0.730	0.742	0.753	0.764	0.764	0.773	0.782	0.791	0.799	0.806	0.814	0.820	0.827	0.833	0.840	0.845	0.851	0.857
9	0.610	0.628	0.642	0.656	0.668	0.678	0.688	0.698	0.698	0.706	0.714	0.722	0.729	0.736	0.743	0.749	0.755	0.761	0.766	0.771	0.776	0.781
10	0.566	0.582	0.596	0.608	0.619	0.628	0.638	0.646	0.646	0.654	0.661	0.668	0.675	0.681	0.687	0.693	0.698	0.704	0.709	0.714	0.718	0.723
11	0.531	0.546	0.558	0.569	0.580	0.589	0.597	0.605	0.605	0.612	0.619	0.626	0.632	0.638	0.643	0.649	0.654	0.659	0.663	0.668	0.672	0.676
12	0.503	0.516	0.528	0.539	0.548	0.557	0.565	0.572	0.572	0.579	0.585	0.592	0.597	0.603	0.608	0.613	0.618	0.622	0.627	0.631	0.635	0.639
13	0.479	0.492	0.503	0.513	0.522	0.531	0.538	0.545	0.545	0.552	0.558	0.564	0.569	0.574	0.579	0.584	0.588	0.593	0.597	0.601	0.605	0.609
14	0.460	0.472	0.483	0.493	0.501	0.509	0.516	0.523	0.523	0.529	0.535	0.540	0.546	0.551	0.555	0.560	0.564	0.568	0.572	0.576	0.580	0.584
15	0.444	0.456	0.466	0.475	0.484	0.491	0.498	0.504	0.504	0.510	0.516	0.521	0.526	0.531	0.536	0.540	0.544	0.548	0.552	0.556	0.559	0.563
16	0.431	0.442	0.452	0.461	0.469	0.476	0.483	0.489	0.489	0.495	0.500	0.505	0.510	0.515	0.519	0.524	0.528	0.532	0.535	0.539	0.542	0.546
17	0.419	0.430	0.440	0.449	0.457	0.464	0.470	0.476	0.476	0.482	0.487	0.492	0.497	0.501	0.506	0.510	0.514	0.518	0.521	0.525	0.528	0.531
18	0.410	0.421	0.430	0.439	0.446	0.453	0.459	0.465	0.465	0.471	0.476	0.481	0.486	0.490	0.494	0.498	0.502	0.506	0.509	0.513	0.516	0.519
19	0.402	0.413	0.422	0.430	0.437	0.444	0.451	0.456	0.456	0.462	0.467	0.472	0.476	0.481	0.485	0.489	0.493	0.496	0.500	0.503	0.506	0.509
20	0.395	0.406	0.415	0.423	0.430	0.437	0.443	0.449	0.449	0.454	0.459	0.464	0.468	0.473	0.477	0.481	0.484	0.488	0.491	0.495	0.498	0.501
21	0.390	0.400	0.409	0.417	0.424	0.431	0.437	0.443	0.443	0.448	0.453	0.457	0.462	0.466	0.470	0.474	0.478	0.481	0.485	0.488	0.491	0.494
22	0.385	0.395	0.404	0.412	0.419	0.426	0.432	0.437	0.437	0.443	0.447	0.452	0.457	0.461	0.465	0.469	0.472	0.476	0.479	0.482	0.485	0.489
23	0.381	0.391	0.400	0.408	0.415	0.421	0.427	0.433	0.433	0.438	0.443	0.448	0.452	0.456	0.460	0.464	0.468	0.471	0.474	0.478	0.481	0.484
24	0.367	0.377	0.386	0.393	0.400	0.406	0.412	0.417	0.417	0.422	0.427	0.431	0.436	0.440	0.443	0.447	0.451	0.454	0.457	0.460	0.463	0.466
25	0.375	0.385	0.394	0.402	0.409	0.415	0.421	0.427	0.427	0.432	0.437	0.441	0.446	0.450	0.454	0.457	0.461	0.465	0.468	0.471	0.474	0.477
26	0.373	0.383	0.392	0.400	0.407	0.413	0.419	0.424	0.424	0.430	0.435	0.439	0.443	0.448	0.452	0.455	0.459	0.462	0.466	0.469	0.472	0.475
27	0.371	0.381	0.390	0.398	0.405	0.411	0.417	0.423	0.423	0.428	0.433	0.437	0.442	0.446	0.450	0.454	0.457	0.461	0.464	0.467	0.470	0.473
28	0.370	0.380	0.389	0.397	0.404	0.410	0.416	0.422	0.422	0.427	0.432	0.436	0.441	0.445	0.449	0.453	0.456	0.460	0.463	0.466	0.469	0.472

desired DBH/totalheight ratio. It should be pointed out that the authors, in the latter case, studied plantations at 8 old, thus, much younger than the plantations in this present study. Adegbeihn (1982) reports that form factor, for 7 years old teak plantations, remained independent of the effect of spacing. Subsequently, the artificial form factor for teak was determined from the artificial form factor model and the equations of relative diameter D0.3 and D0.5. It is presented in Table 4 and its variation may be observed in Figure 3.

It may be observed that in trees with a diameter lesser than 10 cm, the form factors are greater than 0.60. These form factors decline with an increasing diameter, until remaining approximately constant at 0.47. Such stabilisation was also observed by Higuchi (1979) and Drescher et al. (2010) in teak stands in the regions of Cáceres and Santo Antônio do Leverger respectively, in the state of Mato Grosso. Nevertheless, a reduction in form factor values does not necessarily imply tapering of the trunks, because this factor does not directly represent the geometric form of the plant; it is only a reduction factor for the calculation of volume (Burger et al., 1979). A reduction in the form factor curve is also similar when its evolution is assessed as a function of age. Figueiredo et al. (2014) evaluated the form factor for *Araucaria angustifolia* and found that the values were greater than 1.0 up to

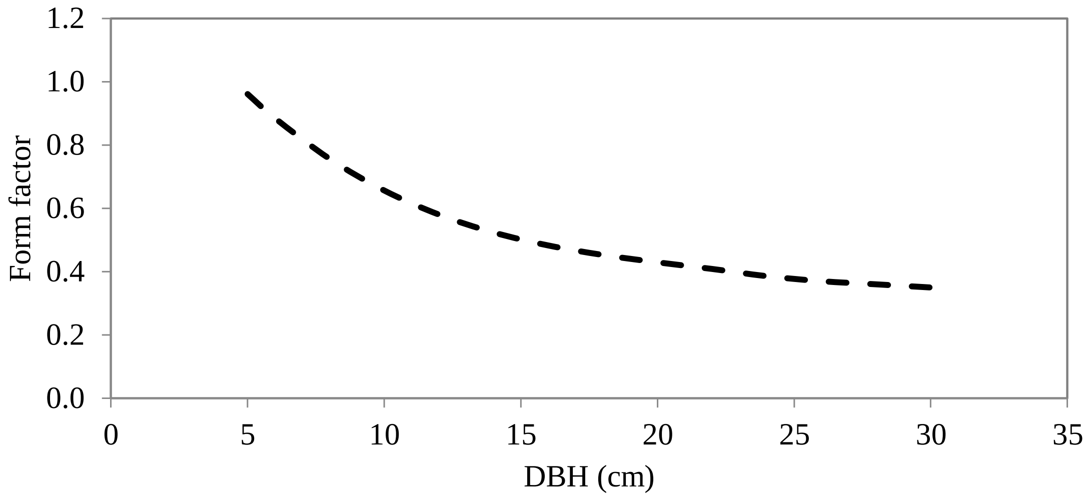

Figure 3. Relationship between the artificial form factor and diameter at a height of 1.3 m (DBH).

4 years of age. The authors' explanation for this finding is that tree volume is greater than cylinder volume at the beginning of development; indeed, the greatest volume is found between the base and the diameter at a height of 1.3 m (DBH). Starting at 4 years of age, the form factor begins to decline, reaching its lowest value (0.58) at 18 years of age. Through the study of form factors, it is possible to observe trends in trees where diameter increases over time, acquiring a more cylindrical form, as also observed in *P. elliottii* by Drescher et al. (2001). Figueiredo et al. (2005) reported a declining form factor in teak as tree age increases.

