Growth, physiological, nutrient uptake efficiency and shade tolerance responses of cacao genotypes under different shades

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Highlights:

- Significant interactions observed between shade and cacao genotypes
- Nutrient use efficiency increased with reduced shade
- Fourteen cacao genotypes were tolerant to high shade
- Intraspecific difference observed for growth, physiology and nutritional traits at different shade levels

Keywords: *Theobroma cacao*, light, abiotic stress, physiology, plant nutrition

Abstract

Cacao is an understory plant cultivated under full-sun monocultures to multi-strata agroforestry systems, where cocoa trees are planted together with fruit, timber, firewood, and leguminous trees, or grown within thinned native forests. Under agroforestry systems of cultivation cacao is subjected to excess shade due to high density of shade trees, and over grown or unmanaged pruning of shade trees. Cacao is tolerant to shade, and maximum photosynthetic rate occurs around irradiance of 400 μmol m^{-2} s^{-1} but excess shade reduces the irradiance further which is detrimental to photosynthesis and growth functions. Intra specific variation is known to exist in cacao for the required saturation irradiance. A greenhouse study was implemented with 58 cacao genotypes selected from four geographically diverse groups: (i) wild cacao from river basins of the Peruvian

Amazon, (PWC), (ii) Peruvian farmers' collection (PFC), (iii) Brazilian cacao collection (BCC) and (iv) national and international cacao collections (NIC). All the cacao genotypes were subjected to 50% and 80% shade where photosynthetic photon flux density (PPFD) was 1000 and 400 µmol m^{-2} s^{-1} respectively. Intra specific variations were observed for growth, physiological and nutritional traits and tolerance to shade. Cacao genotypes tolerant to shade were: UNG-77 and UGU-130 from PWC; ICT-2173, ICT-2142, ICT-2172, ICT-1506, ICT-1087, and ICT-2171 from the PFC; PH-21, CA-14, PH-990 and PH-144 from BCC; and ICS-1, ICS-39, UF-613 and POUND-12 from NIC. Genotypes that tolerate excess shade might be useful plant types to maintain productivity and sustainability in agroforestry systems of cacao management.

1. Introduction

Beyond the basic concept of shade tolerance, which is defined as the minimum light required for a plant to survive and develop in different strata, this factor involves a wide range of effects that are connected with several aspects of the plant life cycle as well as with ecosystem dynamics [1-4]. Adaptation of species to shade is still poorly understood [5], and the positive or negative effects are sometimes contradictory, which is the case of cacao [6].

Cacao (*Theobroma cacao*) is an understory plant [7,8] cultivated under different cropping systems: from full-sun monocultures to multi-strata agroforestry systems, where cocoa trees are planted together with fruit, timber, firewood, and leguminous trees, or within thinned forests [7,8]. The use of shade trees is a common agricultural and sustainable practice in cacao production systems [9-16]. These trees act as a protection barrier against stressful environmental conditions such as extreme temperature, solar radiation, drought, and intense rainfall and wind [6,7,17,18].

Several benefits have been attributed to cacao growing within shaded agroforestry systems such as control of diseases or insect attacks, maintaining soil fertility, enhancing nutrient cycling, reducing soil erosion and deforestation, increasing tree diversity, mitigating climatic changes thorough C sequestration, helping to reduce the use of pesticides and fertilizer applications [13, 19-26].

However, some authors report that lower yields have been observed in shaded cacao systems, mostly related to climate conditions (i.e. precipitation and temperature) [27-30]. Even so, farmers can get more profitable net revenues because of the lower maintenance cost (compare to conventional systems) and higher prices obtained per kilogram of cocoa beans [31].

In other situations, cacao yield is affected due to the presence of too many trees, trees with large canopies and poorly managed tree canopy structure [24,32]. Under such conditions the longevity of the plantation could also be affected [33], therefore it is advantageous to adopt cacao genotypes that maintain their productivity even under reduced irradiance.

A definition of an optimum shade level for cocoa is needed [7,17,33] as well as a selection of the appropriate shade species to avoid the detrimental effects of shade [6]. It is also important to mention that the need of shade may not be required in all cacao-growing regions especially in island and heavy cloud cover ecosystems [6].

In tropical forests, understory plants receive a photosynthetic photon flux density (PPFD) of between 5 and 25 μmol $m^{-2}s^{-1}$ or 1 to 2% of the irradiance obtained above the tree canopy level but they also intermittently get high levels of PPFD in the form of sunflecks [34-40]. Miyaji et al. [41] reported that the light intensity (at full daylight) above the cacao canopy shaded by trees in Bahia, Brazil was between 30 and 100% and between 4 and 10% at ground level. In another study conducted in Alto Beni (Bolivia); Niether et al. [18], measured the light levels of various cacao systems and determined a PPFD between 1580 and 2028 μmol m^{-2} s^{-1} above the canopy. In contrast, in an open sun monoculture, irradiance varied between 985 and 1546 μmol m^{-2} s^{-1} depending on canopy pruning (before and after, respectively). Consequently, light levels have significant influence on growth, physiological traits and nutrition of cacao [33,42-44].

It has been reported that maximum photosynthesis in cacao occurs at a PPFD of 350 to 550 μmol m^{-2} s^{-1}, which is about 20 to 25 % of the intensity of full sunlight [42, 45-47]. In some cacao genotype, an increase of PPFD from 50 to 400 μmol m^{-2} s^{-1} entailed an increase of the net photosynthetic rate (P_N) by about 50%, but further increases (up to 1500 μmol m^{-2} s^{-1}) had no effect, indicating that very little radiant energy is required to support efficient P_N in cacao [43].

Variations in morphological characteristics among different genotypes have been reported in cacao [48,49]. At the same time, these characteristics have been influenced by the level of irradiance [10, 11, 43, 50]. Baligar et al. [43] reported that in juvenile cacao genotypes increasing PPFD from 65 to 190 μmol m^{-2} s^{-1} increased shoot and root growth, mineral nutrition and net assimilation rate. Thus, identification of plant traits for growth and physiological parameters that are influenced by light quality and intensity (PPFD) will help to identify cacao genotypes that could perform well under a range of light intensities (or shade levels).

The aim of this research was to evaluate the effects of shade (PPFD, Photosynthetic Photon Flux Density) on growth, physiological, macronutrients and micronutrients use efficiency and shade tolerance responses of national (wild, domesticated) and international cacao genotypes.

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2. Material and methods

2.1 Experimental conditions

2.1.1 Cacao genotypes

The greenhouse experiment was implemented at the Instituto de Cultivos Tropicales-ICT (Tropical Crops Institute), Tarapoto, San Martin, Peru. A total of 58 cacao genotypes from the germplasm bank located at "El Choclino" experimental station was selected from four geographic origins: (i) wild cacao from river basins of Peruvian Amazon collection (PWC), (ii) Peruvian farmers' collection (PFC), (iii). Brazilian cacao collection (BCC) and (iv). National and International cacao collection (NIC). Several expeditions were under taken by ICT during 2008 to collect wild cacao genotypes from the river basins of Aypena (AYP), Pastaza (PAS), Ungumayo (UGU) and Ungurahui rivers (UNG); on the other hand, Peruvian farmers' collections were made by ICT during 2002 to 2004 in the Provinces of Mariscal Cáceres and Tocache of San Martin department at North-East, Peru. Brazilian clones were from CEPLAC/CEPEC (Comissao Executiva do Plano da Lavoura Cacaueira/Centro de Pesquisas de Cacau) Ilhues/Itabuna Bahia Brazil and International clones were from CFC/ICCO/Biodiversity Clones, University of Reading, Reading, UK. (Table 1).

Table 1. List of 58 cacao genotypes used for this experiment and obtained from 4 different origins maintained at ICT germplasm bank.

Wild cacao genotypes from River basins of Peru Amazon (PWC)			Peruvian farmers' cacao genotypes (PFC)			Brazilian genotypes (BCC)			National & International genotypes (NIC)		
N°	**Genotype**	**Origin**	**N°**	**Geno type**	**Origin**	**N°**	**Genotype**	**Origin**	**N°**	**Genotype**	**Origin**
1	AYP-15	Aypena	1	ICT-1026	Mariscal Cáceres - Juanjui	1	BN-34	Fazenda Boa Nova	1	CCN-10	Ecuador
2	AYP-20	Aypena	2	ICT-1087	Mariscal Cáceres - Juanjui	2	BS-01	Fazenda Bom Sossego	2	CCN-51	Ecuador
3	AYP-22	Aypena	3	ICT-1092	Mariscal Cáceres - Juanjui	3	CA-14	Fazenda Canta Galo	3	EET-400	Ecuador
4	PAS-91	Pastaza	4	ICT-1112	Mariscal Cáceres - Juanjui	4	CEPEC-2002	Centro de pesquisa do cacau	4	H-10	Peru
5	PAS-93	Pastaza	5	ICT-1189	Mariscal Cáceres - Juanjui	5	CP-49-C10	Centro de pesquisa do cacau	5	ICS-1	Trinidad and Tobago
6	PAS-100	Pastaza	6	ICT-1251	Mariscal Cáceres - Juanjui	6	CP-53-C10	Centro de pesquisa do cacau	6	ICS-6	Trinidad and Tobago
7	PAS-105	Pastaza	7	ICT-1281	Mariscal Cáceres - Juanjui	7	IPIRANGA-1	Cidade de Ipiranga	7	ICS-39	Trinidad and Tobago
8	UGU-112	Ungumayo	8	ICT-1292	Mariscal Cáceres - Juanjui	8	PH-09	Fazenda Porto Hibrido	8	ICS-95	Trinidad and Tobago
9	UGU-126	Ungumayo	9	ICT-1506	Mariscal Cáceres - Juanjui	9	PH-15	Fazenda Porto Hibrido	9	IMC-67	Peru
10	UGU-130	Ungumayo	10	ICT-2142	Tocache	10	PH-16	Fazenda Porto Hibrido	10	POUND-12	Peru
11	UNG-53	Ungurahui	11	ICT-2161	Tocache	11	PH-17	Fazenda Porto Hibrido	11	SCA-6	Peru
12	UNG-73	Ungurahui	12	ICT-2171	Tocache	12	PH-21	Fazenda Porto Hibrido	12	TSH-565	Trinidad and Tobago
13	UNG-76	Ungurahui	13	ICT-2172	Tocache	13	PH-144	Fazenda Porto Hibrido	13	TSH-1188	Trinidad and Tobago
14	UNG-77	Ungurahui	14	ICT-2173	Tocache	14	PH-990	Fazenda Porto Hibrido	14	UF-613	Costa Rica
			15	ICT-2653	Tocache				15	UF-667	Costa Rica

2.1.2 Shade (PPFD) levels

The greenhouse was constructed and aligned in an east-west direction to evaluate the response of cacao genotypes to two levels of shade (Figure 1). The greenhouse was divided into two sections and covered with different light transmissibility plastic screens (Raschel mesh, from Arborizaciones EIRL®) to achieve different levels of shade. The first section provided 80% shade with a PPFD of 400±50 μmol m^{-2} $s^{-1,}$ and the second section provided 50% shade with PPFD of 1000±50 µmol m^{-2} s^{-1}. The PPFD in each section of the greenhouse was measured by a MQ-200 Quantum sensor (Apogee Instruments, Logan, UT USA). At both ends of greenhouse exhaust and inlet fans were installed to circulate air from inside to outside.

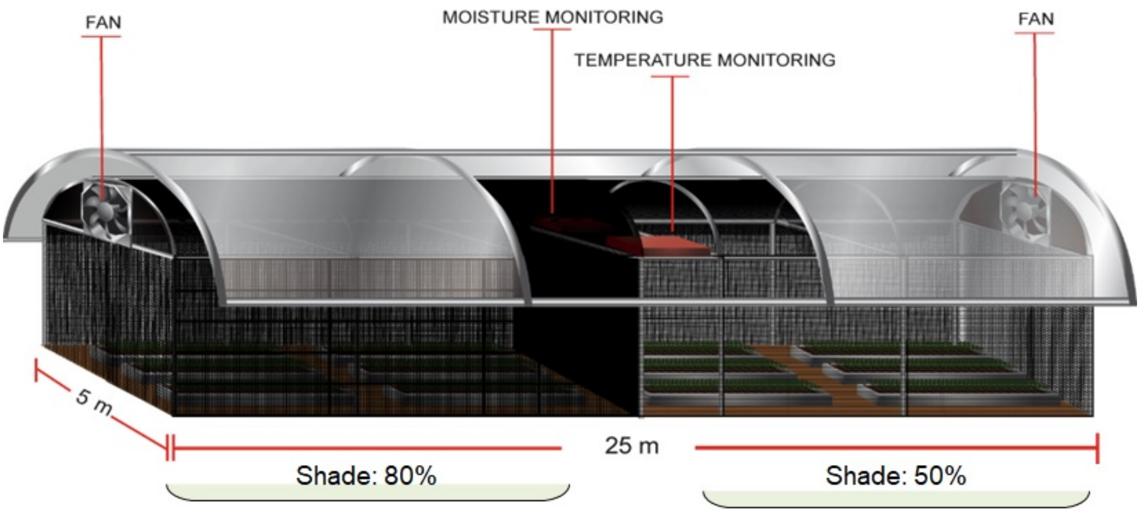

Figure 1. Greenhouse constructed at the ICT (Tropical Crops Institute) Tarapoto Peru to evaluate response of 58 cacao genotypes response to two levels of shade: 50% (PPFD of 1000 ± 50 µmol m^{-2} s^{-1}) and 80% (PPFD of 400 ± 50 µmol m^{-2} s^{-1}).

2.1.3 Plant growth conditions

The rooted clonal cuttings from plagiotropic branches of various genotypes were prepared in the greenhouse. Terminal apical shoots with 3 or 4 leaves from each of the genotypes were cut, making a bevel cut, at the base of the branches (¾ from the leaf area) and dipped into plant rooting hormone (Hormodin3®, 0.8% indole-3-butyric acid, IBA) to induce roots. These cuttings were transplanted into polyethylene bags containing 2 kg of agricultural soil (sand = 50.96%, silt = 22%, clay = 27.04%) previously fertilized with 60N :50P :90 K kg ha^{-1} applied as urea, calcium dihydrogen phosphate and potassium chloride respectively. Dolomitic lime (1MT ha^{-1}) was added to raise pH 6.0. Plants were maintained in the greenhouse at 28 °C, 80% relative humidity, with minimum light PPFD 50±5 µmol·m^{-2}·s^{-1}, soil moisture was maintained at the field capacity until root formation and proper pest control methods were adapted. At the end of 4 months growth, seedlings were transplanted into plastic pots containing 5 kg of sandy loam soil previously fertilized with urea (30 mg N kg^{-1}), $Ca(H_2PO_4)_2$ (25 mg P kg^{-1}), KCl (45 mg K kg^{-1}) and Dolomitic lime (2.5 g kg^{-1}). Soil physicochemical properties of the sandy loam soil used for the study were: 72% sand, 18% clay, 1.55 g cm^{-3} bulk density, 58% porosity, pH 6.1, organic matter 1.77%, CEC 4.44 cmol kg^{-1}, N (0.08%), P (4 mg kg^{-1}), K (75 mg

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kg^{-1}), Ca (1.22 $cmolc^{+}$ kg^{-1}) and Mg (0.60 $cmolc^{+}$ kg^{-1}), determined using the methods of Silva [51].

All the rooted genotypes seedlings were divided into two equal groups and subjected to two shade (PPFD) levels (Fig 1). Plants were grown for 6 months at different shades and soil moisture during growth was maintained at field capacity (-33 KPa) by watering with deionized water every other day and soil moisture status was monitored with a soil moisture tensiometer (2724 ARL Jet Fill tensiometer, Soilmoisture Equipment Corp, Santa Barbara, CA USA). During growth of the plants, the greenhouse provided a mean temperature of 30.0 °C as well as relative humidity of 63%. The experiment was conducted using a split-plot design with three replications under complete random distribution, two shade treatments were the main plots (50% and 80% shade) and 58 cacao genotypes were the subplots.

2.2 Biometric parameters

At the time of harvest, shoot length was measured from the base of the stem to the apex of the plant (cm) and stem diameter was measured with a ruler and digital Vernier (mm) respectively. At harvest stems, leaves and roots were separated; leaf and root area were measured in cm^2 by image analysis (Assess 2.0: Image Analysis Software for Plant Disease Quantification, APS, Saint Paul, MN USA) [52]. All plant parts were washed with tap water, dipped in 1% HCl and rinsed in distilled water, placed in paper sachets and oven dried at 60°C for 72 hours, until reaching a constant weight.

2.3 Physiological parameters

A week before harvest three mature leaves were selected randomly per genotype/pots and per shade treatment to record stomatal conductance (in mmol m^{-2} s^{-1}) using a SC-1 leaf porometer (Decagon Devices, Pullman, WA USA). Leaf chlorophyll content or “greenness” (in SPAD index) was measured by a chlorophyll meter, SPAD 502, (Spectrum Technologies, East Plainfield, IL USA).

Water use efficiency (WUE, g $plant^{-1}$ L^{-1}) was calculated as follows:

Eq.1. $WUE = \frac{SDW}{Total\ water\ used\ during\ entire\ growth}$

where:

SDW = Shoot dry weight, g $plant^{-1}$

Total water used during entire growth period = 18.0 L (50% shade) and 11.7 L (80% shade) $plant^{-1}$

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2.4 Nutrient uptake parameters

2.4.1 Concentration of nutrients

Oven dried shoots of all genotypes were ground to pass through a 1-mm sieve and 500 mg of sample was digested in 10 ml of 65% HNO_3 [53] and the concentration of macronutrients (K, Ca, Mg) and micronutrients (Cu, Fe, Mn and Zn) in the digest were determined by atomic absorption spectrophotometry (AAS, Varian model "Spectra 55B", Victoria, Australia) and P by the ascorbic-molybdate color development method [51]. Total N was determined by the Kjeldahl method (digestion with 5 ml of H_2SO_4 95%) both N and P were detected by a spectrophotometer (Spectronic 20D, Thermo Fisher, Waltham, MA USA) [51]. Concentrations are presented as the mean values from three replicates and expressed in g kg^{-1} for macronutrients and mg kg^{-1} for micronutrients.

2.4.2 Nutrient uptake (U)

The nutrient uptake (U) or element content was calculated as follows:

Eq.2. $U = \frac{Element\ concentration \times SDW}{1000}$

where:

Element concentration= in g kg^{-1} (for macronutrients) or mg kg^{-1} (for micronutrients)

SDW (Shoot dry weight) = in g plant^{-1}

U (or content) = g plant^{-1} (macronutrients) or mg plant^{-1} (micronutrients)

2.4.3 Nutrient uptake efficiency (NUE)

Nutrient uptake efficiency (NUE), which is used to differentiate plant species, genotypes and cultivars for their ability to absorb and utilize nutrients for maximum yields [54, 55] was calculated as follows:

Eq.3. $NUE = \frac{1}{Element\ concentration} \times 1000$

where:

Element concentration = in g kg^{-1} for macronutrients and in mg kg^{-1} for micronutrients

NUE = in g shoot g^{-1} of any given macronutrient or in g shoot mg^{-1} of any given micronutrient

2.5 Shade tolerance index (STI)

Shade tolerance is the ability of a tree to survive and develop under light limited conditions [2]. To classify which cacao genotypes are tolerant or not tolerant, STI was calculated as described in the following equation:

Eq.4. $STI = \frac{Totol\ dry\ biomass\ \text{at 80\% shade}}{Total\ dry\ biomass\ \text{at 50\% shade}}\ x\ 100$

where:

Total (shoot + root) dry biomass (g $plant^{-1}$) at 80% shade (PPFD 400 μmol m^{-2} s^{-1}), this represents low light or heavy shade

Total (shoot + root) dry biomass (g $plant^{-1}$) at 50% shade (PPFD 1000 μmol m^{-2} s^{-1}), this represents high light or low shade

Genotypes were classified into 3 groups: sensitive to shade (STI % ≤ 40), medium shade tolerant (STI % > 40 but ≤ 60) and tolerant to shade (STI % > 60).

2.6 Statistical analysis

Statistical analyses of all the parameters, performed with Infostat ver. 2020 [56], consisted of a split-plot design under complete random distribution in order to compare means across two or more independent variables, in this case shade as a main plot and cacao genotypes as subplots. Normality and homogeneity of each parameter were tested by the Shapiro-Wilk test and Q-Q plot. When the effect of interactions between factors was not statistically significant (α>0.05) and the effect of a factor was significant, the analysis was extended to a Scott-Knott test used to compare means of each parameter.. Double bar graphs were made for NU and NUE, where the results of each nutrient was represented with comparisons between treatments: Shade-50% and Shade-80%, average for each origin. For NUE the "x" axis was presented in a logarithmic scale to facilitate the vision of the results. "ns" indicated the results were not significant (P>0.05) Standardized data were also assessed by Principal Component Analysis (PCA) using R software version 1.2.5042, to evaluate the general correlations between biometric, physiological parameters and nutrient uptake (NU) of cacao seedlings for genotypes sensitive to shade and tolerant to 50% and 80% shade.

3. Results and Discussion

Plants have the ability to adjust their morphology and physiology to a particular light environment. In the case of tropical and subtropical tree species, they have developed species-specific morphological and physiological features allowing them to optimize the capture of scarce solar radiation [36]. Larger leaf areas with anatomical properties associated with increased photosynthetic efficiency as well as an accumulation of anthocyanin are some of the responses of trees to low and high irradiance levels [36, 50], whereas root dry matter remains invariable, suggesting little anatomical or chemical changes [57].

Several authors have reported negative effects of shade on cacao growth [30,58], whereas greater chlorophyll contents [7] and higher photosynthetic rates have been observed in plants under light shade but related to water regime, highlighting the genotypic differences as a response to shade factor [9,59]. The following sections summarize the results obtained in this study for 58 cacao genotypes subjected to 50% and 80% shade conditions.

3.1 Growth parameters influenced by shade levels

The interactions between cacao genotypes and shade levels for biometric parameters were significant (*$P \leq 0.05$*), except for leaf area (Table 2), but for this parameter, significant difference was found between the shade levels and across genotypes. These findings agree with a previous study conducted by Daymond et al. [60], where leaf area varied significantly in eight cacao clones (AMAZ 15/15, ICS-1, IMC-47, MAN 15/2, SC-1, SCA-6, SPEC 54/1, UF-676) exposed to different levels of irradiance (0 to 696 µmol m^{-2} s^{-1}). As highlighted by Acheampong et al. [9], genotypic differences in leaf area under the different shade levels suggest differential partitioning of assimilates in response to light in terms of final biomass.

In most cases, mean values of shoot length, leaf area, root area and shoot/root ratio per genotype were higher at 80% shade, while stem diameter, root dry weight and total dry biomass were higher at 50% shade. The minimum values of leaf and root area were observed for the AYP-20 genotype at 50 and 80% shade. For stem diameter the lowest value of 6.45 mm (80% shade) was recorded for the UGU-130 genotype. The minimum values of shoot length were 23.07 (50% shade) and 33.07cm (80% shade) for PH-09 and CA-14 (BCC), respectively (Table 2).

Table 2. Growth parameters (mean values per plant) of cacao genotypes from different origins subjected to two levels of shade (50% and 80%).

Genotype	SL (cm)		SD (mm)		LA (cm^2)		RA (cm^2)		RDW (g)		BDW (g)		S/R	
	50%	80%	50%	80%	50%	80%	50%	80%	50%	80%	50%	80%	50%	80%
PWC														
AYP-15	33.33c*	45.27b	10.72b	8.19d	706.92b	860.25b	239.83d	208.15d	3.75e	1.14e	25.49d	11.91g	6.22b	9.41a
AYP-20	38.27c	51.27b	10.84b	8.90c	388.71b	701.45b	108.34d	151.63d	6.13d	2.64e	30.97c	16.03f	4.07c	5.25b
AYP-22	37.50c	48.77b	8.94c	7.88d	1245.41a	1032.92a	306.99c	325.15c	5.06d	1.94e	26.02d	6.63g	4.39b	2.47d
PAS-100	27.67c	36.80c	9.91c	7.49d	845.88b	959.96b	190.21d	222.01d	5.72d	1.93e	29.19d	13.37g	4.48b	5.96b
PAS-105	32.67c	36.13c	9.57c	8.07d	596.09b	937.02b	300.61c	388.76b	4.97d	1.40e	28.44d	9.57g	4.74b	5.97b
PAS-91	31.63c	46.90b	11.84a	8.05d	1173.48a	1228.88a	290.09c	433.64a	10.71b	2.72e	42.38a	13.33g	3.07d	4.19c
PAS-93	43.37c	59.97a	11.38b	8.39d	722.72b	1045.25a	197.86d	315.98c	9.17c	2.22e	28.71d	12.68g	2.18d	4.71b
UGU-112	31.43c	44.37b	10.08c	7.75d	531.56b	838.70b	239.69d	337.40b	6.92c	1.31e	29.12d	9.78g	3.93c	6.57b
UGU-126	30.57c	41.03c	10.97b	7.67d	733.08b	1057.4a	210.60d	281.22c	5.91d	1.41e	32.25c	14.17g	4.71b	9.18a
UGU-130	23.37c	35.77c	8.64d	6.45d	666.84b	1152.7a	214.62d	209.03d	3.57e	1.64e	21.34e	13.19g	5.19b	7.34a
UNG-53	35.60c	42.87c	9.09c	7.64d	990.34b	1040.46a	251.17c	361.67b	6.46c	2.65e	26.13d	11.37g	3.04d	3.27c
UNG-73	31.83c	45.07b	10.63b	7.24d	658.56b	1235.49a	324.58c	365.62b	9.76b	3.30e	39.59a	18.07f	3.16c	4.75b
UNG-76	36.60c	49.33b	10.39b	7.10d	559.94b	944.88b	192.31d	192.97d	9.15c	3.37e	39.42a	21.75e	3.42c	5.48b
UNG-77	36.90c	40.87c	9.39c	9.55c	1108.74a	1300.43a	314.03c	350.42b	9.93b	5.67d	32.28c	21.70e	2.32d	2.83d
PFC														
ICT-1026	45.00b	50.73b	12.43a	9.03c	749.85b	1246.95a	261.78c	376.03b	10.41b	2.50e	36.27b	11.78g	2.59d	4.49b
ICT-1087	45.87b	51.83b	12.63a	10.30b	904.52b	1102.92a	295.09c	496.99a	10.04b	5.59d	41.94a	26.77d	3.22c	3.87c
ICT-1092	35.40c	68.47a	13.00a	9.06c	1040.10a	1202.26a	287.74c	373.28b	9.03c	2.78e	40.35a	17.19f	3.65c	5.29b
ICT-1112	42.27c	55.10a	12.03a	7.88d	985.50b	1392.01a	342.00b	391.44b	12.65a	5.37d	40.01a	20.91e	2.16d	2.89d
ICT-1189	38.93c	50.67b	12.90a	10.04c	1207.02a	1253.8a	292.81c	341.51b	12.64a	4.31d	40.61a	20.04e	2.22d	3.65c
ICT-1251	38.53c	51.60b	11.47b	9.72c	812.41b	1162.32a	346.18b	416.42a	9.14c	3.20e	33.01c	16.04f	2.62d	4.09c
ICT-1281	36.93c	37.27c	11.02b	8.22d	656.73b	1221.94a	360.48b	379.81b	9.78b	2.59e	34.20b	16.46f	2.54d	5.72b
ICT-1292	43.03c	54.90a	13.15a	9.02c	968.91b	1057.95a	293.15c	371.26b	12.05a	3.96e	37.69b	15.65g	2.16d	3.03d
ICT-1506	38.97c	49.00b	11.42b	10.88b	776.12b	1038.42a	304.42c	460.27a	9.42b	3.67e	31.95c	21.17e	2.48d	4.77b
ICT-2142	34.90c	46.20b	11.20b	9.26c	776.68b	822.84b	322.66c	449.18a	10.17b	3.37e	28.95d	19.44e	1.87d	4.89b
ICT-2161	35.00c	46.10b	11.78a	10.13c	616.93b	983.81b	257.92c	472.67a	10.04b	3.60e	32.34c	16.35f	2.22d	3.88c
ICT-2171	41.53c	55.10a	13.27a	8.58d	881.30b	999.13a	285.55c	336.09b	12.46a	5.54d	35.91b	22.19e	1.89d	3.00d
ICT-2172	37.60c	65.43a	13.65a	8.70d	1437.02a	1412.61a	249.74c	307.74c	12.27a	4.64d	34.35b	22.83e	1.80d	3.92c
ICT-2173	47.40b	48.93b	10.27b	8.49d	785.07b	1255.67a	277.44c	349.77b	7.54c	4.18d	28.69d	21.09e	2.98d	4.04c
ICT-2653	32.10c	47.23b	11.93a	9.76c	879.42b	1035.99a	337.94b	307.94c	11.57a	3.67e	37.41b	13.43g	2.24d	2.67d
BCC														
BN-34	37.70c	35.57c	8.54d	6.65d	763.10b	1058.05a	334.53b	340.96b	9.25c	3.63e	32.60c	16.18f	2.53d	3.49c
BS-01	34.50c	41.73c	8.72d	9.83c	879.54b	1079.15a	338.36b	402.63a	7.60c	3.38e	31.68c	15.64g	3.29c	3.63c
CA-14	36.73c	33.07c	9.65c	7.66d	818.76b	834.93b	272.47c	361.36b	6.47c	2.49e	26.66d	17.44f	3.43c	6.07b
CEPEC-2002	36.20c	40.43c	10.72b	8.25d	632.53b	970.48b	302.44c	288.82c	7.95c	3.27e	29.00d	10.96g	2.60d	2.31d
CP-49-C10	36.27c	35.23c	10.52b	7.58d	763.34b	1118.17a	332.71b	390.72b	7.69c	3.00e	34.63b	12.96g	3.51c	3.37c
CP-53-C10	31.40c	37.13c	9.79c	7.62d	812.02b	950.53b	290.11c	343.77b	7.25c	2.62e	32.38c	11.66g	3.64c	3.60c
IPIRANGA-1	31.73c	56.00a	10.05c	7.93d	1117.13a	1166.97a	340.01b	345.07b	7.41c	2.87e	30.37c	10.88g	3.62c	3.71c
PH-09	23.07c	46.63b	9.92c	8.33d	687.77b	1002.07a	307.15c	270.79c	8.20c	1.30e	33.86b	9.39g	3.19c	6.54b
PH-144	45.33b	54.07a	11.99a	9.63c	977.80b	1016.84a	252.06c	343.32b	7.66c	3.03e	34.97b	21.61e	3.59c	6.19b
PH-15	34.73c	51.40b	9.79c	8.56d	872.39b	969.05b	439.71a	450.62a	5.22d	2.55e	25.58d	13.40g	4.01c	4.33b
PH-16	29.53c	34.07c	9.62c	7.53d	817.56b	980.65b	368.82b	371.42b	9.13c	3.21e	29.15d	14.06g	2.29d	3.36c
PH-17	31.20c	35.57c	9.09c	7.16d	801.06b	1115.94a	251.80c	257.28c	7.50c	3.52e	25.56d	12.82g	2.48d	2.65d
PH-21	39.97c	53.20a	9.34c	8.27d	791.30b	1077.63a	302.73c	221.90d	4.24d	3.06e	26.84d	17.85f	5.33b	4.87b
PH-990	35.47c	40.30c	9.41c	7.83d	943.25b	1185.36a	318.69c	332.32b	5.43d	2.99e	27.45d	17.21f	4.73b	5.09b
NIC														
CCN-10	31.27c	41.33c	13.43a	10.48b	1024.44a	1184.12a	298.51c	442.30a	11.02a	3.63e	34.39b	17.13f	2.14d	3.77c
CCN-51	40.10c	45.33b	12.06a	10.56b	1294.43a	1393.24a	404.42a	494.13a	11.30a	5.70d	40.18a	22.28e	2.61d	2.91d
EET-400	39.27c	50.03b	10.93b	8.55d	965.54b	988.93b	240.07d	308.90c	9.76b	3.07e	32.60c	17.21f	2.38d	4.69b
H-10	50.05b	63.10a	12.26a	8.05d	720.73b	1335.75a	474.94a	372.16b	8.15c	3.81e	27.16d	13.09g	2.31d	2.52d
ICS-1	41.30c	42.93c	12.14a	9.32c	917.33b	1140.95a	272.13c	407.49a	8.67c	4.18d	29.37d	22.22e	2.38d	4.35b
ICS-39	41.47c	53.90a	12.40a	8.53d	816.56b	1054.92a	305.41c	372.95b	7.33c	3.34e	32.80c	20.93e	3.53c	5.63b
ICS-6	36.40c	45.57b	12.14a	8.21d	801.38b	914.53b	328.02c	453.16a	7.56c	3.91e	29.79d	15.83g	2.96d	3.06d
ICS-95	44.80b	37.93c	11.03b	8.32d	967.35b	1182.73a	284.28c	345.16b	8.20c	2.63e	31.51c	15.46g	2.86d	5.09b
IMC-67	42.27c	44.83b	13.49a	8.78c	1022.91a	1281.34a	364.60b	472.07a	11.37a	3.07e	33.77b	14.30g	2.07d	3.75c
POUND-12	42.07c	45.97b	12.00a	9.55c	1038.90a	1117.07a	341.77b	452.85a	7.03c	3.80e	28.47d	17.28f	3.07d	3.56c
SCA-6	35.77c	39.83c	11.01b	10.22b	943.69b	1315.00a	269.91c	412.50a	7.27c	2.80e	28.18d	13.68g	2.98d	4.15c
TSH-1188	35.83c	44.40b	12.36a	8.74c	737.38b	1054.80a	345.92b	430.10a	10.29b	3.49e	32.50c	17.62f	2.19d	4.10c
TSH-565	33.77c	42.07c	11.86a	9.17c	902.88b	1262.33a	289.53c	433.21a	9.52b	3.00e	35.75b	12.18g	2.75d	4.10c
UF-613	33.27c	38.87c	11.79a	8.36d	815.09b	1070.66a	303.36c	442.48a	7.51c	3.77e	28.87d	18.53f	2.85d	3.95c
UF-667	38.17c	43.33c	11.63b	8.63d	1319.35a	1527.33a	395.79b	434.70a	10.39b	3.80e	35.91b	19.70e	2.52d	4.21c
Media PWC	33.62d	44.60b	10.17b	7.88d	780.59d	1023.99b	241.50d	295.97c	6.94c	2.38e	30.81b	13.83d	3.92b	5.53a
Media PFC	39.56c	51.90a	12.14a	9.27c	898.51c	1145.91a	300.99c	388.69a	10.61a	3.93d	35.58a	18.76c	2.44d	4.01b
Media BCC	34.56d	42.46b	9.80b	8.06d	834.11d	1037.56b	317.97b	337.21b	7.21c	2.92e	30.05b	14.43d	3.45c	4.23b
Media NIC	39.05c	45.30b	12.04a	9.03c	952.53c	1188.25a	327.91b	418.28a	9.02b	3.60d	32.08b	17.16c	2.64d	3.99b
Media Total	**36.79B****	**46.15A**	**11.07A**	**8.58B**	**868.47B**	**1110.27A**	**297.69B**	**361.54A**	**8.50A**	**3.23B**	**32.19A**	**16.11B**	**3.09B**	**4.42A**

Source of variation	*df*	*Pv*						
Shade (S)	1	0.0004	0.0001	0.0001	<0.0001	<0.0001	<0.0001	0.0001
Genotype (G)	57	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G*S	57	0.0072	0.0003	0.6657	0.0008	<0.0001	<0.0001	0.0006

SL: shoot length, SD: stem diameter, LA: leaf area, RA: root area, RDW: root dry weight, BDW: total dry weight, S/R: shoot/root rate. (*) Different lower case letters on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, $P \leq 0.05$). (**) Different capital letters on the right of each value in line of each variable indicate a significant difference between shade level (Scott & Knott test, $P \leq 0.05$). PWC: wild cacao from river basins of Peruvian Amazon collection, PFC: Peruvian farmers' collection, BCC: Brazilian cacao collection, NIC: National and International cacao collection. Different lower case letters in parenthesis on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, ($P \leq 0.05$).

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As reported by Galyuon et al. [12], cacao grown at full sunlight (1800 µmol m^{-2} s^{-1}) and shade (900 µmol m^{-2} s^{-1}) had different morphology. At full sunlight, leaf size, internode length, total leaf area and dry matter per plant were significantly reduced, while leaf thickness and leaf number per plant were increased compared to plants grown in shade. Besides, cacao plants growing between 35 and 55% shade have a leaf area higher than those grown under heavy shade whereas stem dry matter accumulation was lower as the level of shade increased [9].

Da Silva Branco et al. [10] evaluated the effects of four levels of shade (no shade, 50% 29% and 22% of the incident of radiation) and two levels of water regime (control and flooded plants) on cacao seedlings performance. They reported that responses of shoot length, stem diameter, leaf number, total leaf area, specific leaf area, root biomass and total biomass varied by genotype, treatment or combined effects. For example, leaf number and leaf area were reduced by increasing light intensity, on the other hand root length and collar diameter didn't vary when exposed to different shade levels.

Similarly, stem diameter can be wider in cacao monocultures, followed by agroforestry and successional agroforestry systems in a long-term field trial in Alto Beni, Bolivia [18]. Nevertheless, the production system (full-sun monoculture, agroforestry system, both under organic and conventional farming, and a highly diverse successional agroforestry system under organic farming) didn't have an effect on root length, surface area, specific root length, specific root area or diameter [61]. But in the highly successional agroforestry system, root volume and biomass were higher than those found in the agroforestry system [61]. These results are in agreement with our findings (Table 2). An investment in root growth is most relevant in plants under high rather than low light intensity to provide large surface for evapotranspiration and maintain cell turgor pressure [10].

A maximum shoot/root ratio was recorded for the AYP-15 cacao genotype (PWC) for both shade levels. The highest values of root dry weight 12.65 and 5.70 g $plant^{-1}$ were noted for the genotypes ICT-1112 (PFC) and CCN-51 (NIC) at 50% and 80% shade, respectively. The PAS-91 genotype had the highest total dry weight-- with 42.38 g $plant^{-1}$ (50% shade) and ICT-1087 with 26.77 g $plant^{-1}$ (80% shade) (Table 2). Baligar et al. [43] observed an increase in biomass accumulation in cacao roots, leaves, stems and shoots in a greenhouse experiment when PPFD increased from 65 to 190 µmol m^{2} s^{-1} (high to medium shade) that also entailed a reduction in leaf area and leaf specific area. Recently Baligar et al. [44] reported that in seven genetically different cacao genotypes increasing PPFD from 100 to 400 µmol m^{-2} s^{-1} increased shoot and root growth, relative growth rate (RGR) and net assimilation rates (NAR). Moreover, cacao grown under two shaded systems, one with N-fixing legume trees and one with different shade trees,

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compared to a monoculture system, had higher stem density, shoot:root ratio, above-ground and root biomass but similar tree height and a lower stem diameter [19].

3.2 Physiological parameters influenced by shade

The interaction between cacao genotypes and shade levels was significant for the physiological parameters (Tables 3).

An increase in chlorophyll content (SPAD index) was observed in cacao seedlings under 80% shade compared to 50% shade, with some exceptions across genotypes. By contrast, overall stomatal conductance was higher in plants grown at 50% shade, however there were some exceptions in genotypes of the PFC and NIC (Table 3)

The maximum level of chlorophyll content at 50% shade was 34.70 SPAD for UGU-130 genotype, whereas at 80% shade it was 34.50 SPAD for AYP-15, both from the PWC. The range of observed stomatal conductance was between 104.57 and 363.03 mmol $m^{-2}s^{-1}$ at 50% shade for UGU-112 and PH-21 respectively, and between 47.33 and 260.67 mmol $m^{-2}s^{-1}$ at 80% shade for UGU-126 and H-10, respectively (Table 3).

With respect to chlorophyll content, shade leaves in cacao often exhibit greater total chlorophyll concentrations per unit mass than sun leaves. De Almeida and Valle [62] and Daymond and Hadley [59] reported a consistent decline in chlorophyll content with increasing light in three cacao genotypes and it can vary quite considerably over time. