

INFLUENCE OF ENVIRONMENTAL FACTORS ON CACAO BIOMETRIC ATTRIBUTES

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This study aimed to investigate the influence of different sites of cacao (*Theobroma cacao* L.) cultivation, represented by different soils and cropping systems, on biometrics attributes of pods and beans. The 12 study sites are in the cacao region of Bahia, Brazil, in the humid zone. The variation and the statistical differences between the means of these attributes clearly indicate the influence of genotype and environment interaction. Attributes pod wet biomass, beans with mucilage wet biomass and number of beans were positively correlated with the cultivation sites represented by Argisol Red-Yellow Eutrophic cambisolic and Argisol Red-Yellow Dystrophic Cohesive abrupt soils. The highest values of length and dry weight of dry cacao beans correspond to Argisol Red-Yellow Eutrophic cambisolic. The dystrophic soils are related to lower dry weight values of cacao beans. The selection of cacao cropping sites by biometric attributes also highlighted the Argisol Red-Yellow Eutrophic cambisolic and Argisol Red-Yellow Dystrophic Cohesive abrupt soils. Understanding the variability of cacao biometric attributes emphasizes the importance of using technologies for achieving sustainable production of cacao with quality.

Key words: *Theobroma cacao* L., plant biometrics, post-harvest, cacao quality.

Influência de fatores ambientais sobre atributos biométricos do cacau. Este estudo teve como objetivo investigar a influência de diferentes locais de cultivo de cacau (*Theobroma cacao* L.), representados por solos e diferentes sistemas de cultivo, sobre atributos biométricos de frutos e amêndoas. Os 12 locais de estudo estão na região cacaueira da Bahia, Brasil, na zona úmida. A variação e as diferenças estatísticas entre as médias destes atributos indicam claramente a influência da interação genótipo e ambiente. Os atributos biomassa úmida de frutos, biomassa úmida de amêndoas com mucilagem e número de amêndoas foram positivamente correlacionados com os locais de cultivo representados pelos solos Argissolo Vermelho-Amarelo Eutrófico cambissólico e Argissolo Vermelho-Amarelo Distrófico coeso abrupto. Os maiores valores de comprimento e peso seco de amêndoas de cacau correspondem ao Argissolo Vermelho-Amarelo Eutrófico cambissólico. Os solos distróficos estão relacionados com menores valores de peso seco de amêndoa de cacau. A seleção de locais de cultivo de cacau por atributos biométricos também destacou o Argissolo Vermelho-Amarelo Eutrófico cambissólico e Argissolo Vermelho-Amarelo Distrófico coeso abrupto. A compreensão sobre a variabilidade de atributos biométricos do cacau enfatiza a importância do uso de tecnologias para uma produção sustentável do cacau de qualidade.

Palavras-chave: *Theobroma cacao* L., biometria de plantas, pós-colheita, qualidade do cacau.

Introduction

Cacao (*Theobroma cacao* L.) is a tropical species whose agricultural production directly involves approximately six million people (FAO, 2003). Cacao beans are the main raw material for the manufacture of chocolate (Beckett, 2009). However, cacao is still produced by small farmers with a daily income equivalent to US \$ 1.25, below the absolute poverty line (Cocoa Barometer, 2015). To reverse this panorama is primarily needed to invest in agricultural education and finance projects that enable producers to add value to the crop. In this actions it is important the joint engagement of cacao science and governmental institutions. For example, among current technological and environmental challenges facing the world cacao production (FAO, 2003; WCF, 2014) can be cited: increased production and productivity by deployment of higher yielding and tolerant cultivars of the main diseases and pests (Pereira, 2001; Ayestas et al., 2013; Dias, 2001; Lopes et al., 2011; Monteiro & Ahnert, 2012; Muniz et al., 2013), phytotechnical use of technology and agricultural mechanization in the stages of planting, pruning, harvesting and post-harvest processing (Adzimah; Asiam, 2010; Bentley et al., 2004; Caires et al., 2014; Icco, 2009; Somarriba, 2004; Wood & Lass, 1985), irrigation (Carr & Lockwood, 2011), the use of crop residues (Sodré et al., 2012), intercropping (Icco, 2010; Müller & Gama-Rodrigues, 2012; Ruf, 2015), and quality certifications related to environmental services and the nutritional quality of the produced beans (Afoakwa, 2010; Amores et al., 2009; Badrie et al., 2015; Jacobi et al., 2015; Obeng & Aguilar, 2015).

In this technological context, biometric characterization of pods and cacao beans is important for studies of production and productivity of the crop and may contribute to the selection of genetic materials with better agricultural performance (Alexandre et al., 2015; Dias & Resende, 2001; Kobayashi et al., 2001). The high genetic variability in cacao requires these agronomic components in assessment strategies that should be considered by breeding programs (Lopes et al., 2011).

Cacao genotypes tolerant to witches' broom, disease caused by the fungus *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora, have been propagated as the main strategy to control the disease

(Pereira, 2001; Lopes et al., 2011; Monteiro & Ahnert, 2012). This research is based on Porto Híbrido 16 (PH-16), a clonal variety selected from a population of interclonal crosses between cacao trees of the Forastero (Amazon) and Trinitario groups (whose parents are unknown), located at Porto Híbrido farm in São José da Vitória, Bahia, Brazil (Moreau Cruz et al., 2013).

The objective of this study was to investigate the influence of different sites, represented by different soils and cropping systems on biometrics attributes of pods and cacao beans.

Materials and Methods

Sites

The 12 study sites are located in the cacao region of Bahia (Table 1) in the humid zone, according to the climatic classification of Thornthwaite: B4r A', B3r A', B2r A', B2r B', B1r A', B1r' A', B1w A' (SEI, 2014). These sites (Table 1), are cultivated with PH-16 under different cropping systems, different averages density of shade trees per hectare in different soils according to the Brazilian System of Soil Classification (SiBCS) (2006) and its correspondence in Soil Survey Staff (2006) (Figure 1; Table 1).

Soil and cacao pods sampling

Soil is the factor that stratifies the environment of the sample source and the experimental units are the cacao trees grafted with PH-16 from which pods were collected.

Each study site with approximately one hectare was divided into three collection areas, characterized by the same soil and same cropping system (Table 2). Pods were collected at a distance of approximately 100 m from the soil identifying points for SiBCS (EMBRAPA, 2006) in the three collection areas (Table 2). Thus, the origin of each sample of pods and beans corresponds to a properly identified and classified soil in each study site, as shown in the example of Table 2.

The beans and beans mucilage were obtained from a composed sample of 50 mature pods (Table 2).

Cacao sampling occurred on November of 2008, during the second harvest period (August to January).

Table 1 - Summary information about the study sites (soils) cultivated with PH-16 cacao genotype in the cacao region of Bahia, Brazil

Site	Geographic Coordinates	City	Acronym of the SiBCS ¹	Soil Classification	Soil Taxonomy	Cropping Systems	Average density of shade trees/ha
1	13° 40' 30" S, 39° 14' 27" W	Nilo Peçanha	LAd cam	Latosol Yellow Dystrophic cambisolic	Hapludox	Cacao ² x Rubber Tree ³	150
2	13° 44' 38" S, 39° 30' 10" W	Gandú	PVAd	Argisol Red-Yellow Dystrophic tipic	Hapludult	Cacao ² x Erythrina ⁴	60
3	13° 45' 21" S, 39° 20' 25" W	Pirai do Norte	PVAd	Argisol Red-Yellow Dystrophic abrupt	Hapludult	Cabruca ⁵	60
4	13° 46' 07.0" S, 39° 17' 52.0" W	Ituberá	LAd	Latosol Yellow Dystrophic tipic	Typic Hapludox	Cacao ² x Rubber Tree ³	350
5	13° 51' 08" S, 39° 17' 54" W	Ituberá	LVAd	Latosol Red-Yellow Dystrophic tipic	Typic Hapludox	Cacao ² x Rubber Tree ³	400
6	14° 31' 14" S, 39° 15' 45" W	Uruçuca	PVAe cam	Argisol Red-Yellow Eutrophic cambisolic	Hapludalf	Cabruca ⁵	50
7	14° 51' 36" S, 39° 14' 42" W	Itabuna	CXd	Cambisol Haplic Dystrophic tipic	Dystropept	Cabruca ⁵	35
8	14° 51' 47" S, 39° 06' 47" W	Ilhéus	LVAd arg	Latosol Red-Yellow Dystrophic argisolic	Hapludox	Cabruca ⁵	70
9	15° 17' 04" S, 39° 28' 43" W	Arataca	PAd lat	Argisol Yellow Dystrophic latosolic	Hapludult	Cabruca ⁵	35
10	15° 23' 08" S, 39° 26' 04" W	Camacan	PVAd	Argisol Red-Yellow Dystrophic tipic	Hapludult	Cabruca ⁵	35
11	15° 23' 15.1" S, 39° 25' 48.6" W	Camacan	PVA ali	Argisol Red-Yellow Alitic tipic	Hapludult	Cabruca ⁵	35
12	16° 29' 02" S, 39° 23' 56" W	Porto Seguro	PVAd coe	Argisol Red-Yellow Dystrophic Cohesive abrupt	Hapludult	Cacao ² x Rubber Tree ³	400

¹SiBCS - Brazilian System of Soil Classification (EMBRAPA, 2006). ²*Theobroma cacao* L. ³*Hevea brasiliensis* (Willd. Ex Adr de Juss) Muell. Arg. ⁴*Erythrina fusca* Lour. ⁵Cabruca is an ecological system of agroforestry cultivation where cacao trees are grown under native trees of the Atlantic Forest of South of Bahia (Lobão et al., 2007).

Table 2 - Summary information of pods and cacao beans sampling

Site ¹	SiBCS ²	Cropping Systems	Collection Area (100 m radius)	Simple Sample		
				Pod ³ (Post-Harvest processing)	Pod ⁴ (biometrics)	Bean (biometrics)
1	LAd cam	Cacao x Rubber Tree	1	50	15	90
			2	50	15	90
			3	50	15	90
...
12	PVAd coe	Cacao x Rubber Tree	1	50	15	90
			2	50	15	90
			3	50	15	90

¹Site: area of approximately 1 hectare. ²SiBCS: Brazilian System of Soil Classification (EMBRAPA, 2006). ³Total number of pods used for cacao post-harvest processing. ⁴Number of pods used in biometric evaluation.

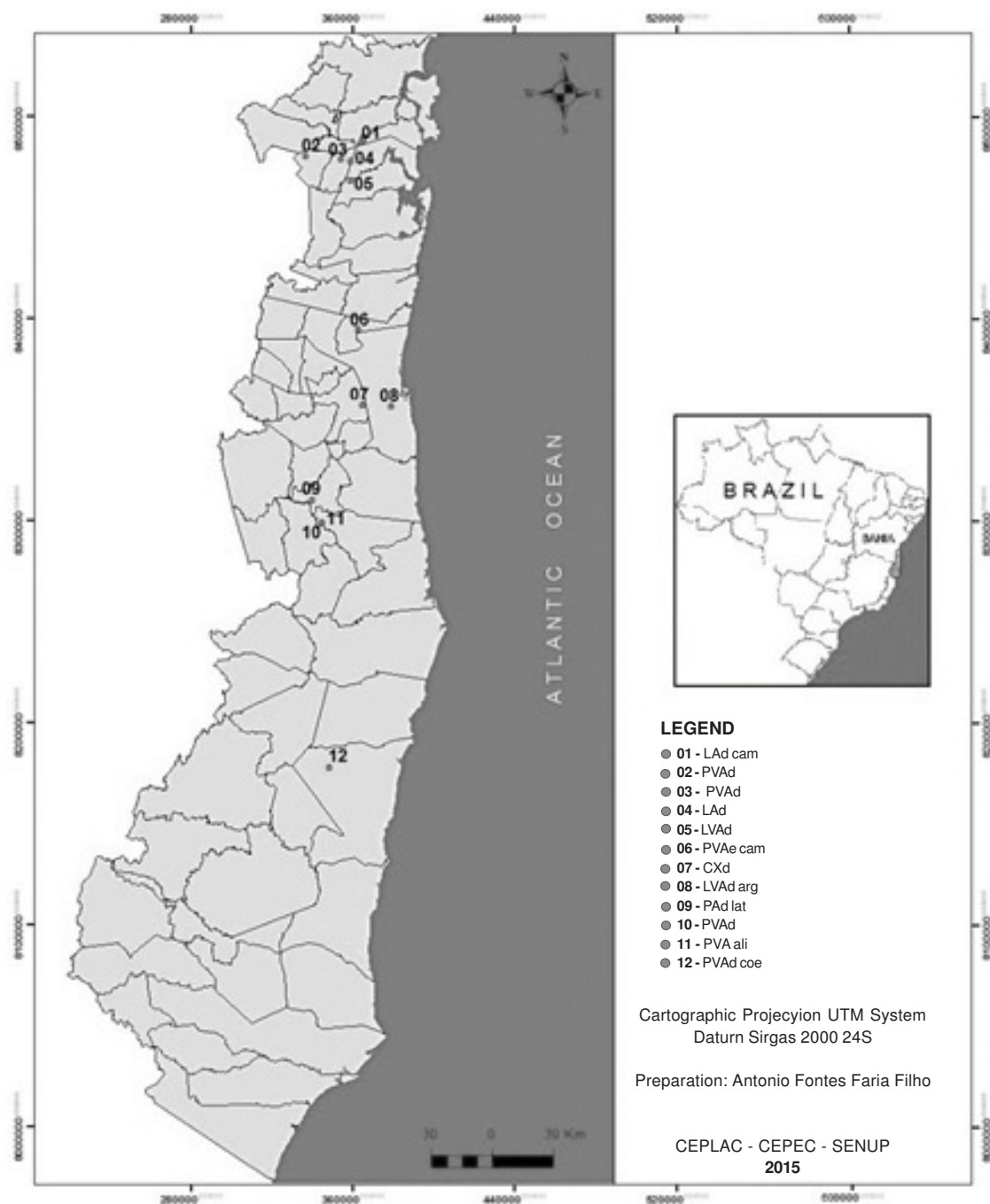


Figure 1 - Map with the geographic scope of the study sites, represented by 12 soils cultivated with PH-16 cacao genotype.

Biometric characterization of pods

15 pods chosen of the 50 pods collected were used to represent the three repetitions of each study site to make biometric characterization (Table 2). The attributes: number of beans per pod, pod wet biomass, husk wet biomass, content of pod (beans with mucilage and placenta) wet biomass were evaluated. The wet biomass was determined with a semi-analytical balance.

Biometric characterization of beans

The physical dimensions of length, width and thickness were determined with a digital pachymeter of 200 mm in the cacao beans.

The dry cacao beans weight from moisture between 6-7% was determined by semi-analytical balance.

The moisture content of cacao beans was determined gravimetrically, using an oven with air circulation at a temperature of 105°C to constant weight according to the AOAC method 977.10 (2005).

Statistical Analysis

Statistical procedures used in this study were performed in the R Core Team program (2013). Package 'stats': Shapiro-Wilks normality test, Bartlett homoscedasticity test (R Development Core Team, 2013). Package 'nortest': Kolmogorov-Smirnov normality test (Lilliefors correction) (Gross & Ligges, 2012). Package 'MASS': Box-Cox transformation (Ripley et al., 2015). Package 'ExpDes': Analysis of Variance (ANOVA) and Scott-Knott test (Ferreira, Cavalcanti, & Nogueira, 2013). Package 'Lattice': Graphics (Sarkar, 2015). Package 'bpca': Biplot applied to Principal Component Analysis (Faria et al., 2013).

Selection of sites by cacao biometric attributes

The scores and sums of scores to select the sites related to the best cacao production characteristics were obtained from the groups generated by multiple mean comparisons by the Scott-Knott test.