Conclusion

The equation derived from Model 2 is efficient for estimating the form factor of *T. grandis*. The identity test of models indicated that plantation spacing did not significantly affect the parameters of the model. Thus, a single equation may be employed to estimate form factor in different planting spaces. As expected, from a dendrometric point of view, *T. grandis* trunks become more cylindrical with an increasing diameter; stabilisation occurs starting at a diameter of 27 cm and a height of 25 m, with a form factor of 0.47.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Present status of the black rot disease of tea in Bangladesh

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A survey was carried out in 2015 at Bangladesh Tea Research Institute (BTRI) to ascertain the impact of varieties, topography, age of plants and shade condition on the incidence and severity of Black rot disease in tea. Data were collected monthly from 300 tea bushes selected randomly for each attribute with three replications. Data were recorded on the incidence and severity of the diseases by observing the typical symptom by using 0-5 scoring scale. Disease incidence and disease index was computed by following a referenced formula. In all the cases of attributes, the maximum level of incidence with Black rot disease was found in September but severity with the same was found in July. The incidence and severity of Black rot disease were significantly higher in hellock (23.52 and 16.64%) areas as compared to flat and tillah areas. Age of tea plants had no variation with severity of the disease but a significant variation was found with the incidence. Highest incidence (22.64%) was found in immature tea bushes. More amount of disease was observed in shaded areas as compared to unshaded areas. Seed tea bush (22.64 and 17.07%) was found to be more predisposed to Black rot disease than clones (20.59 and 16.38%). The findings of this study will help to understand the pattern of distribution of the disease in the commercial tea fields so that the planters can escape the diseases easily.

Key words: Incidence, severity, black rot, tea.

INTRODUCTION

Tea is an important cash crop of Bangladesh. It is one of the largest agro-based industries in the country. There are 169 tea estates having about 59.609 thousand hectares of land under tea plantation producing about 63.86 million kg of made tea with average yield of 1239 kg per hectare during 2014 (PDU, 2015). About 2.67% of this production is exported and Bangladesh earns a substantial amount of foreign exchange of US$ 2,867 thousand during 2013-2014 (PDU, 2015). The geographical location of tea growing area is restricted only to some green specks between 21° 30′ and 26° 15′ north latitude and between 89° 0′ and 92° 41′ east longitude (Ahmed, 2005). Tea ecosystem is a complex agro-ecosystem. It comprises tea, shade trees, green

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crops, forest, etc. The intensive mono culture of a perennial crop like tea over an extensive and contiguous area in apparently isolated ecological zones in Bangladesh has formed virtually a stable ecosystem which provided unlimited opportunity for perpetuation and spread of endemic and introduced diseases (Alam, 1999). The architecture of tea plantation, variability of plant types and the systemic interaction of various agro-techniques, intercultural operation, etc. imposes a significant impact on development of diseases. In Bangladesh, the average yield of tea per hectare is quite low as compared to other tea growing countries of the world. Many factors are associated with such low yield. The loss of tea in Bangladesh tea due to various pests, diseases and weeds has been estimated to be about 10-15% (Sana, 1989). Tea being a perennial crop is prone to attack by many pests and diseases. The majority of the diseases in tea are of fungal origin. More than 400 pathogens cause various diseases in tea (Chen and Chen, 1990) viz. foliage, stem and root. Among the diseases, Black rot is a most destructive leaf disease of tea caused by *Corticium theae* Bernard (Ali, 1992). Black rot of tea is of primary nature and is responsible for direct reduction of crop yield (Ali, 1992). The disease attacks the maintenance leaves just below the plucking table. Infected leaves do not falloff but remain hanging and attached to the next leaf by means of small pads of mycelium at the point of contact. Dense shade, bad drainage and sanitation, high humidity etc. are usually considered predisposing factors for the prevalence of the disease. Hellock areas and North tillah slopes are also conductive to the disease (Ali, 1992). Tunstall and Sarmath (1947) recorded a loss in the yield up to 50% on a bush attacked by Black rot when left untreated for four seasons consequently. Tea zone of Bangladesh divided into three units, viz. Tillah- a low hill, are normally up to 300 ft high steeply rounded. Flat are categorised as high flat and low flat. These are slightly undulating to undulating (Sana, 1989). Hellock is the place in between two adjacent tillah.

Microclimate of an area under tea plantation is greatly influenced by the architectures of plantation. In the same area, there are tillah, flat and hellock. Such topographical diversities directly affect penetration of solar radiation, humidity, temperature and air circulation in an area, thus influences plant growth as well as diseases development (Islam and Ali, 2010). There still remain many gaps in the authors' knowledge on the process of different diseases in tea and understanding of the complex relationships between various dimensions. So, this research work was conducted to define the pattern of distribution and severity of Black rot disease in the commercial tea fields towards the appropriate control strategies.

