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Growth and Nutritional Responses of Juvenile Wild and Domesticated Cacao Genotypes to Soil Acidity

César O. Arévalo-Hernández [1,2,*], Enrique Arévalo-Gardini [1,2], Abel Farfan [3], Martin Amaringo-Gomez [1], Andrew Daymond [4], Dapeng Zhang [5] and Virupax C. Baligar [5]

1 Instituto de Cultivos Tropicales, Tarapoto 22000, Peru
2 Agronomy Faculty, Universidad Nacional Autónoma de Alto Amazonas (UNAAA), Yurimaguas 16501, Peru
3 Bioversity International, Av. La Molina 1895, Distrito de La Molina Lima 12, Lima 15023, Peru
4 School of Agriculture, Policy and Development, University of Reading, Reading RG6 6UR, UK
5 Beltsville Agricultural Research Center, U.S. Department of Agriculture/Agricultural Research Service, Beltsville, MD 20705, USA
* Correspondence: cesar.arevaloh@gmail.com

Abstract: Cacao is an important tropical crop and requires high-fertility soils for better growth and productivity; nevertheless, soils where this crop is grown are, in general, acidic and low in fertility. Therefore, germplasm with tolerance to soil acidity is desirable for cacao genetic improvement. The objective of this study was to evaluate cacao germplasm for early growth, nutrient concentration, and potential tolerance to soil acidity. A greenhouse experiment was conducted to evaluate 60 cacao genotypes with diverse geographic origins. Cacao seedlings were grown for six months in acid soil with and without lime. Growth parameters and the total concentration of N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn were measured in shoots after harvest. Our results indicate that the best early growth predictors of acid soil tolerance are the number of leaves and root area. N, Ca, Mg, and K uptake may have a potential role in tolerance to soil acidity. Finally, the results revealed a large difference among cacao genotypes in terms of their responses to acid soil stress, which led to the selection of ten genotypes: CCN-51, PH-21, CCN-10, PAS-91, ICT-1087, ICS-95, UF-667, TSH-565, PH-144, ICT-1189 that are potentially tolerant to soil acidity and could be used for breeding acid soil-tolerant cacao varieties.

Keywords: abiotic stress; fertility; calcium; macro nutrient effects

Citation: Arévalo-Hernández, C.O.; Arévalo-Gardini, E.; Farfan, A.; Amaringo-Gomez, M.; Daymond, A.; Zhang, D.; Baligar, V.C. Growth and Nutritional Responses of Juvenile Wild and Domesticated Cacao Genotypes to Soil Acidity. *Agronomy* **2022**, *12*, 3124. https://doi.org/10.3390/agronomy12123124

Academic Editor: Ying Ma

Received: 3 November 2022
Accepted: 7 December 2022
Published: 9 December 2022

1. Introduction

Cacao is a perennial crop native to the Amazon Basin, and Peru is one of the main countries that has the highest genetic diversity of this crop, contributing more than 60% of cacao varieties cultivated by farmers worldwide [1].

Cacao is one of the main agricultural commodities, having a world production of 4.72 million tons of cocoa beans, produced by five to six million smallholder producers [2,3]. Cocoa production is mainly conducted in tropical regions where high temperature and humidity are characteristics of these agroecosystems. In Peru, many crops are produced by small farmers with less than 3 ha of terrain [4], and cacao is no exception. The small cacao plantations increase the production cost, including agricultural products such as fertilizers, lime, and pesticides, which in many cases make cacao farming unprofitable.

Cacao requires high fertility to obtain high productivity. However, soils where cacao is grown are, in general, acidic with low fertility [5]. Soil acidity is one of the main constraints in cacao production worldwide. Soil acidification is a natural process in most tropical regions due to high precipitation regimes that lead to the loss of basic cations and organic matter through erosion and excess nutrient leaching. Moreover, long-term cacao farming generates a loss of nutrients through harvested beans, and in some areas, the use of ammonium-based fertilizers (NH_4) increases soil acidification [6,7]. Aluminum and

manganese uptake are important growth-limiting factors in many acid soils where soil pH < 5.5, and the problem is more serious in soils with strong subsoil acidity that are difficult to lime. The toxicity of Al and Mn produces shoot and root stunting and reduced growth rate [8]. In general, acid soils are characterized by low fertility, low base saturation, low concentrations of Ca and Mg, low P, and high Al [9]. Soil acid conditions can affect the root tip and acquisition of nutrients by affecting cell division and elongation, water uptake and germination [10,11].

Soil acidification can be diminished by sustainable management practices. In general, acid soils can be managed using lime and gypsum (subsurface) applied to reach the desired pH value and high base saturation. Nevertheless, in most cacao-producing countries, the cost of liming is high, and cacao farmers often have difficulty acquiring these products due to legal restrictions from their use in cocaine production (especially in Peru). In this scenario, using acid-tolerant cacao clones provides an alternative strategy for acid soil management. In general, the current program of cacao genetic improvement has been focused on productivity rather than tolerance to abiotic stresses. Tolerance to acidity may be defined as the ability of a plant to grow better, produce a higher amount of dry matter, and present fewer symptoms of nutrient deficiency at high soil acidity levels while producing higher yields [7].

Cacao genotypes are known to differ widely in their tolerance to soil acidity and Al [12–16]. Genotypes with a high nutrient uptake efficiency under soil acidity stress may have an advantage in adapting to mineral-stressed soil ecosystems of the cacao growing regions.

Plants have several mechanisms to tolerate acidity that can be separated into internal and external mechanisms. The external mechanisms are those involving exclusion of acidity such as organic acid exudation for chelation of soil Al^{3+} and H^{+} [7,10,17], while the internal mechanisms involve tolerance within the cells such as genetic polymorphism [18] and increased activity of root H^{+}-ATPase [19]. Moreover, the expression of transcription factors such as the STOP1 gene can regulate the Al-activated malate transporter AtALMTl and can regulate the internal pH [17,20,21].

Superior genotypes with tolerance to soil acidity could be used as rootstock to graft high-yielding scions. Such genotypes can also be used to breed superior cacao cultivars that not only have the adaptability to acid soils but also have high yield potentials.

This research was conducted with 60 cacao genotypes from different geographical origins commonly cultivated in the Latin-American Amazon. The clones were grown in unlimed acid soil and limed soils to (i) explore the tolerance of cacao clones for soil acidity, (ii) determine the macro and micronutrients uptake in the different cacao genotypes under acidic conditions, and (iii) identify cacao genotypes with tolerance to soil acidity constraint. The outputs could be useful to farmers to produce economically viable cacao plantations in areas with soil acidity constraints.

2. Materials and Methods

2.1. Localization and Cacao Clones

This study was conducted in 2015 (February-September) in the Juan Bernito Experimental Station belonging to the Instituto de Cultivos Tropicales (ICT), (6°28′37.3″ S, 76°19′54.6″ W; 500–530 m.a.s.l), which is located in the district of La Banda de Shilcayo, department and province of San Martin (Figure 1). This area is also reported by Arevalo-Gardini et al. [22] to have an average annual precipitation of 1250 mm, an annual mean temperature of 26 °C, and 87% relative humidity.

Figure 1. Location of Instituto de Cultivos Tropicales facilities in La Banda del Shilcayo district, adjacent to the main city of Tarapoto in the San Martin region, Peru.

Cacao genotypes explored for this study were obtained from the ICT germplasm bank ubicated in the El Choclino Experimental Station. In total, 60 cacao genotypes from four geographic regions were selected and the list is presented in Table 1.

Table 1. List of cacao genotypes (n = 60) from four different geographical origin used for the experiment with soil acidity tolerance.

n	Wild Cacao Genotypes from the River Basins of Peruvian Amazon	Peruvian Grower's Cacao Genotypes (ICT Collection)	Brazilian Cacao Genotypes	National and International Cacao Genotypes
1	AYP-15	ICT-1112	BN-34	CCN-10 *
2	AYP-20	ICT-1292	BS-01	CCN-51
3	AYP-22	ICT-2171	CA-14	EET-400
4	PAS-91	ICT-1092	CEPEC 2002	H-10
5	PAS-93	ICT-2172	CP-2005 C10	IMC-67
6	PAS-100	ICT-2653	CP-49 C10	POUND-12
7	PAS-105	ICT-2161	CP-53 C10	SCA-6
8	NUC-156	ICT-2142	IPIRANGA-1	ICS-1
9	UGU-112	ICT-1506	PH-09	ICS-6
10	UGU-126	ICT-1026	PH-15	ICS-39
11	UGU-130	ICT-2173	PH-16	ICS-95
12	UNG-53	ICT-1087	PH-17	TSH-565
13	UNG-73	ICT-1281	PH-21	TSH-1188
14	UNG-76	ICT-1251	PH-144	UF-613
15	UNG-77	ICT-1189	PH-990	UF-667

* EET-400 from Ecuador. H-10, IMC-67, POUND-12, and SCA-6 from Peru. ICS-1, ICS-6, ICS-39, ICS-95, TSH-565, and TSH-1188 from Trinidad and Tobago. UF-613 and UF-667 from Costa Rica.

2.2. Cacao Seedling Propagation

Cacao seedlings were produced in the ICT greenhouse by asexual propagation using rooted cuttings. Terminal apical shoots were used, and the base was treated with 0.8% indole-3-butyric acid (IBA) for inducing rooting. Cuttings were transferred to a substrate containing a mixture of sandy clay loam soil and coarse sand (3:1) for propagation. This mixture was fertilized with N, P, and K (CH_4N_2O, $CaH_4P_2O_8$ and KCl, 60 N:50 P_2O_5:90K_2O kg ha^{-1}). The substrate was then saturated, and pots and cuttings were covered with plastic for 2 months to favor root differentiation and growth and reduce evapotranspiration. During this process, soil moisture was maintained at adequate levels to promote root formation. Then, juvenile cacaos were acclimated for 2 weeks by using foliar fertilizer 20-20-20 (Abonofol®) and the hormone Citogib®.

Soil samples of the substrate (before and after liming) of 500 g were taken, air dried, ground with a soil grinder, sieved through a 2-mm diameter mesh, and stored at room temperature before analysis.

2.3. Acidity-Stress Pot Experiment

The experiment was conducted as a complete random design (CRD) in a split-plot arrangement with three replications, where the main plots considered were the control (NL, without lime) and amended soil (L, with lime), and the subplots included the 60 genotypes.

Testing soil was prepared as reported in Section 2.2 and divided into two groups. One group was maintained at natural conditions of acidity (NL, pH = 4.46) while the other group (L) was amended with $Ca(OH)_2$ and incubated for one month (30 days before planting) to achieve a pH of 5.8 in the soil.

After three months of growth, acclimated rooted cacao seedlings of each genotype were subdivided into two groups and transplanted into plastic pots containing 5 kg of limed and unlimed soil. Juvenile plants were cultivated for six months in a greenhouse with protection from rain and with a PPFD of 850–900 μmol m^2s^{-1} (50% of shade). During the experiment, soil water levels were preserved at 90% of field capacity.

2.4. Determination of Soil Physicochemical Properties

Physical and chemical properties of the soil such as pH, E.C., organic matter, textural fractions (sand, clay, and lime), exchangeable bases (Ca, Mg, Na, and K), exchangeable acidity, available P, and CEC were determined before and after liming the soil. The chemical methods used for assessing soil characteristics are reported in previous publications [23,24].

Soil texture analysis was performed with the Bouyoucos method, using 1 M L^{-1} of NaOH as a dispersant. Soil pH (1:2.5 H_2O) was measured with a potentiometer; electrical conductivity (EC) with a conductivity meter and organic matter (OM) concentration with the Walkley and Black method by titration.

CEC and base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were determined using extraction with 1 M NH_4OAc and after, determined in flame atomic absorption spectrophotometer—FAAS. To determine exchangeable acidity (Al^{3+}, H^+), Yuan's method [25] was used. Available P was extracted with the Olsen method (0.5 M $NaHCO_3$ pH 8.5) and determined in a UV-VIS spectrophotometer. The selected microelements (Cu, Fe, Mn, Zn) were extracted by DTPA and then analyzed by AAS. For soil chemical analysis, reference material AG-1 from SPC-Science was used to assure quality control.

2.5. Determination of Biometric Parameters and Tolerance Index in Cacao Seedlings

At the end of the experiment, plant growth variables were measured based on every single plant. These parameters include shoot length (from the base of the stem to the apex of the plant, in cm), maximum root length (main root, from the base of the stem, opposite to the apex, in cm), stem and crown diameter (in mm and cm, respectively), the number of branches and leaves, and the total dry biomass (roots and shoots, in g). Moreover, foliar and root area (in cm^2) were determined by image analysis with the software Assess 2.0®.

For the total dry biomass, roots were separated from the aerial part (stem and leaves), washed with tap water, rinsed with 1% HCl and distilled water, then deposited in envelopes and dried at 60 °C for 72 h or until reaching a constant weight.

Three indices were calculated to determine the tolerance: the acidity tolerance index (ATI) based on the shade tolerance index [22], the genotypic tolerance index (GTI), [26] and the corrected acidity tolerance index (CATI); the formulae used for calculation of each index are presented as follows:

$$ATI\ (\%) = \frac{B_0}{B_1} \times 100$$

B_0: Total dry biomass of each genotype without lime
B_1: Total dry biomass of each genotype with lime

$$GTI\ (\%) = \frac{G_0}{G_{0*}} \times \frac{G_1}{G_{1*}} \times 100$$

G_0: Total dry biomass of each genotype without lime
G_{0*}: Mean total dry biomass of all assessed genotypes without lime
G_1: Total dry biomass of each genotype with lime
G_{1*}: Mean total dry biomass of all assessed genotypes with lime

$$CATI\ (\%) = \frac{B_0}{B_1} \times \frac{B_0}{B_{0max}} \times 100$$

B_0: Total dry biomass of each genotype without lime
B_1: Total dry biomass of each genotype with lime
B_{0max}: Maximum value of total dry biomass observed above all genotypes in conditions without lime.

2.6. Physiological Variables

A week before the end of the experiment, mature leaves from each genotype were selected and both stomatal conductance (mmol m^{-2} s^{-1}) and leaf chlorophyll (SPAD) concentration were measured. Both variables were measured with an SC-1 leaf porometer (Decagon Devices, Pullman, WA, USA) and a SPAD 502-Plus (Konica Minolta, Inc., Tokyo, Japan), respectively.

2.7. Determination of Macro and Micronutrients Concentrations and Uptake in Cacao Shoots

Dried shoots (stem and leaves) were used for the calculation of dry weight, and then mixed, milled (20 mesh), and reserved to perform chemical analysis. A sample of 0.5 g of dried shoots was used to determine N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn. Total N was performed by the Kjeldahl method. For the other elements, a wet digestion procedure with concentrated HNO_3 (70%) was performed in a hot block at 120 °C for 6 h. P was determined by the ascorbic-molybdate color development method in a UV-VIS spectrophotometer, whereas Ca, Mg, K, Cu, Fe, Mn, and Zn were determined by AAS (atomic absorption spectrophotometry) using the Spectra 55B from Varian. Concentrations are presented as the mean values of three replicates from each repetition.

For the calculation of macro and micronutrient uptake, the following formula was used:

$$Uptake\left(mg\ plant^{-1}\right) = \frac{Element\ concentration \times Dry\ weight\ (g)}{f}$$

Element concentration = g kg^{-1} for macronutrients and mg kg^{-1} for micronutrients
f = 1 for macronutrients and 1000 for micronutrients.

For the calculation of macro and micronutrient uptake efficiency (NUE) the following formula was used [22]:

$$NUE = \frac{1000}{Element\ concentration}$$

Element concentration = g kg^{-1} for macronutrients and mg kg^{-1} for micronutrients
NUE = g shoot g^{-1} for macronutrients and g shoot mg^{-1} for micronutrients.

2.8. Statistical Analysis

All statistical analysis was calculated with R software, version 4.1.2 [27]. Biometric variables and all chemical elements at the end of the experiment (after the addition of lime) were compared by an analysis of variance (ANOVA), and if differences were significant ($p < 0.05$), the mean values were compared by the Scott–Knott clustering algorithm for each cacao genotype. Moreover, to explore the relationship between all nutrient uptake and the index of tolerance to soil acidity between the different cacao genotypes, a principal component analysis (PCA) was performed.

3. Results

3.1. Soil Physical and Chemical Attributes before and after Liming

The physical (texture class) and chemical attributes of the soil before and after liming are presented in Table 2. The soil used for the experiment was of sandy loam textural class with a pH of 4.46. The EC was 0.08 dS m^{-1}, and no presence of carbonates was observed. Macronutrients and organic matter concentration were low (1.77%). The CEC increased slightly because of liming on acidic soils. Nevertheless, K, Ca, P, and Mg increased by more than 40, 200, 100, and 300%, respectively. In contrast, the concentrations of Al and micronutrients decreased after liming and fertilization. After liming, pH reached a value of 5.88; also, base saturation levels were more than 60%, and aluminum saturation was low, values considered adequate for cacao production [28,29].

Table 2. Attributes of the soil used for the experiment with cacao genotypes in a greenhouse before and after liming.

Variables	Before Liming	After Liming
pH	4.46	5.88
EC dS/m	0.08	0.37
O.M.%	1.77	1.57
$CaCO_3$%	<0.3	<0.3
N%	0.08	0.07
P mg kg^{-1}	2.19	4.13
Ca cmolc kg^{-1}	1.22	3.37
Mg cmolc kg^{-1}	0.6	1.69
K cmolc kg^{-1}	0.19	0.25
Al cmolc kg^{-1}	2	0.00
Effective CEC cmolc kg^{-1}	4.44	5.31
CEC pH 7.0	6.34	7.88
Base saturation%	31.70	67.38%
Al saturation%	45.05%	0.00%
Fe mg kg^{-1}	141.1	110.3
Cu mg kg^{-1}	1.5	0.7
Mn mg kg^{-1}	6.2	4.4
Zn mg kg^{-1}	1.2	0.9
Texture class	sandy loam	sandy loam

3.2. Biometric and Physiological Characteristics of Cacao Plants under Acid Soils

The results of the effects on soil acidity for biometric characteristics (height, diameter, number of leaves, leaf, and root area, as well as shoot and root biomass) and physiologic characteristics (stomatal conductivity and chlorophyll concentration) of cacao plants are

presented in Table 3 and results for each clone on ESI-Table S1 (Electronic Supplementary information-ESI). In general, significant differences were observed ($p < 0.05$) in eight (out of the 13) biometric characteristics, where plants grown in limed soil outperformed the no-liming treatment. However, five biometric characteristics, including shoot length, crown diameter, number of branches, stomatal conductance, and chlorophyll content, had no significant difference between the two treatments. Nonetheless, there were no biometric characteristics that performed better in the no-liming treatment (Table 2, ESI-Table S1).

Table 3. Effect of soil acidity on biometric characteristics (height, diameter, number of leaves, leaf, and root area, as well as shoot and root biomass) and physiologic characteristics (stomatal conductivity—gs and chlorophyll concentration).

Variables	Units	Limed (n = 60)	Unlimed (n = 60)	p
Shoot length	cm	50.74 ± 9.61	49.86 ± 10.11	0.3978
Root maximum length	mm	76.81 ± 16.48	67.81 ± 15.54	<0.001
Stem diameter	cm	11.99 ± 1.41	11.40 ± 1.24	<0.001
Crown diameter	cm	40.23 ± 4.02	40.02 ± 5.59	0.6937
Number of branches	count	7.07 ± 2.69	6.51 ± 2.79	0.059
Number of leaves	count	41.42 ± 11.99	38.02 ± 11.29	<0.001
Leaf area	cm^2	1114.30 ± 310.80	875.10 ± 244.72	<0.001
Root area	cm^2	364.61 ± 97.72	293.45 ± 74.01	<0.001
Shoot weight	g	31.89 ± 5.22	29.48 ± 6.08	<0.001
Root weight	g	12.27 ± 3.39	11.05 ± 3.13	<0.001
Shoot/root	ratio	38.55 ± 14.97	27.77 ± 8.60	<0.001
gs *	mmol m^{-2} s^{-1}	244.52 ± 115.93	232.46 ± 96.32	0.2835
Chlorophyll	SPAD	12.63 ± 3.25	12.35 ± 2.73	0.3841

* gs: Stomatal conductance.

Significant genotype differences were observed among the tested cacao clones (ESI-Table S1). The cacao genotypes with the higher maximum root lengths (mm) were UF-667 (110.10 ± 9.81), PH-09 (99.43 ± 15.31), CCN-51 (96.93 ± 20.43), and CP-49-C10 (95.80 ± 21.40). The clones with the highest shoot biomass (g) values were ICT-1087 (41.65 ± 2.52), PH-990 (41.05 ± 2.87), ICT-2173 (39.10 ± 0.57), and CCN-51 (37.90 ± 0.64). The genotypes with the highest root biomass values were CCN-51 (18.57 ± 1.16), IPIRANGA-1 (17.80 ± 1.44), ICS-6 (17.16 ± 1.09), and PH15 (16.74 ± 1.16). The plants with the highest leaf areas were ICT-112 (1625.3 ± 480.9), UF-667 (1527.3 ± 319.5), CP-49-C10 (1522.6 ± 963.3), and ICT-2142 (1492.6 ± 585.2), while genotypes CCN-10 (533.6 ± 158.6), ICT-2142 (524.5 ± 99.7), ICT-1087(496.9 ± 53.9), and CCN-51 (494.1 ± 43.7) showed the highest root areas (ESI-Table S1).

3.3. Concentration and Uptake of Macro-Micro Nutrients in Juvenile Cacao Genotypes Grown on Limed and Unlimed Acid Soil

The effects of soil acidity on the uptake of macronutrients (N, P, K, Ca, and Mg) and the selected micronutrients (Fe, Cu, Mn, and Zn) are presented in Table 4, and results for each clone on ESI are in Table S2. Significant differences ($p < 0.05$) were observed for 14 (out of 17) measured macro- and microelements between plants grown in limed and unlimed soils. The exceptions include N and Fe uptake, which did not present significant differences ($p > 0.05$) between these treatments in either concentration and uptake of nutrients. In addition, Cu showed a significant difference in concentration but not uptake (Table 3, ESI-Table S2).

Table 4. Effect of soil acidity on concentration, uptake and nutrient use efficiency of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn in juvenile cacao plants.

Variables	Unit	Concentration		
		Lime (*n* = 60)	**Unlimed (*n* = 60)**	***p***
N *	dag kg^{-1}	1.08 ± 0.16	1.13 ± 0.16	0.300
P	dag kg^{-1}	0.08 ± 0.04	0.06 ± 0.03	<0.001
K	dag kg^{-1}	2.31 ± 0.49	1.82 ± 0.5	<0.001
Ca	dag kg^{-1}	1.51 ± 0.61	1.16 ± 0.21	<0.001
Mg	dag kg^{-1}	0.84 ± 0.34	0.59 ± 0.1	<0.001
Cu	mg kg^{-1}	5.38 ± 3.4	6.42 ± 2.44	<0.001
Fe	mg kg^{-1}	112.36 ± 42.1	133.15 ± 56.19	<0.001
Mn	mg kg^{-1}	120.7 ± 71.84	1172.32 ± 421.78	<0.001
Zn	mg kg^{-1}	38.2 ± 16.41	52.95 ± 12.13	<0.001
		Uptake		
N	mg $plant^{-1}$	343.37 ± 79.23	334.47 ± 88.54	0.3152
P	mg $plant^{-1}$	24.09 ± 14.18	17.96 ± 7.82	<0.001
K	mg $plant^{-1}$	674.09 ± 177.12	579.33 ± 194.56	<0.001
Ca	mg $plant^{-1}$	484.6 ± 223.02	339.07 ± 79.04	<0.001
Mg	mg $plant^{-1}$	269.15 ± 128.03	173.31 ± 45.56	<0.001
Cu	mg $plant^{-1}$	1.7 ± 1.16	1.84 ± 0.7	0.144
Fe	mg $plant^{-1}$	35.78 ± 14.31	38.88 ± 18	0.072
Mn	mg $plant^{-1}$	39.28 ± 26.11	348.23 ± 143.71	<0.001
Zn	mg $plant^{-1}$	12.07 ± 5.52	15.41 ± 4.04	<0.001
		Nutrient Use Efficiency		
N	g shoot g^{-1}	95.88 ± 19.66	91.23 ± 17.73	0.003
P	g shoot g^{-1}	1635.52 ± 911.82	1919.40 ± 1000.92	<0.001
K	g shoot g^{-1}	59.87 ± 20.37	45.08 ± 8.74	<0.001
Ca	g shoot g^{-1}	87.47 ± 65.15	88.40 ± 14.78	<0.001
Mg	g shoot g^{-1}	148.86 ± 88.67	175.15 ± 29.41	<0.001
Cu	g shoot mg^{-1}	274.56 ± 191.12	185.85 ± 111.65	<0.001
Fe	g shoot mg^{-1}	10.47 ± 5.80	14.66 ± 74.19	<0.001
Mn	g shoot mg^{-1}	11.37 ± 7.31	1.00 ± 0.53	<0.001
Zn	g shoot mg^{-1}	29.65 ± 11.18	20.15 ± 6.88	<0.001

* N: nitrogen, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, Cu: copper, Fe: iron, Mn: manganese, Zn: zinc.

For the nutrients studied, concentration, NUE, and uptake showed similar patterns. N uptake was similar in both treatments (with and without lime), and the genotypes that showed higher values for N uptake were ICT-2173 (477.92 ± 102.26 mg $plant^{-1}$), PH-990 (474.00 ± 84.83 mg $plant^{-1}$), PH-144 (463.77 ± 44.88 mg $plant^{-1}$), CCN-51 (462.45 ± 26.12 mg $plant^{-1}$), and ICT-1089 (455.03 ± 59.52 mg $plant^{-1}$). For P, uptake was higher in limed soil, and the genotypes that showed higher values were PH-16 (57.42 ± 7.32 mg $plant^{-1}$), UGU-112 (57.25 ± 9.38 mg $plant^{-1}$), AYP-15 (54.13 ± 5.19 mg $plant^{-1}$), and SCA-6 (52.74 ± 8.48 mg $plant^{-1}$). Overall, genotypes grown on limed soil had higher K concentration and uptake, and genotypes: ICT-2173 (1172.7 ± 33.61 mg $plant^{-1}$), PAS-91(1082.45 ± 217.38 mg $plant^{-1}$), TSH-1188 (971.22 ± 82.69 mg $plant^{-1}$), CCN-51 (968.71 ± 98.78 mg $plant^{-1}$), and H-10 (966.57 ± 378.88 mg $plant^{-1}$) recorded the highest K uptake.

For Ca and Mg, concentrations and uptake were higher in limed soil, and the genotypes that showed higher values of Ca uptake were ICT-2173 (1275.2 ± 59.74 mg plant^{-1}), PAS-91 (871.23 ± 140.11 mg plant^{-1}), PH-990 (808.00 ± 120.57 mg plant^{-1}), PAS-93 (797.78 ± 97.13 mg plant^{-1}), and PH-16 (763.52 ± 214.18 mg plant^{-1}), while the genotypes with a higher Mg uptake were ICT-2173 (718.89 ± 56.15 mg plant^{-1}), PAS-91 (592.46 ± 77.63 mg plant^{-1}), H-10 (496.44 ± 180.08 mg plant^{-1}), PAS-93 (469.07 ± 39.66 mg plant^{-1}), and PH-990 (459.81 ± 46.58 mg plant^{-1}). Curiously, several cacao genotypes collected in the Pastaza region of Peru (PAS) have higher Ca and Mg uptake, indicating a possible high requirement or ability to uptake Ca by these genotypes that could be related to their mechanism of tolerance to soil acidic conditions.

For micronutrients, in general, genotypes showed higher uptake in unlimed soil than in limed soil. In the case of Cu, the genotypes that showed higher Cu uptake in mg plant^{-1} were ICT-1087 (7.08 ± 2.38), ICT-1189 (4.02 ± 2.46), ICT-2171 (3.88 ± 0.54), and IMC-67 (3.14 ± 1.57). For Fe, the genotypes that showed higher uptake in mg plant^{-1} were CA-14 (89.94 ± 2.31), PAS-91 (74.61 ± 23.43), PAS-93 (67.85 ± 16.29), UF-667 (65.27 ± 16.58), and UGU-112 (64.19 ± 4.5). For Mn, the genotypes with higher uptake in mg plant^{-1} were EET-400 (656.15 ± 119.49), ICS-95 (606.05 ± 217.83), ICT-1189 (554.44 ± 45.48), CP-2005-C10 (553.47 ± 72.71), and ICT-1292 (543.72 ± 9.37). For Zn, the genotypes with higher uptake in mg plant^{-1} were UNG-77 (33.01 ± 27.52), PAS-105 (23.26 ± 1.59), AYP-22 (21.23 ± 4.57), ICS-95 (20.54 ± 3.49), and CEPEC-2002 (20.45 ± 5.06).

3.4. Comparison of Tolerance Indices to Acidic Soils in Juvenile Cacao

The calculations for ATI (%), GTI (%), and CATI (%) for the 60 cacao genotypes are presented in ESI-Table S3, where the clones were classified as non-tolerant (ATI or CATI < 85%; GTI < 100%) and tolerant (ATI or CATI > 85%; GTI > 100%). The results calculated with ATI (%), GTI (%), and CATI (%), are presented in Table 5. When the classification was based on ATI (%), 42 genotypes were considered tolerant (70%), and 18 were considered non-tolerant (30%). If the classification is based on GTI (%), the threshold was more restrictive; therefore, 34 genotypes were considered tolerant (56.7%), and 26 were considered non-tolerant (43.3%). Nevertheless, when the classification was based on CATI (%), only ten genotypes were considered tolerant (16.7%), and 50 were non-tolerant (83.3%).

ATI, however, only considers the tolerance to acidity but ignores the overall biomass in the studied genotypes. Therefore, in that list, we have genotypes with low production of biomass but high tolerance to acidity. In the case of GTI, it considers the effect of the biomass, being more selective than ATI but uses central tendency measures (means) for calculation. This has an undesired effect of increasing values for low-biomass clones and diminishing values for high-production biomass clones under high stress.

On the other hand, CATI is the more selective index. It chooses clones with high tolerance to acidity but also considers biomass production related to the maximum value, avoiding the problem of lowering or increasing index values in high or low biomass-producing clones, making it a better choice when working with species that can tolerate high acidity in the soil. Finally, based on the CATI index (%), ten genotypes were selected as tolerant clones, and we suggest their use in acid soils in cacao-growing regions since they are considered for the relative production of plant biomass. The genotypes selected are the following: CCN-51, PH-21, CCN-10, PAS-91, ICT-1087, ICS-95, UF-667, TSH-565, PH-144, and ICT-1189.

Table 5. Clones selected as tolerant, and non-tolerant (medium and sensitive) with the application of the ATI (acid tolerance index), CATI (corrected acid tolerance index), and GTI (genotypic tolerance index) for selecting cacao genotypes tolerant to soil acidity.

Tolerance Indices	Tolerant	Non-Tolerant
ATI *	ICT-1506, TSH-565, PAS-91, UGU-112, ICT-1281, TSH-1188, PAS-93, CCN-51, BN-34, PH-09, ICT-1251, PH-21, CP-49-C10, CA-14, CCN-10, EET-400, ICT-1189, PH-144, IMC-67, SCA-6, PH-15, AYP-22, ICT-2161, CP-53-C10, ICT-1087, ICT-1026, ICT-1112, UF-613, ICS-95, ICS-39, ICT-1292, UF-667, POUND-12, ICT-2142, BS-01, PAS-100, PAS-105, ICT-1092, ICS-6, CEPEC-2002, NUC-156, PH-16	PH-17, UNG-53, AYP-15, H-10, ICT-2172, ICT-2653, ICS-1, CP-2005-C10, PH-990, ICT-2171, UNG-77, ICT-2173, IPIRANGA-1, UGU-130, UNG-73, AYP-20, UGU-126, UNG-76
CATI	CCN-51, PH-21, CCN-10, PAS-91, ICT-1087, ICS-95, UF-667, TSH-565, PH-144, ICT-1189	CA-14, PAS-93, PH-09, SCA-6, BN-34, ICT-1026, CP-49-C10, EET-400, PH-15, ICT-1281, ICS-39, BS-01, ICT-1506, TSH-1188, CP-53-C10, UGU-112, ICT-1112, ICT-1251, PH-990, ICT-2142, PH-16, IMC-67, AYP-22, AYP-15, UF-613, ICT-1292, ICT-2172, ICT-2161, ICS-6, ICT-2173, CEPEC-2002, UNG-53, ICT-2171, ICT-1092, PH-17, IPIRANGA-1, UNG-77, PAS-105, CP-2005-C10, H-10, PAS-100, ICT-2653, POUND-12, NUC-156, ICS-1, UNG-73, UGU-126, UGU-130, AYP-20, UNG-76
GTI	ICT-1087, CCN-51, CCN-10, UF-667, PH-990, TSH-565, IPIRANGA-1, PH-21, PH-144, PAS-91, PH-16, ICT-1026, CP-49-C10, ICS-95, ICT-2171, PH-15, ICT-2173, PH-09, SCA-6, ICT-1189, ICT-2172, ICT-2142, UNG-77, BN-34, CA-14, AYP-15, ICS-39, ICT-1292, ICS-6, CEPEC-2002, BS-01, ICT-1112, PAS-93, CP-53-C10	EET-400, CP-2005-C10, H-10, ICT-2161, UNG-53, TSH-1188, IMC-67, PH-17, ICT-2653, UNG-73, PAS-105, ICT-1281, UF-613, AYP-22, ICT-1506, ICT-1092, ICT-1251, ICS-1, POUND-12, UGU-112, UGU-126, PAS-100, NUC-156, UGU-130, UNG-76, AYP-20

* ATI: acid tolerance index; CATI: corrected acid tolerance index, GTI: genotypic tolerance index.

3.5. Relationship between Biometric Parameters and Nutrient Uptake in Different Clones

To explore the relationship between acidity-tolerant and acidity-sensitive cacao genotypes, principal component analyses were performed on biometric parameters and nutrient uptake in limed and unlimed soil, and the result is presented in Figure 2. In the case of plants grown in unlimed acid soil, a relatively good model was obtained through PC1, PC2, and PC3, where the variations were 48.3, 11.2, and 9.1%, respectively, and the accumulated variance of the model was 68.5%. For the plants grown in the limed soil, the results for variance in PCA1, PC2, and PC3 were 22.7, 15.5, and 13.5%, with an explained variation of 51.7%. The representative points of each genotype are presented as sensitive and tolerant clones (as described in Table 2) and mapped in the space for the first two principal components (PC1 and PC2).

Our main interest was to explore the correlations between CATI and the other evaluated variables since it is our main predictor of acidity tolerance and will assist the selection of the main explanatory variables for acidity tolerance. In the case of limed soil conditions, CATI was positively correlated with Fe and N uptake and to a minor degree with shoot/root ratio (S/R), height, root and foliar area, and number of leaves. In unlimed acid soil conditions, CATI was positively correlated with N, K, Ca, Mg, Mn, and Zn uptake and to a minor degree with the number of leaves and foliar area. In general, foliar area, number of leaves, and N uptake seem to be good indicators for selecting plants since these variables were positively correlated with CATI in both limed and unlimed acid soil conditions.

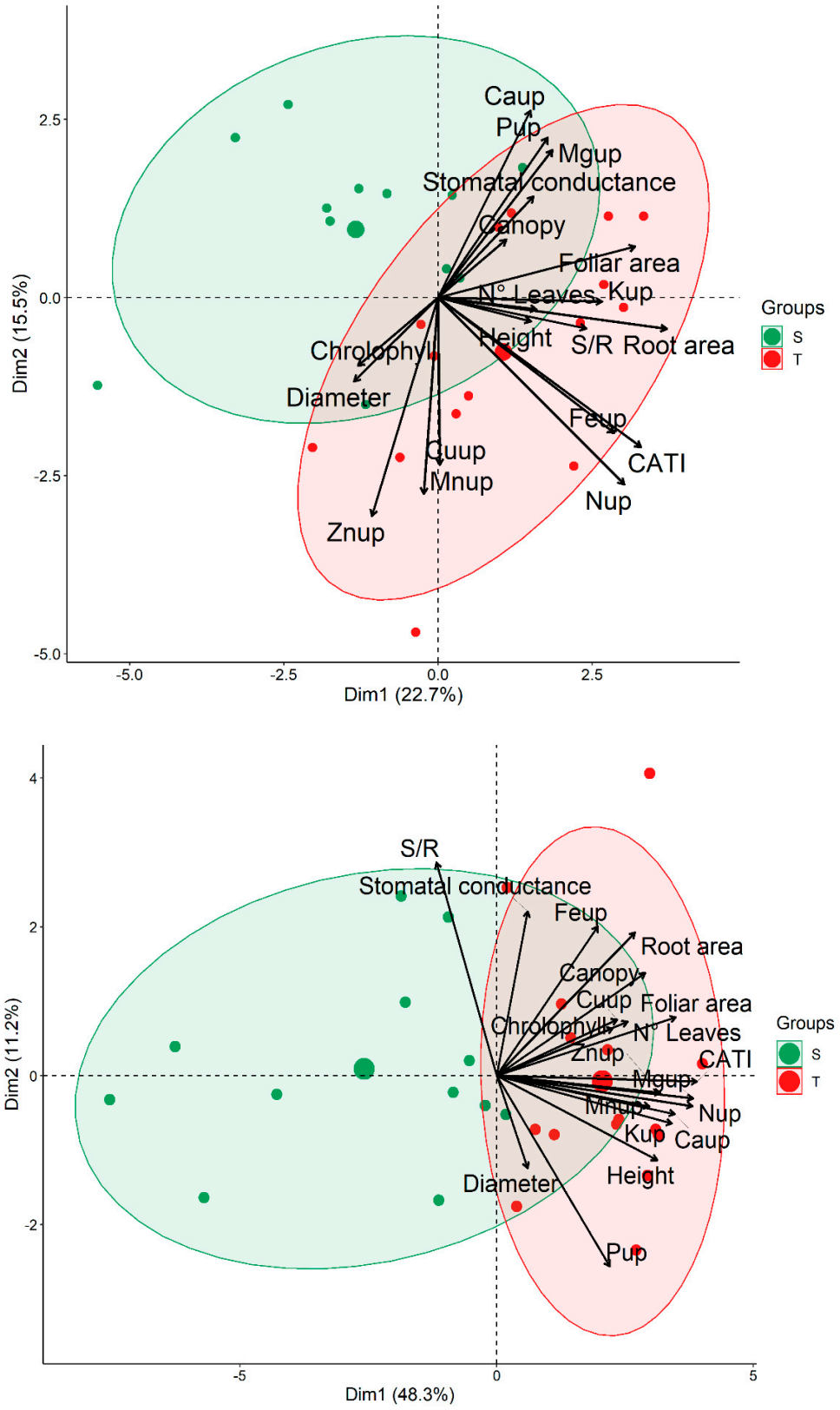

Figure 2. Results biplot of principal component analysis for assessing relations between growth parameters, nutrient uptake, and CATI on tolerant and sensitive clones under limed soil (**top**) and unlimed acid soil (**bottom**). Nup: nitrogen uptake; Pup: phosphorus uptake; Kup: potassium uptake; Caup: calcium uptake; Mgup: magnesium uptake; Cuup: copper uptake; Feup: iron uptake; Mnup: manganese uptake; Znup: zinc uptake.

4. Discussion

4.1. Responses of Cacao Plants under Acid Soils

Overall, cacao plants in limed treatment performed better for biometric and physiological characteristics. In general, in comparison to other tropical crops, cacao responds well to fertilization [28]. Literature in cacao research has been mostly focused on aluminum toxicity rather than H^+ rhizotoxicity, and information is scarce on that subject. However, H^+ toxic effects should not be forgotten, as they can produce similar symptoms as those observed in toxicity from Al^{3+} [17]. Therefore, in this paper, while discussing the effects of soil acidity, we took both Al^{3+} and H^+ toxicity into consideration.

Acid soils are considered a worldwide problem and affect the general growth and production of different crop species. In general, soil acidity is divided into active acidity (pH), exchangeable acidity (Al^{3+}), and potential acidity or total acidity (Al^{3+} + H^+). Domination of Al^{3+} or H^+ may directly affect crop tolerance to acid soils [17]. Acidity can diminish growth, root elongation, and biomass production, as observed in our study and others, in cacao and other tropical crops [6,12,15,30,31]. One of the main effects of Al^{3+} in plants is the inhibition of root elongation, resulting in stubby root tips, making plants more susceptible to drought, or limiting nutrient acquisition [32]. Some of the reported effects of acidity (mainly aluminum), as well as other previous studies in cacao, are decreased shoot and root dry weight, reduced stem height, root length and root volume [12–15]. Nevertheless, the present results on height and diameter were like those reported by Dogbatse et al. [15], who observed no significant differences in height and diameter in different soils that had pH values ranging from 4.21 to 5.64.

4.2. Nutrition and Tolerance to Soil Acidity of Cacao Plants

In general, plants that grow in unlimed acidic soil conditions have higher concentrations of micronutrients, because of the high availability of these elements in acidic soils, in particular Mn [33]. High concentrations of Mn can have toxic effects on plants and can also affect the general growth and development of cacao.

Acidification has many sources and one of the most important ones is the replacement of exchangeable Ca^{2+}, Mg^{2+}, and K^+ by H^+ and Al^{+3}, consequently, metal toxicity (Al and Mn primarily) and nutrient deficiencies (i.e., P) occur in acid soils that limit plant survival and productivity [7,17]. In general, P has been one of the most studied nutrients in acid soils due to its general deficiency; its deficiency is related to metabolic, physiological, and productivity-limiting problems in different crops, and cacao is no exception [15,17,29]. Our results indicate that liming acid soil improves macro nutrient acquisition by cacao and liming reduces micronutrient absorption, especially Mn, which was observed in toxic concentrations (>500 mg kg^{-1}) as stated by Marschner [34]. Several studies in cacao focused on concentrations and uptake of macronutrients (N, P, K, Ca, Mg, and S) in acidic and non-acidic soils and showed that plants with less Al^{3+}, have a higher uptake of N, P, K, Ca, Mg, and S [12,13,15,31,35]. In the case of micronutrient uptake, they are less studied in cacao. The consensus is that liming or soil with higher pH induces cacao to uptake fewer micronutrients, especially Mn, as shown in the present study and other studies with acid soils in cacao [13,35]. It is known that soil pH is responsible for the availability of nutrients and can affect the soil CEC [33]. In general, in acid soils, a low absorption of Ca is observed. However, some of the clones that were more tolerant to acidity also had higher absorption of Ca. Calcium is known to alleviate acidity-induced toxicity in plants by several mechanisms, such as displacement of Al from the surface of the cell membrane (SCM), restoration of the Ca on the SCM and, ionic competition between Ca and Al in root cells [6,36], which could be the mechanisms explaining tolerance of acidity in these cacao genotypes. Both ATI and GTI indices are commonly used for estimating tolerance to acidity [37,38]. However, CATI is a more selective index for acidity tolerance in plants.

Cacao propagation programs have long used rootstocks to overcome abiotic and biotic constraints limiting cacao growth [39]. The main characteristics of desirable rootstocks are

their vigor (rapid growth or biomass production) and pest resistance, especially to Cocoa wilt or *Phytophthora*, as shown in IMC-67 [39,40].

4.3. Relationship between Biometric, Physiology and Nutrition and Tolerance to Soil Acidity of Cacao Plants

In general, foliar area, number of leaves and N uptake seem to be good indicators for selecting plants since these variables were positively correlated with CATI in both limed and unlimed acid soil conditions.

These results agree with other published studies in cacao, which emphasized the importance of biometric parameters for assessing tolerance to acid conditions in soils [12,13,31]. These correlations also suggest that the most critical nutrients under acid soil conditions may be related to better nutrition of N, Ca, Mg, and K, which could be optimized by fertilizer application via foliar means and may have a direct effect on tolerance to acidity on cacao clones, but further research should be conducted to confirm this observation.

N is the nutrient in highest demand by agricultural and forest crops worldwide. Its deficiency limits growth and yield and is also a major problem in acid soils [41,42]. The two main sources for N are NH_4^+ and NO_3^-. In acid soils, where Al^{+3} is highly available, the tolerance of plants to Al may be related to NH_4^+ over NO_3^- preference [43,44]. In the case of cacao, previous work has shown that its preference is NH_4^+ [45]. This may be one of the factors associated with the ability of these genotypes to tolerate acidity since NH_4^+ alleviates Al toxicity in acid soils [44,45].

Ca, Mg, and K are nutrients that are commonly in low concentrations in acid soils and have direct implications on tolerance to plants to abiotic stresses such as soil acidity, drought, and salinity [17,34]. Potassium (K) is the most abundant nutrient and is essential for overall growth, development, and productivity, and it is the most exported nutrient by cacao crops [46,47]. K in adequate levels enhances photosynthetic efficiency and nutrient uptake and maintains adequate leaf turgor [48]. The movement of K across the membranes of cells has a direct implication in the cascade of responses to stimuli in plants for diverse types of stress such as drought and flooding that are common in Amazon regions [48,49].

Ca and Mg are essential as structural or regulatory components of the plants, related to the capacity of coordination, which provides stable reversible molecular linkages, and they have a role as a secondary messenger for physiological responses to environmental and developmental stimuli [34,50,51]. As stated in Section 3.3, Ca alleviates toxicity produced by soil acidity, and in general, calcium is added when amending acid soils to elevate pH and reduce overall acidity in the soil, which enhances productivity and tolerance to stress in different tropical plants such as oil palm, coffee, cacao, and maize [52–54].

5. Conclusions

Acidity is a worldwide problem to produce crops in tropical regions, and in many parts of the world, access to amendments to correct soil acidity is limited. Planting acidity-tolerant genotypes offer an alternative solution to alleviate the stress of soil acidity. In the present study, 60 cacao genotypes from different geographic origins were grown in ulimed acid and limed soil conditions to evaluate their tolerance to soil acidity. Growth and physiological parameters as well as the concentrations of nutrients varied significantly among these genotypes. Better growth parameters were obtained under limed conditions. Furthermore, acid conditions revealed different cacao behavior responses to acidity stress, which enabled the selection of ten acidity-tolerant cacao genotypes: CCN-51, PH-21, CCN-10, PAS-91, ICT-1087, ICS-95, UF-667, TSH-565, PH-144, ICT-1189. These genotypes are potentially useful as parental clones for breeding programs or as rootstock. Finally, our results indicated that the best early growth predictors of this acid soil tolerance are the number of leaves and the root area, and that under acid conditions, N, Ca, Mg, and K uptake may have a special role in cacao acid soil tolerance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123124/s1. Table S1. Effect of soil acidity (Mean ± sd) on biometric characteristics (height, diameter, number of leaves, leaf and root area, shoot and root biomass) and physiological characteristics (stomatal conductivity and chlorophyll concentration) for each clone assessed. Table S2. Effect of soil acidity (Mean ± sd) on the concentration and uptake of macronutrients (N, P, K, Ca, Mg) and micronutrients (Cu, Fe, Mn, and Zn) for each cacao genotype assessed. Table S3. Results of acid tolerance index (ATI), corrected acidity tolerance index (CATI), and genotypic tolerance index (GTI) in % of 60 cacao clones submitted to soil acidity.

Author Contributions: C.O.A.-H. planned the experiments, carried out the soil and plant analyses, conducted the experiment and statistical analysis, contributed to the manuscript. E.A.-G. conceived the original idea and planned the experiments. A.F. conducted the cacao propagation experiment. M.A.-G. conducted the cacao propagation experiment. A.D. and D.Z. helped shape the research, analysis, and manuscript. V.C.B. conceived the study and was in charge of the overall direction and planning. All authors provided critical feedback in the writing process. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The project 428-PNICP-PIAP-2014/FINCYT and the project NACA 58-1275-7-112F and 58-1275-2-090F of USDA-ARS-ICT partially supported the lab and greenhouse activities of this research.

Data Availability Statement: Data presented in this study are available on request from the corresponding author.

Acknowledgments: We thank the Intemational Cocoa Quarantine Centre, University of Reading, UK for providing the Intemational Clones used in this research; also, we thank Raul Valle of CEPLAC/CEPEC (Comissao Executiva do Plano da Lavoura Cacaueira/Centro de Pesquisas de Cacau), Ilheus/Itabuna, Bahia, Brazil for providing the Brazilian clones used for this research. We also appreciate the technical and logistical facilities for the field research and laboratory analytical facilities, provided by Instituto de Cultivos Tropicales [Tropical Crops Institute]-ICT/CITE Cacao y Otros Cultivos Tropicales [Productive Innovation and Technology Transfer Centre (CITE) of Cacao and Other Tropical Crops]. We thank Marshall Elson for excellent review of the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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