Results and Discussion

Pods biometrics

The mean pod wet biomass of 653 g found in this study (Table 3) was similar to the mean of 655.6 g observed in another study with the same cacao genotype (Moreau Cruz et al., 2013). The cacao pod

has many morphologically difference between genetic materials (Kobayashi et al., 2001). It is subjected to environmental influences and physiological changes during the various stages of growth and development, when changes occur in their biometric attributes (ALmeida & Dias, 2001; Britto and Silva, 1983; Lopes, 2000; Machado & Almeida, 1989). In this evaluation, it is hypothesized that the higher the wet biomass from ripe pod, the higher the increase in production associated with the weight of beans (Table 3).

In a study with the same clone, it was observed a mean cacao husk wet biomass value of 475.8 g (Moreau Cruz et al. 2013), lower than the mean value of 527 g found in this work (Table 3). Biometric attributes of the cacao husk are heavily influenced by physiological maturation processes and it is an important component of pod wet biomass (Britto & Silva, 1983; Machado & Almeida, 1989). Our work highlights the cacao bean wet biomass as an important production component in the biomass partitioning. This study highlights the group of lowest means of husk wet biomass (Table 3), because husk wet biomass is not considered a positive characteristic for post-harvest processing.

Pod wet biomass content has an overall mean of 121g (Table 3). In a study conducted with the same genotype (Moreau Cruz et al., 2013) there is no information about the pod wet biomass content of this component, however, adding the means of beans with mucilage (pulp) and placenta wet biomasses gives an approximate value of 175.4 g, nearly 30% higher than the value observed in this study (Table 3). It is considered that the highest cacao pod wet biomass content (Table 3) is associated with an increase in final weight of the dry beans. Cacao pod wet biomass is also related also with environmental conditions like rain, humidity and temperature (Britto & Silva, 1983).

In Argisol Red-Yellow Dystrophic Cohesive abrupt production of fresh beans was approximately twice the amount produced in LAd - Yellow Latosol dystrophic tipic (Table 3). This result suggests that the soil eutrophic character was responsible for the difference in production, but environmental factors are also important to define the productivity and quality of cacao beans (Britto & Silva, 1983; Dantas, 2011; Pinto, 2013).

It is observed that both ANOVA coefficients of variation for wet biomass attributes, pod, husk and content of pod (Table 3) are approximate values, which

Table 3 - Summary of analysis of variance, Scott-Knott test and descriptive analysis of the wet biomass of pod, husk and content of pod of PH-16 cacao clone

Source	DF	Pod	Husk ---- g ----	Content of Pod ¹
		Mean Square		
Soil ²	11	83255**	74593**	4399**
Error	168	19292	13704	678
Total	179			
CV (%)		21,2	22,2	21,5
Soil		Mean ± Standard Deviation (n = 15)		
01 LAd cam		681 ± 117 a	541 ± 105 a	134 ± 24 a
02 PVAd		720 ± 119 a	591 ± 116 a	126 ± 17 b
03 PVAd		573 ± 159 b	459 ± 136 b	108 ± 33 b
04 LAd		611 ± 102 b	520 ± 90 a	83 ± 16 c
05 LVAd		757 ± 185 a	621 ± 161 a	127 ± 32 a
06 PVAd cam		711 ± 152 a	555 ± 138 a	151 ± 19 a
07 CXd		674 ± 132 a	547 ± 11 a	123 ± 31 a
08 LVAd arg		535 ± 139 b	420 ± 127 b	109 ± 26 b
09 PAd lat		599 ± 147 b	467 ± 119 b	130 ± 34 a
10 PVAd		742 ± 152 a	631 ± 130 a	108 ± 26 b
11 PVA ali		673 ± 145 a	545 ± 128 a	124 ± 27 a
12 PVAd coe		564 ± 92 b	432 ± 77 b	128 ± 20 a
		Overall mean (n = 180)		
Minimum		310	240	53
Mean ± Standard Deviation		653 ± 152	527 ± 135	121 ± 30
Maximum		1060	950	192

¹Soil: 01 LAd cam - Latosol Yellow Dystrophic cambisolic, 02 PVAd - Argisol Red-Yellow Dystrophic tipic, 03 PVAd - Argisol Red-Yellow Dystrophic abrupt, 04 LAd - Latosol Yellow Dystrophic tipic, 05 LVAd - Latosol Red-Yellow Dystrophic tipic, 06 PVAd cam - Argisol Red-Yellow Eutrophic cambisolic, 07 CXd - Cambisol Haplic Dystrophic tipic, 08 LVAd arg - Latosol Red-Yellow Dystrophic argisolic, 09 PAd lat - Argisol Dystrophic latosolic, 10 PVAd - Argisol Red-Yellow Dystrophic tipic, 11 PVA ali - Argisol Red-Yellow Alitic tipic, 12 PVAd coe - Argisol Red-Yellow Dystrophic Cohesive abrupt. DF - Degrees of Freedom. CV - Coefficient of Variation. Significance levels by test F: (**) = 1% of error.

can display a standard for the variability of biometric attributes of PH-16.

The overall mean of the beans with mucilage wet biomass corresponds to 92 g (Table 4), lower than the mean of 148,4 g observed in another study with PH-16 (Moreau Cruz et al., 2013).

The variation of the beans with mucilage wet biomass (CV = 24.6%) (Table 4) can be related to biometrics and physiological differences of the pod at harvest, particularly related to the hydration and beans mucilage. The final weight of the dry cacao beans is related to wet biomass of in natura beans, being an important production component (Beckett, 2009; Britto and Silva, 1983; Engels et al., 1980; Lopes, 2000; Sánchez et al., 1996).

Placenta wet biomass content observed in this study corresponds to 29 g (Table 3) was higher than the mean value of 27 g for the same genotype (Moreau Cruz et al., 2013). For processing, the placenta is removed so that the beans are fermented with mucilage (Wood, 2001). This study highlights the group of lowest means of placenta wet biomass (Table 3), considering that the higher weight of placenta in natura suggests that the pods are not in the ripening ideal point to post-harvest process.

In the same cacao clone (Moreau Cruz et al., 2013) was observed an mean of 43 beans per pod, approximate value to the mean of 40 beans observed in this study (Table 4). This study highlights the group

Table 4 - Summary of analysis of variance, Scott-Knott test and descriptive analysis of the beans with mucilage and placenta wet biomass, and number of beans of PH-16 cacao clone

		Beans with mucilage ----- g -----	Placenta	Number of beans
		Mean Squared		
Soil ¹	11	4045**	165**	179**
Error	168	515	37	58
Total	179			
CV (%)		24,6	21,2	18,8
Soil ¹		Mean ± Standard Deviation (n = 15)		
01 LAd cam		105 ± 23 b	30 ± 4 a	42 ± 7 a
02 PVAd		95 ± 18 b	31 ± 5 a	39 ± 8 b
03 PVAd		82 ± 28 c	25 ± 7 b	38 ± 9 b
04 LAd		56 ± 15 d	27 ± 4 b	33 ± 8 b
05 LVAd		93 ± 27 b	33 ± 8 a	40 ± 7 b
06 PVAd cam		120 ± 15 a	31 ± 7 a	46 ± 4 a
07 CXd		95 ± 28 b	29 ± 6 a	40 ± 10 b
08 LVAd arg		86 ± 23 c	24 ± 7 b	41 ± 10 a
09 PAD lat		103 ± 28 b	26 ± 6 b	38 ± 8 b
10 PVAd		75 ± 21 c	33 ± 7 a	39 ± 9 b
11 PVA ali		96 ± 24 b	30 ± 6 a	40 ± 6 b
12 PVAd coe		104 ± 15 b	25 ± 5 b	47 ± 4 a
		Overall mean (n = 180)		
Minimum		30	13	18
Mean ± Standard Deviation		92 ± 27	29 ± 7	40 ± 8
Maximum		153	45	54

¹Soil: 01 LAd cam - Latosol Yellow Dystrophic cambisolic, 02 PVAd - Argisol Red-Yellow Dystrophic tipic, 03 PVAd - Argisol Red-Yellow Dystrophic abrupt, 04 LAd - Latosol Yellow Dystrophic tipic, 05 LVAd - Latosol Red-Yellow Dystrophic tipic, 06 PVAd cam - Argisol Red-Yellow Eutrophic cambisolic, 07 CXd - Cambisol Haplic Dystrophic tipic, 08 LVAd arg - Latosol Red-Yellow Dystrophic argisolic, 09 PAD lat - Argisol Dystrophic latosolic, 10 PVAd - Argisol Red-Yellow Dystrophic tipic, 11 PVA ali - Argisol Red-Yellow Alitic tipic, 12 PVAd coe - Argisol Red-Yellow Dystrophic Cohesive abrupt. DF - Degrees of Freedom. CV - Coefficient of Variation. Significance levels by test F: (**) = 1% of error.

of higher means of number of beans, because the number of beans is an important component of production used in cacao breeding (Beckett, 2009; Britto & Silva, 1983; Engels et al., 1980; Sánchez et al., 1996).

The statistical differences of the ANOVA F test showed a variation of around 20% between biometric variables of the PH-16 cacao clone as a function of cultivation sites represented by soil (Table 4). However, these statistical differences (Table 4) can be related to several factors that were not considered in this study, for example, pod yield per plant (nutrient partitioning and biomass), competition of light, water and nutrients between plants, plant age, fertility and soil management, disease and pest attacks, and other genetic, environmental and phytotechnical factors that

interfere with the physiological and cacao dendrometric characteristics (Almeida & Valle, 2007; Britto and Silva, 1983; Engels et al., 1980; Lopez Baez, 1995; Machado and Almeida, 1989; Sánchez et al., 1996).

Positive correlations between pod wet biomass and husk wet biomass ($r = 0.98$) (Figure 2A) and with the placenta wet biomass ($r = 0.98$) (Figure 2C) were found. A positive correlation between the pod wet biomass and content of pod wet biomass ($r = 0.58$) (Figure 2B) was also observed.

There is biological evidence that the husk wet biomass of PH-16 correspond to approximately 80% of the total pod wet biomass (Table 3). The high correlation between these attributes (Figure 2A) may indicate that both are subject to the same influence of

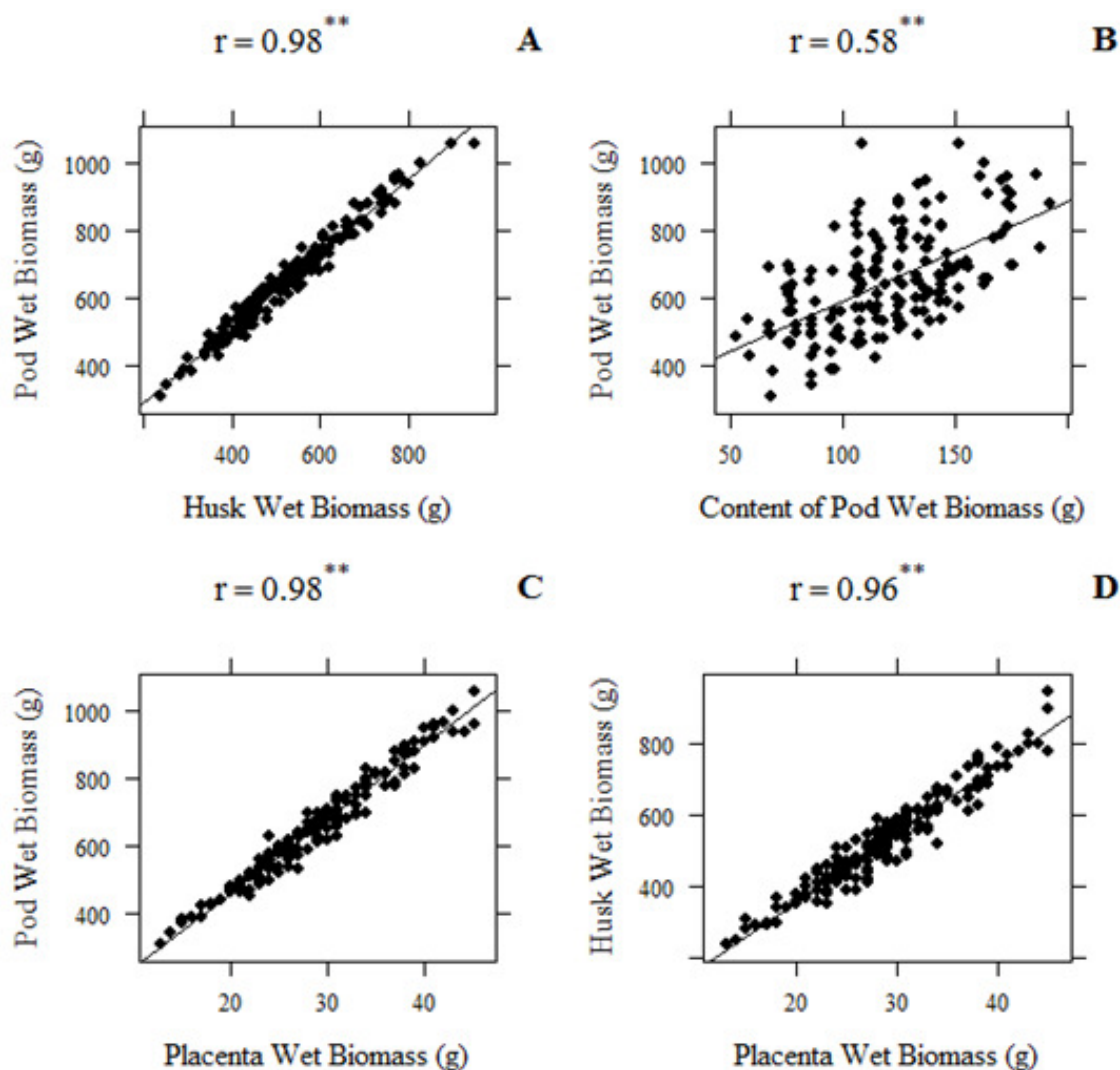


Figure 2 - Correlation between cacao pods biometrics attributes of PH-16 clone (r = Pearson correlation coefficient; **Significance at 1% level; $n = 180$).

environmental factors related to the physiology of growth. This information is important for breeding and nutritional management of cacao trees, because they relate to the partition of nutrients and biomass. These biometric attributes may be decisive for the choice of genotypes with different agricultural potential, particularly regarding cacao quality.

The husk wet biomass also showed a positive correlation with placenta wet biomass ($r = 0.95$) (Figure 2D). With regard to partitioning of nutrients the husk has largely of the nutrients directed by cacao trees is growing of the pod, showing the large export of nutrients for this plant organ (drain) (Pinto, 2013; Sodr  et al., 2012). The placenta is the conductive part of

pod nutrients to the beans (Muniz et al., 2013), and a high correlation with the husk (Figure 2D) may indicate that both are important in the partition of nutrients and biomass in cacao trees.

Primarily, the content of pod wet biomass is positively correlated with beans with mucilage wet biomass ($r = 0.97$) (Figure 3A), followed by positive correlation with number of beans (Figure 3C) ($r = 0.67$) and, finally, with placenta wet biomass ($r = 0.59$) (Figure 3B). It was also observed that beans with mucilage wet biomass was positively correlated with the number of beans ($r = 0.70$) (Figure 3D). According to this correlation (Figure 3D), pods with more beans tend to have higher beans with mucilage wet biomass

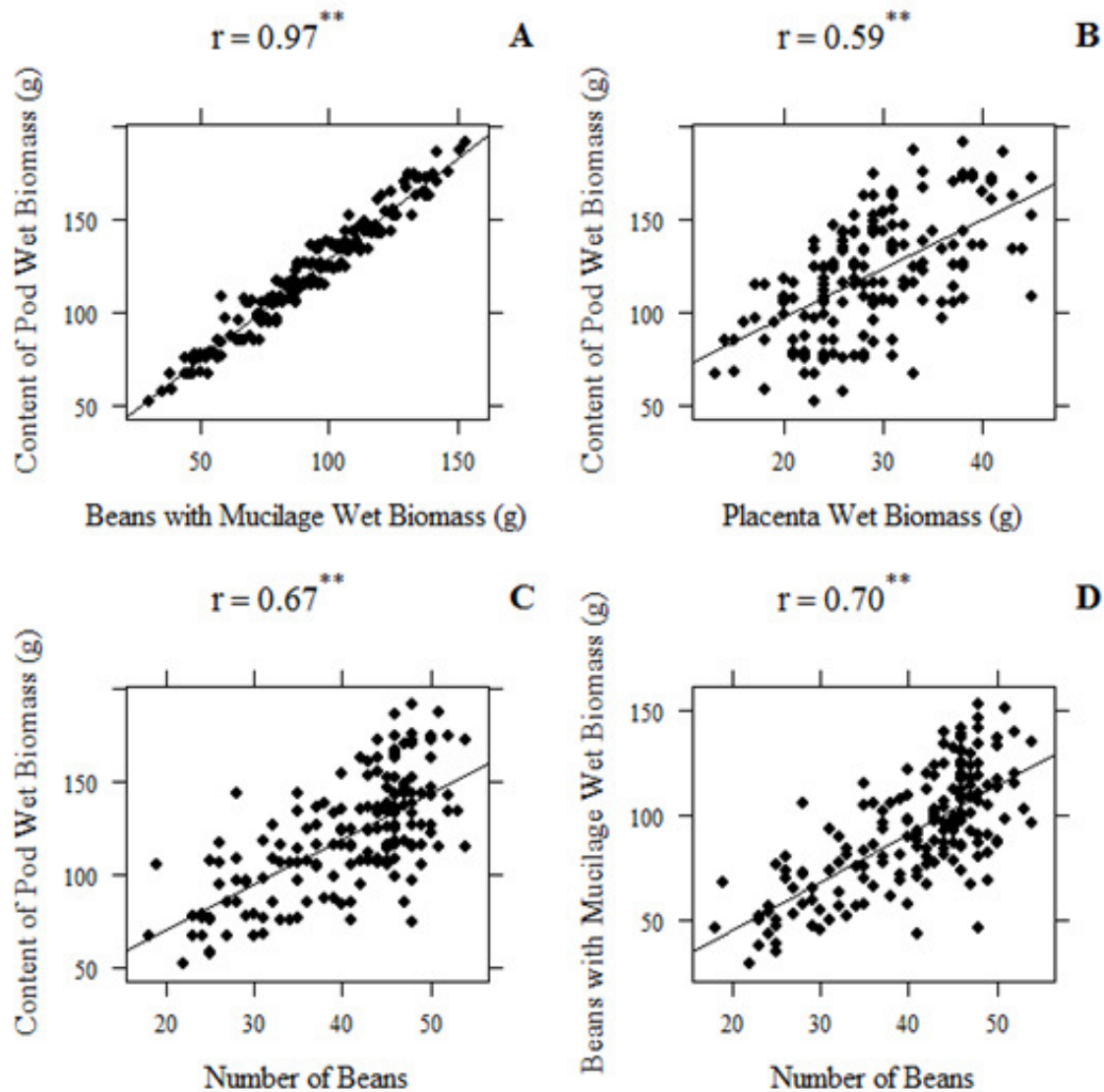


Figure 3 - Correlation between cacao pods biometrics attributes of PH-16 clone (r = Pearson correlation coefficient; $**$ Significance at 1% level; $n = 180$).

as verified in Tables 3 and 4, following an opposite relationship with respect to the groups of means of wet biomass of the pod and husk. However, because they are partial values of the pod wet biomass total value; these interpretations were also based on the graphical analysis of the correlations (Table 3). Among some attributes, there was wide graphic dispersion of sample observations and lower correlation coefficients (Figures 3B; 3C). This can be explained by the great variability of biometric in cacao pods (Lopez et al., 2011).

In higher plants, both dry matter as well as nutrient partitioning are phenomena of growth and development

closely related to genetic factors (Taiz & Zeiger, 2013). The husk of the Forastero group is thicker than of the Criollo group, and the Trinitarian cacao husk shows intermediate thickness (Bartley, 2005). PH-16 is a hybrid of Forastero with Trinitarian groups (Moreau Cruz et al., 2013). The value of the ratio pod wet biomass and number of beans in Cacao Common (Forastero group) approaches 15 (Loureiro, 2012), whereas in this study, the value observed for the ratio in PH-16 was approximately 17 (Tables 3 and 4). The ratio between pod wet biomass and number of seeds may mean plant nutrients invested in biomass conversion is particular directed to the seeds (beans) (Marenco & Lopes, 2009;

Taiz & Zeiger, 2013). However, not only the number of beans must be considered an important component of plant production, but also the bean weight, especially after processing, when they will be marketed (Garcia, 1985; Garcia, 1973; Santana, 1981).

Principal components analysis (PCA) biplots of cacao pods biometric attributes of PH-16 clone are shown in Figures 4 and 5. It is observed a correlation structure similar to the structure of bivariate correlations in Figures 2 and 3.

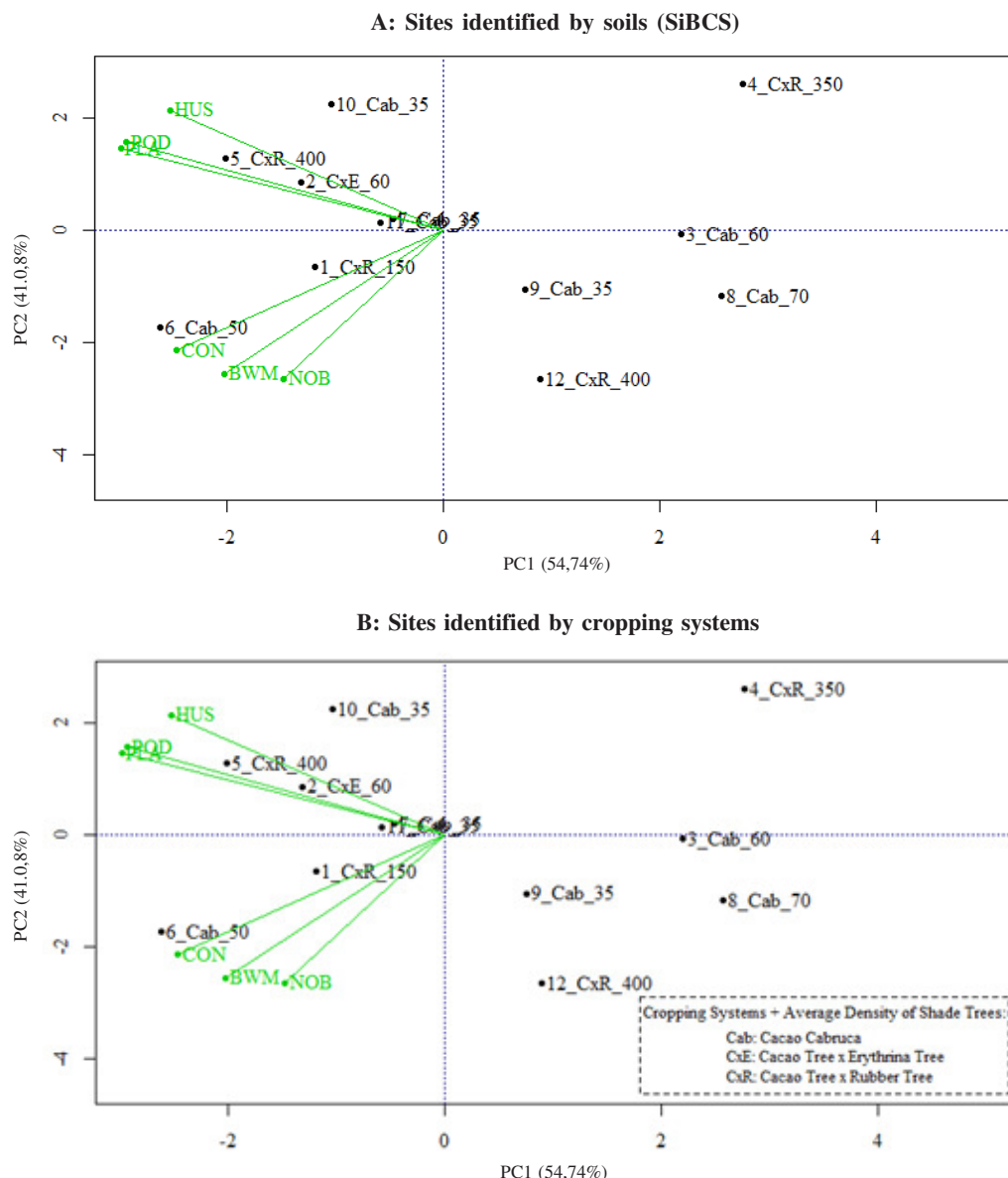


Figure 4 - Principal Component Analysis Biplots. Factors indicate the relative weight of the variables on the axes. Cacao pods biometric attributes of PH-16 clone: pod wet biomass (POD), husk wet biomass (HUS), content of pod (beans with mucilage and placenta) wet biomass (CON), beans with mucilage wet biomass (BWB), placenta wet biomass (PLA), number of beans (NOB). Places represented by soils (Brazilian System of Soil Classification - SiBCS): Latosol Yellow Dystrophic cambisolic (1_LAd cam), Argisol Red-Yellow Dystrophic tipic (2_PVAd), Argisol Red-Yellow Dystrophic abrupt (3_PVAd), Latosol Yellow Dystrophic tipic (4_LAd), Latosol Red-Yellow Dystrophic tipic (5_LVAd), Argisol Red-Yellow Eutrophic cambisolic (6_PVAe cam), Cambisol Haplic Dystrophic tipic (7_Cxd), Latosol Red-Yellow Dystrophic argisolic (8_LVAd arg), Argisol Dystrophic latosolic (9_PAd lat), Argisol Red-Yellow Dystrophic tipic (10_PVAd), Argisol Red-Yellow Alitic tipic (11_PVA ali), Argisol Red-Yellow Dystrophic Cohesive abrupt (12_PVAd coe). Numbered soils according to the longitudinal direction North-South.

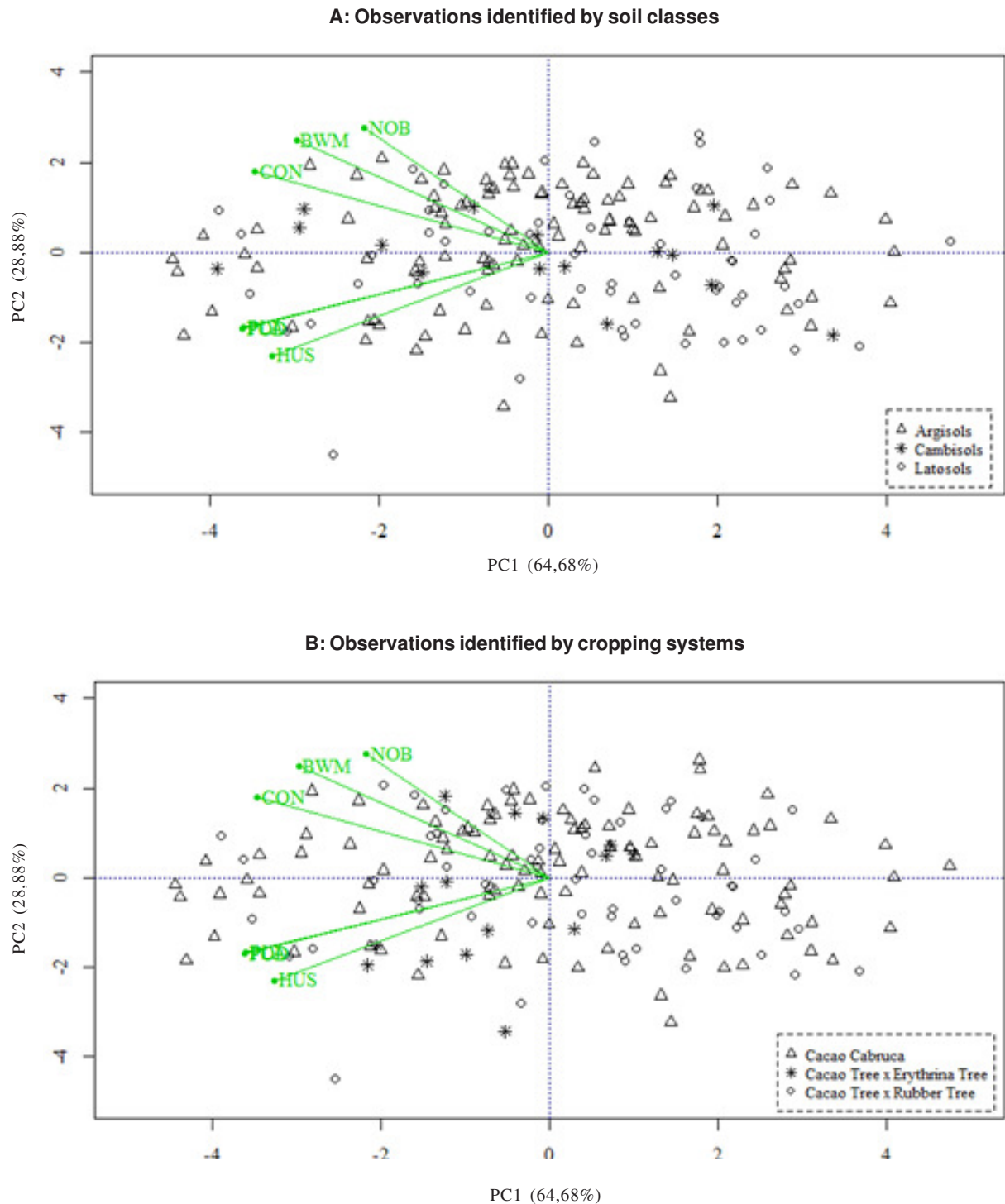


Figure 5 - Principal Component Analysis Biplots. Factors indicate the relative weight of the variables on the axes. Cacao pods biometric attributes of PH-16 clone: pod wet biomass (POD), husk wet biomass (HUS), content of pod (beans with mucilage and placenta) wet biomass (CON), beans with mucilage wet biomass (BWM), placenta wet biomass (PLA), number of beans (NOB). Observations identified by soil classes (A) and cropping systems (B).

Table 5 is a summary of the PCA of cacao pods biometric attributes of PH-16 clone explained by Biplot graph.

The Biplots represent the cacao pods biometric attributes of PH-16 clone, which vary according to the study sites represented by soils and their classes, different cropping systems, the average density of shade trees per hectare and the geographic coordinates (Figures 4 and 5).

Graphs A and B in Figure 4, have the same structure between variables and objects because they are the same ACP (Table 5). Graphs A and B in Figures 5 also have the same structure between variables and objects (Table 5). The objects shown in A and B were renamed for interpretation purposes (Figures 4 and 5).

The main components represented in Biplot graphs A and B of Figure 4 have eigenvalue higher than 1, and retains 96% of the total variance of the data for interpretation based on the means (Table 5). The CP represented by the graphs A and B of Figure 5 also have eigenvalue higher than 1 (one), retaining about 94% of the total variance of the data (Table 5).

The variables number of beans (NOB), content of pod wet biomass (CON) and beans with mucilage wet biomass (BWM) are positively correlated (Figure 3). Graphically, the site 06 - PVAe cam (Argisol Red-Yellow Eutrophic cambisolic) in Cacao Cabruca system (Cab), with an average density of 50 shade trees per hectare, and the site 12 - PVAd coe (Argisol Red-Yellow Dystrophic Cohesive abrupt) in Cacao x

Rubber Tree intercropping system, with an average density of 400 shade trees, are positively correlated with these variables (Figure 3). Besides the natural fertility, soil management conditions, such as correction of acidity and fertilization, are related to the eutrophic character of the soil; therefore, the correlation of these agronomic characteristics of cacao pods (NOB, CON and BWM) may be associated with good soil management. Site 04 - Lad (Latosol Yellow Dystrophic tipic) showed a negative correlation with these same variables (NOB, CON and BWM) (Figure 3). Sites 10 - PVAd (Argisol Red-Yellow Dystrophic tipic), 05 - LVAd (Latosol Red-Yellow Dystrophic tipic) and 02 - PVAd (Argisol Red-Yellow Dystrophic tipic) were positively correlated with the attributes pod (POD), husk (HUS) and placenta (PLA) wet biomasses. These soils have in common the "Dystrophic tipic" character, showing that low soil fertility favors biometric attributes as HUS and PLA, which are not as important as the attributes related to the production of beans (NOB, CON and BWM).

All observations from biometric variables were also explored in relation to the three soil classes Argisols (Arg), Cambisols (Cam) and Latosols (Lat), and also in relation to the three types of cropping systems Cacao Cabruca (Cab), Cacao x Erythrina Tree (CxE), and Cacao x Rubber Tree (CxR) (Figure 5). Clusters between soils related classes (SiBCS) with cropping systems or longitudinal arrangement of the geographic coordinates (Figures 4 and 5) were not observed. But

Table 5 - Summary of Principal Component Analysis of cacao pods biometric attributes of PH-16 clone

Summary	12 soils and cropping systems with average density of shade trees		All observations by soil types and cropping systems	
	PC1	PC2	PC1	PC2
POD	-0.49	0.30	-0.46	-0.32
HUS	-0.42	0.41	-0.41	-0.43
CON	-0.41	-0.41	-0.44	0.34
BWM	-0.34	-0.49	-0.38	0.47
PLA	-0.50	0.28	-0.46	-0.31
NOB	-0.25	-0.51	-0.28	0.52
Eigenvalue	6.01	5.21	26.36	17.61
Retained Variance	0.55	0.41	0.65	0.29
Accumulated Variance	0.55	0.96	0.65	0.94

POD – pod wet biomass (g), HUS – huks wet biomass (g), CON – content of pod (beans with mucilage and placenta) wet biomass (g), BWM - beans with mucilage wet biomass (g), PLA – placenta wet biomass (g), NOB – number of beans. PC – Principal Component.

even if the level of detail of this study (Figures 4 and 5) were not sufficient to explain these differences directly, indirectly they indicate that biometric attributes of cacao pods are under the influence of genotype x environment interaction (Almeida & Valle, 2007; Dias & Kageyama, 1985; Engels et al., 1980; Garcia, 1973; Icco, 2008; Machado and Almeida, 1989; Monteiro et al., 2011; Sánchez et al., 1996).

Beans biometrics

The highest mean of dry cacao beans length of 18 mm correspond to site 6 represented by Argisol Red-Yellow Eutrophic cambisolic (Table 6); this site is also related with the highest mean of cacao beans dry weight of 1.4 g (Table 6). As already discussed, the eutrophic character of the soil associated with good

fertility conditions is directly correlated with the production of components related to cacao beans, both in wet biomass (Tables 3 and 4; Figure 4) and dry biomass (Table 6). It was observed that the lowest means of dry cacao beans weight are related to soils with dystrophic character (Table 6), showing the relationship between soil fertility and production of cacao beans.

The variation in values of dry cacao beans length shown in this study (Table 6) is similar to the variation observed in another study employing the same genotype (Moreau Cruz et al., 2013). The mean of dry cacao beans width corresponds to 11 mm, this value was lower the mean of 14.3 mm observed in another study with PH-16 clone (Moreau Cruz et al., 2013). Our study analyzed dry cacao beans and another study analyzed in natura cacao beans, the absence of post-harvest

Table 6 - Summary of analysis of variance, Scott-Knott test and descriptive analysis of the biometrics attributes of dry cacao beans of PH-16 clone

Source	DF	Length	Width	Thickness	Dry Weight
			----- mm -----		g
Mean Squared					
Soil ¹	11	14,9**	10**	UR	4,8**
Error	1068	4,8	2,6	UR	1,2
Total	1079				
CV (%)		12,7	14,8	UR	17,1
Soil ¹		Mean ± Standard Deviation (n = 15)			
01 LAd cam		17.6 ± 2.1 a	11.0 ± 1.4 b	6.5 ± 1.2	1.3 ± 0.2 b
02 PVAd		17.0 ± 2.2 b	10.9 ± 1.9 b	6.1 ± 1.0	1.2 ± 0.2 c
03 PVAd		16.6 ± 2.1 b	10.3 ± 1.4 c	6.1 ± 0.9	1.2 ± 0.2 c
04 LAd		17.2 ± 2.1 b	10.9 ± 1.4 b	6.1 ± 1.1	1.2 ± 0.2 c
05 LVAd		16.9 ± 2.3 b	10.6 ± 1.5 c	6.1 ± 1.0	1.2 ± 0.2 c
06 PVAe cam		18.0 ± 2.2 a	11.4 ± 1.8 a	6.7 ± 1.0	1.4 ± 0.2 a
07 CXd		17.6 ± 2.1 a	11.1 ± 1.5 b	5.9 ± 1.3	1.2 ± 0.2 c
08 LVAd arg		17.1 ± 2.4 b	11.6 ± 1.8 a	6.0 ± 1.2	1.2 ± 0.2 c
09 PAd lat		17.4 ± 2.1 a	11.0 ± 1.6 b	6.1 ± 1.1	1.2 ± 0.2 c
10 PVAd		17.8 ± 2.4 a	11.2 ± 1.8 a	6.2 ± 1.0	1.3 ± 0.2 b
11 PVA ali		17.5 ± 2.3 a	10.9 ± 1.7 b	6.3 ± 1.1	1.3 ± 0.3 b
12 PVAd coe		17.6 ± 2.1 a	11.0 ± 1.7 b	6.1 ± 0.9	1.2 ± 0.2 c
Overall mean (n = 180)					
Minimum		10.8	7.3	3.3	0.67
Mean ± Standard Deviation		17.4 ± 2.2	11.0 ± 1.7	6.2 ± 1.1	1.2 ± 0.2
Maximum		23.8	15.4	9.6	1.93

¹Soil: 01 LAd cam - Latosol Yellow Dystrophic cambisolic, 02 PVAd - Argisol Red-Yellow Dystrophic tipic, 03 PVAd - Argisol Red-Yellow Dystrophic abrupt, 04 LAd - Latosol Yellow Dystrophic tipic, 05 LVAd - Latosol Red-Yellow Dystrophic tipic, 06 PVAe cam - Argisol Red-Yellow Eutrophic cambisolic, 07 CXd - Cambisol Haplic Dystrophic tipic, 08 LVAd arg - Latosol Red-Yellow Dystrophic argisolic, 09 PAD lat - Argisol Dystrophic latosolic, 10 PVAd - Argisol Red-Yellow Dystrophic tipic, 11 PVA ali - Argisol Red-Yellow Alitic tipic, 12 PVAd coe - Argisol Red-Yellow Dystrophic Cohesive abrupt. DF - Degrees of Freedom. CV - Coefficient of Variation. UN – Unrealized. Significance levels by test F: (**) = 1% of error.

and all biochemical transformations related were the probable causes of the differences of these values (Table 6).

The overall mean of dry cacao beans weight with 6-7% moisture corresponds to 1.24 g (Table 6). This values are highest than the value of 1.0 g (for samples of 100 cacao beans) recommended by industry (CCCA, 1984), also is recommended that not more than 12% of the cacao beans should have a variance larger or smaller than 1/3 of mean dry weight. However, these and other requirements of the chocolate industry were determined without considering the genetic diversity and biometric variation of cacao genotypes in different growing conditions. Hybrid cacao beans can vary widely regarding their biometric dimensions (Beckett, 2009; Engels et al. 1980; Almeida, 2001; Dias and Rezende, 2001; Sánchez et al., 1996). This result indicates that the dry cacao beans weight of clone PH-16 was slightly

influenced by the soil in which it was being cultivated. The length, width and dry weight and are the seed of morphological and agronomic characteristics as descriptors used in breeding programs to differentiate the cacao genotypes, and are also subject to the degree of development and ripening of pods (Bekele & Butler, 2000; Engels et al., 1980; Lopes, 2000; Bartley, 2005; Mattietto, 2001; Sánchez et al., 1996).

The cacao beans length showed positive correlation with cacao beans width ($r = 0.63$) (Figure 6A) and bean dry weight ($r = 0.58$) (Figure 6B). Besides the length, cacao beans thickness also showed positive correlation with cacao bean dry weight ($r = 0.54$) (Figure 6C). The dry weight of dry cacao beans is an important attribute for the final marketing of cacao (Beckett, 2009; WCF, 2014), and the correlations shown Figure 6, the attributes that represent the cacao beans dimensions are directly related to dry weight.

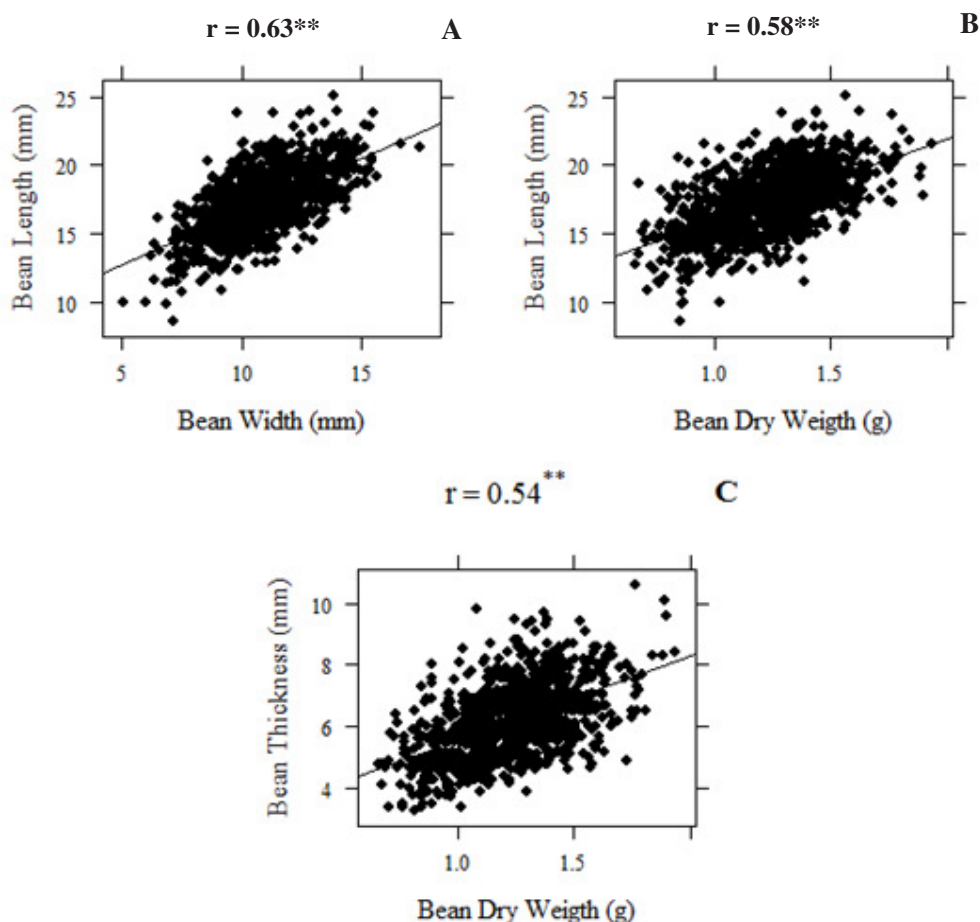


Figure 6 - Correlation between dry cacao beans biometrics attributes of PH-16 clone (r = Pearson correlation coefficient; ** Significance at 1% level; $n = 1080$).

Selection of cropping sites by cacao biometric attributes

The evaluation criteria used in the selection of cacao cropping sites were (Table 7): means of attributes interpreted as good production characteristics received a score of 1 (one), and the means unselected received a score of 0 (zero). For example, values from groups with the highest means of beans with mucilage wet biomass and number of beans were converted to 1, and the other values to 0 (zero). Another example of interpretation, values from groups with the lower means of husk and placenta wet biomass were converted to 1, and the other values to 0 (zero) (Table 7).

According to the sum of the scores of cacao biometrics attributes of PH-16 clone selected from the Scott-Knott test, sites 06 - PVAe cam and 12 - PVAd coe showed the best performance with 7 and 5 the best means of the studied biometric attributes, respectively (Table 7). Sites 02 - PVAd and 04 - LAd have the lowest performances, both with a score of 1 point (Table 7).

This method of evaluation (selection by scores) does not replace the multivariate statistical methods, but, it proved to be able to differentiate levels of study of factor (source of variation) showing similar results of nivariate (Tables 3, 4 and 6) and multivariate analyzes (Figures 4 and 5) objectively. However, it is

necessary to emphasize that this evaluation does not provide the best performance between means that belong to the same group generated by Scott-Knott test because regardless of higher or lower values within the group, the averages were classified with the values 0 (zero) or 1 (one) (Table 7).

Conclusions

Cacao biometric attributes of pods and beans were influenced by different cropping sites.

Attributes content of pod (beans with mucilage and placenta) wet biomass, beans with mucilage wet biomass and number of beans were positively correlated with the cultivation sites represented by Argisol Red-Yellow Eutrophic cambisolic and Argisol Red-Yellow Dystrophic Cohesive abrupt soils.

The highest values of length and dry weight of dry cacao beans correspond to Argisol Red-Yellow Eutrophic cambisolic.

The dystrophic soils are related to lower dry weight values of cacao beans.

The selection of cacao cropping sites by biometric attributes also highlighted the Argisol Red-Yellow Eutrophic cambisolic and Argisol Red-Yellow Dystrophic Cohesive abrupt soils.

Table 7 - Selection of cacao cropping sites by mean groups of biometrics attributes generated by the Scott-Knott test

Soil ¹	Pod ²						Dry Bean ³			Sum
	POD	HUS	CON	BWM	PLA	NOB	LEN	WID	WEI	
01 LAd cam	1	0	1	0	0	1	1	0	0	4
02 PVAd	1	0	0	0	0	0	0	0	0	1
03 PVAd	0	1	0	0	1	0	0	0	0	2
04 LAd	0	0	0	0	1	0	0	0	0	1
05 LVAd	1	0	1	0	0	0	0	0	0	2
06 PVAe cam	1	0	1	1	0	1	1	1	1	7
07 CXd	1	0	1	0	0	0	1	0	0	3
08 LVAd arg	0	1	0	0	1	1	0	1	0	4
09 PAd lat	0	1	1	0	1	0	1	0	0	4
10 PVAd	1	0	0	0	0	0	1	1	0	3
11 PVA ali	1	0	1	0	0	0	1	0	0	3
12 PVAd coe	0	1	1	0	1	1	1	0	0	5

¹Soil: 01 LAd cam - Latosol Yellow Dystrophic cambisolic, 02 PVAd - Argisol Red-Yellow Dystrophic tipic, 03 PVAd - Argisol Red-Yellow Dystrophic abrupt, 04 LAd - Latosol Yellow Dystrophic tipic, 05 LVAd - Latosol Red-Yellow Dystrophic tipic, 06 PVAe cam - Argisol Red-Yellow Eutrophic cambisolic, 07 CXd - Cambisol Haplic Dystrophic tipic, 08 LVAd arg - Latosol Red-Yellow Dystrophic argisolic, 09 PAd lat - Argisol Dystrophic latosolic, 10 PVAd - Argisol Red-Yellow Dystrophic tipic, 11 PVA ali - Argisol Red-Yellow Alitic tipic, 12 PVAd coe - Argisol Red-Yellow Dystrophic Cohesive abrupt. ²Pod: POD – pod wet biomass (g), HUS – husk wet biomass (g), CON – content of pod (beans with mucilage and placenta) wet biomass (g), BWM – beans with mucilage wet biomass (g), PLA – placenta wet biomass (g), NOB – number of beans per pod. ³Dry Beans: LEN – Bean length (mm), WID – Bean width (mm), WEI – Bean dry weight (g).

Understanding the variability of cacao biometric attributes emphasizes the importance of using technologies for achieving sustainable production of cacao quality.

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