METHODOLOGY

A survey was carried out during February to last November' 2015 at the main farm of Bangladesh Tea Research Institute (BTRI) and its research farm to ascertain the impact of varieties, topography, age of plants and shade condition (Figure 2) on the prevalence and severity of the diseases. Surveyed site was situated under Moulvibazar district (Figure 1). Data were collected monthly from 300 tea bushes selected randomly for each attribute. This survey was replicated thrice for each. Data were recorded on the prevalence and severity of the diseases by observing the typical symptom (Figure 3). These were done by using the following 0-5 scoring scale (Islam and Ali, 2011).

The severity of the disease was expressed in percent disease index (PDI), which was computed following a standard formula as described below (Singh, 2000).

$$\text{Percent disease incidence} = \frac{\text{No.of infected plants}}{\text{Total number of plants counted}} \times 100$$

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

Weather parameter during the surveyed period was collected from the weather regional office, BTRI campus, Srimangal, Government of the People Republic of Bangladesh. Data was subjected to analysis of variance by MSTAT computer programe. Mean separation was done by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The Black rot disease was seen in the field in the month of February with very negligible amount. In all cases of attributes, the maximum incidence was found in September but severity with the same was found in July. From the month of April to May, an increasing trend of disease development was found. During this time, temperature, relative humidity and rainfall were also high (Figure 4). The result in Table 1a and b revealed that seed tea bush was found to be more predisposed (22.64 and 17.07%) to Black rot disease than clones (20.59 and 16.38%). All the year round, a significant protagonist of variety was found for infecting the disease only with the incidence (Table 1a and b). Age of tea plants had no variation with severity of the disease but a significant ($p= 0.01$) variation was found with the incidence. Highest incidence (22.64%) was found in immature tea bushes and severity (16.88%) in mature tea (Table 2a and b). Table 3a and b represents the result of topographical variation for infection of the Black rot disease. The maximum and statistically dissimilar incidence was found in hellock areas (23.52%) followed by flat areas (22.52%). Hellock areas has also a great impact on disease severity, which is 16.46% followed by flat areas. But the degrees of severities with all topographical areas were statistically ($p= 0.01$) identical. Another element shade revealed the significantly different impact on disease severity but similar impact on incidence. More amount of disease was observed in shaded areas as compared to unshaded areas. In shaded condition, both incidence and severity were high these were 20.83 and 16.44%, respectively (Table 4).

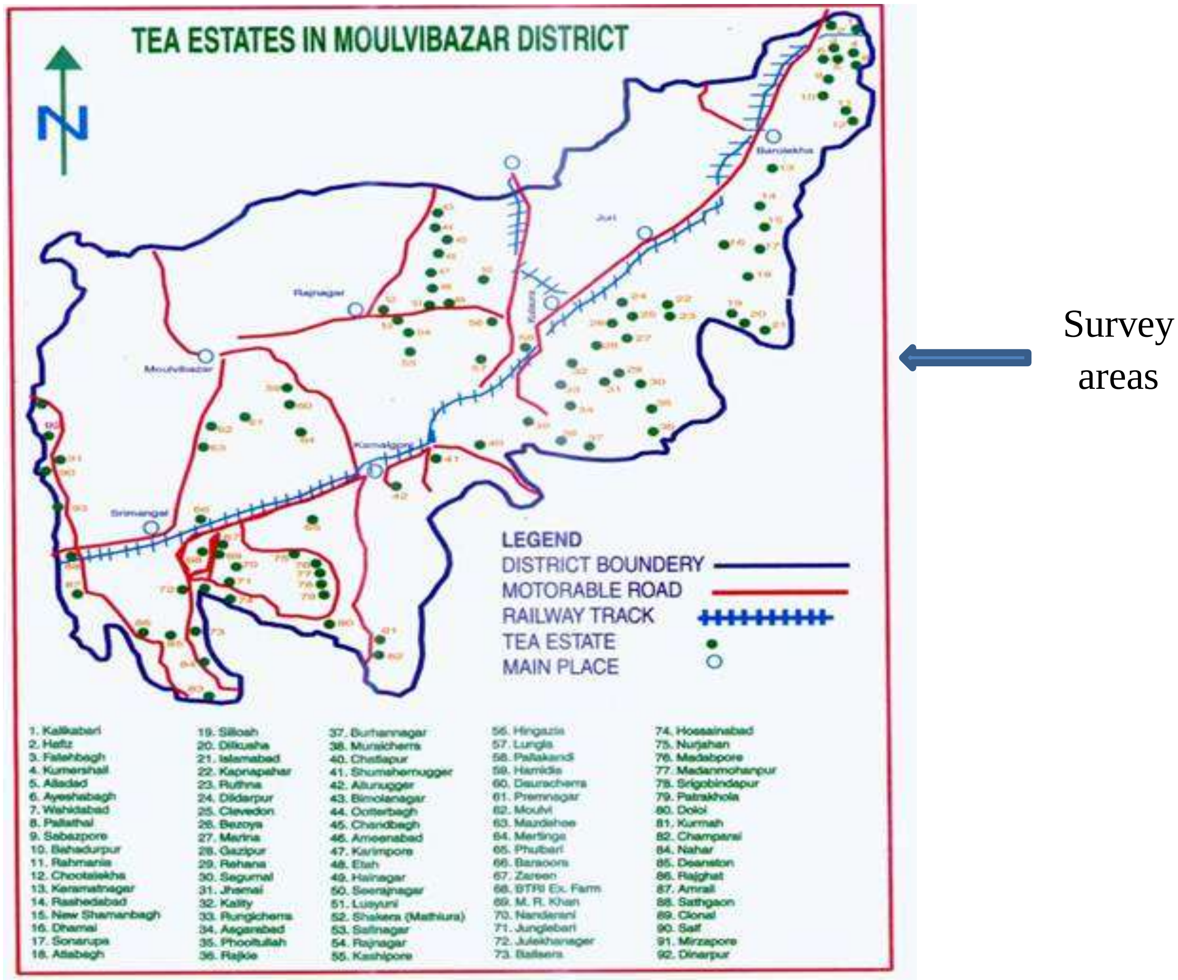

Figure 1. Map of survey areas.

At the end of the monsoon, the disease causing fungus, *Corticium theae* Bernard produced resting bodies in the soil, creaks and crevices of the infected stems. The active mycelium died during the dormant season which produces new mycelium again during the next monsoon (Ali, 1992). The disease became evident after about four weeks following the onset of rains. Mature leaves and adjacent stems were infected by the disease causing organisms (Sarmah, 1960). Islam and Ali (2010) specified as the highest incidence of Black rot was noticed only on seedling and highest in flat areas. Mooi (1965) and Stewart (1990) reported that very young plants are susceptible to late blight, plants of intermediate age are the most resistant, and old plants become more susceptible again. A study conducted on *Chrysanthemum morifolium* found that leaf age affected the number and size of lesions caused by *Pseudomonas cichorii*, with older leaves being less susceptible to infection than younger, immature leaves (Jones et al., 1985). Conversely, Sanchez et al. (2003) found that in coffee (*Coffea arabica*), infection by *P. cichorii* was more frequent on older leaves. In the case of cocoa, coffee or tea plantations, agroforestery systems could also modify pests and disease incidence when compared with mono specific plantations and the effect of shade trees on diseases could have several explanations (Beer, 1987; Beer et al., 1989; Guyot et al., 2008; Schroth et al., 2000). The shade reduces sunlight and particularly UV-B, which plays an important role in some plant diseases such as blister blight of tea (Gunasekera et al., 1997); shade modifies the micro-environmental conditions (reduced temperature, reduced temperature fluctuations, reduced air movements and increased humidity) and creates a “phylloclimate” able to perturb interactions between pathogens and target organs (Chelle, 2005); and shade can also work as a barrier and can limit the splash dispersal of the pathogen (Ntahimpera et al., 1998). Young fruit and leaf tissues of citrus are more susceptible to *Xanthomonas axonopodis* pv. *citri* (citrus canker) than mature tissues (Verniere et al., 2003). The

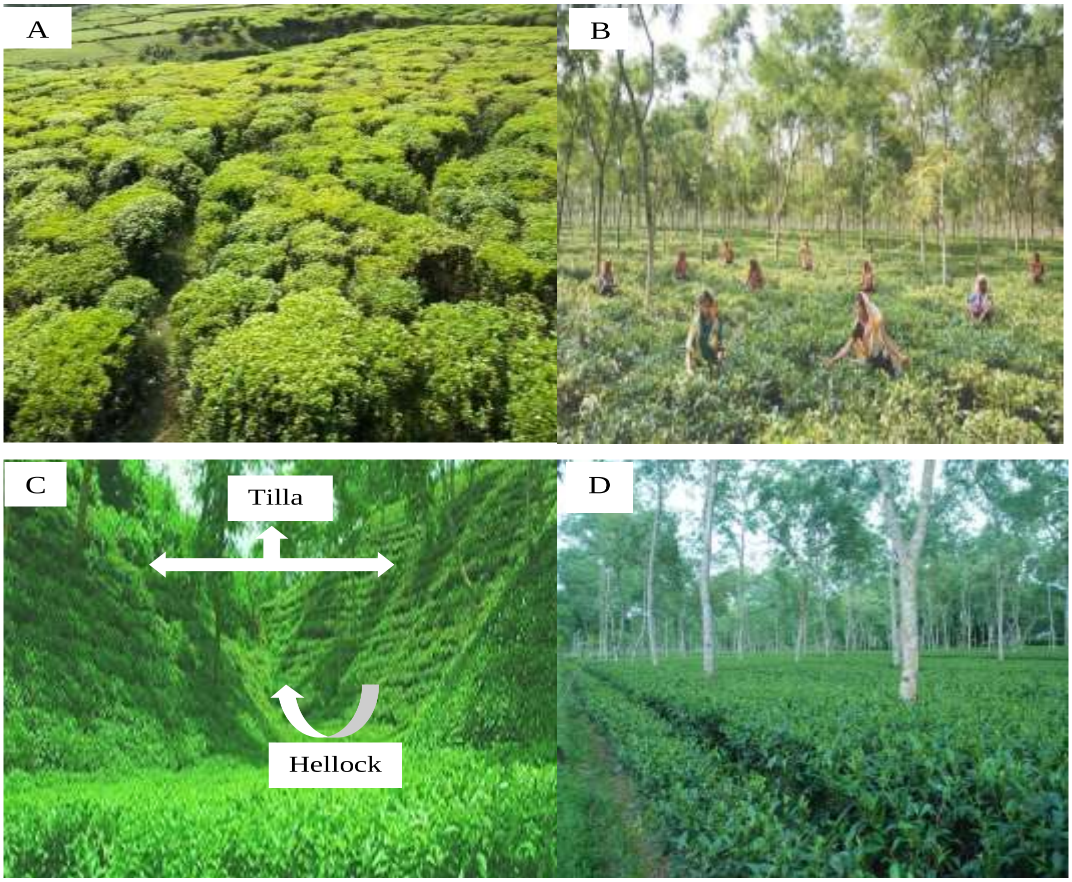

Figure 2. The surveyed fields were mentioned as A = Mature tea plantation with un-shade condition, B = Immature tea plantation in flat areas with sufficient shade, C = Tea plantation in tillah and hellock areas and D = Mature tea plantation in flat areas with sufficient shade.

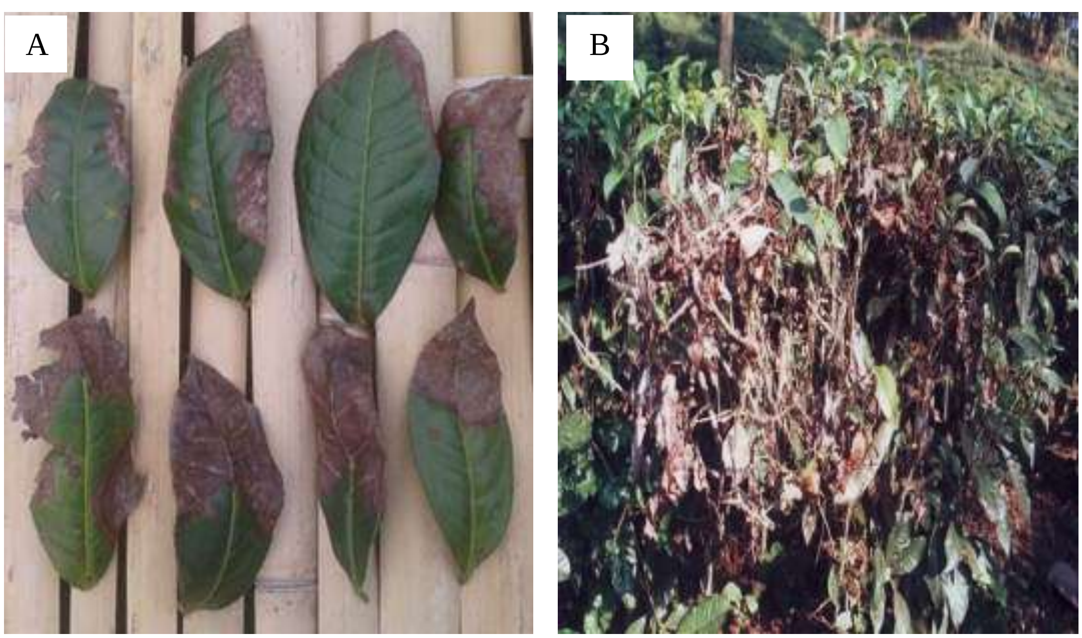

Figure 3. A= Black rot infected lea leaves and B= Black rot infected tea bush.

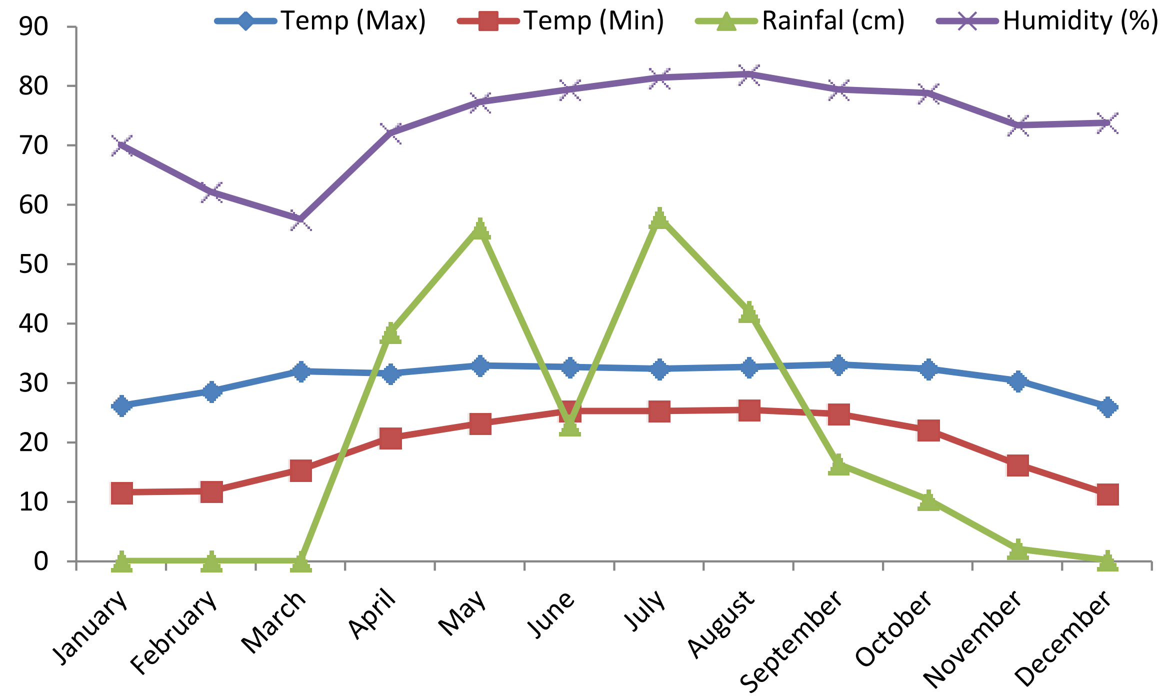

Figure 4. Temperature (°C), rainfall (cm) and humidity (%) during 2015.

Table 1a. Varietal impact on month wise pattern of disease incidence and severity of Black rot disease of tea.

Month	Variety			
	Clone		Seedlings	
	Disease incidence (%)	Disease severity (PDI)	Disease incidence (%)	Disease severity (PDI)
March	8.61^{h}	2.72^{h}	9.47^{h}	3.16^{g}
April	11.01^{g}	4.10^{g}	12.11^{g}	5.62^{f}
May	14.39^{f}	28.50^{c}	15.83^{f}	26.65^{c}
June	21.09^{e}	29.99^{b}	23.19^{e}	31.10^{b}
July	24.66^{c}	33.29^{a}	27.12^{c}	36.00^{a}
August	26.97^{b}	23.18^{d}	29.67^{b}	26.23^{c}
September	27.86^{a}	14.84^{e}	30.64^{a}	12.54^{d}
October	26.90^{b}	6.64^{f}	29.59^{b}	8.91^{e}
November	23.82^{d}	4.13^{g}	26.20^{d}	3.43^{g}

*Numbers followed by the same letters within a column are statistically similar according to a least significant difference test (P < 0.05).

Table 1b. Showing different grades against different infection level

Infection (%)	Grade/scale
Nil	0
1- 20	1
21- 40	2
41- 60	3
61- 80	4
>80	5

intimate mixing of varieties is a proven method in certain cropping systems of introducing diversity to reduce plant disease (Wolfe, 1985, Akem et al., 2000 and Zhu et al., 2000). Variety mixtures may reduce disease in three basic ways (Finckh and Wolfe, 1998; Mundt, 2002): (a) resistance induction, by avirulent spores preventing or delaying infection by adjacent virulent spores; (b) barrier effects, with resistant plants acting as barriers to pathogen spread and (c) dilution of susceptible, where there is an increased distance between plants of the most susceptible component for any particular pathogen

Table 2a. Impact of age on the pattern of disease incidence and severity of Black rot of tea.

Month	Age of tea plant			
	Mature		Immature	
	Disease incidence (%)	Disease severity (PDI)	Disease incidence (%)	Disease severity (PDI)
March	9.47[h]	2.99[g]	8.51[g]	2.93[g]
April	12.11[g]	5.44[f]	11.19[f]	4.98[g]
May	15.83[f]	26.52[c]	14.55[e]	28.42[c]
June	23.19[e]	30.47[b]	20.91[d]	31.26[b]
July	27.12[c]	35.97[a]	24.84[c]	33.91[a]
August	29.67[b]	26.18[c]	26.88[b]	24.13[d]
September	30.64[a]	12.66[d]	27.93[a]	14.25[e]
October	29.59[b]	8.64[e]	27.02[b]	7.39[f]
November	26.20[d]	3.16[g]	24.05[c]	3.84[g]

*Numbers followed by the same letters within a column are statistically similar according to a least significant difference test (P < 0.05).

Table 2b. Variation of incidence and severity of Black rot disease due to variety

Variety	Disease incidence (%)	Disease severity (PDI)
Clone	20.59	16.38
Seedlings	22.64	17.07
LSD (P=0.01)	0.014	1.60
CV (%)	0.02	2.72

Table 3a. Topographical variation on pattern of disease incidence and severity of Black rot.

Month	Topography					
	Tillah		Flat		Hellok	
	Disease incidence (%)	Disease severity (PDI)	Disease incidence (%)	Disease severity (PDI)	Disease incidence (%)	Disease severity (PDI)
March	8.61h	2.06h	9.12f	1.74f	9.19g	3.03f
April	11.01g	4.09g	11.63e	4.22ef	11.74f	5.30ef
May	14.39f	28.49c	15.64d	23.00c	15.68e	25.70c
June	21.09e	29.98b	23.55c	27.82b	23.14d	29.82b
July	24.66c	33.27a	26.82b	32.64a	26.97c	34.79a
August	26.97b	23.20d	30.00a	22.74c	29.41b	24.59c
September	27.86a	14.85e	30.18a	13.36d	30.39a	13.37d
October	26.90b	6.65f	29.67a	7.01e	29.70ab	7.91e
November	23.82d	4.13g	26.13b	3.90f	26.42c	3.63f

*Numbers followed by the same letters within a column are statistically similar according to a least significant difference test (P < 0.05).

Table 3b. Statistical analysis of impact of age on disease incidence and severity

Age of plant	Disease incidence (%)	Disease severity (PDI)
Mature	20.65	16.88
Immature	22.64	16.79
LSD (P=0.01)	0.255	3.81
CV (%)	0.34	6.45

genotype. Variety diversification may also help to reduce pesticide input in conventional systems (Andrivon et al., 2003). The research findings were reviewed with the above findings of other plantations and fruits crops. All these reviewed findings matched the present findings.

Survival and dissemination of the pathogen, nature of infection, mode of penetration of the pathogen etc. are greatly influenced by the various environmental factors. Temperature, humidity, light penetration, ventilation, etc. are the important factors for aggressiveness of the pathogen as well as disease development. These factors varies from one tea section to another section with varies of tea plant architecture, shade condition, topography, age and varieties. The findings of this study will be helpful to understand the pattern of distribution of the disease in the commercial tea fields so that the planters can escape the disease easily. These findings met the objectives of the present study and are also helpful for further study regarding the control of the disease.

Conflict of Interests

The authors have not declared any conflict of interests.

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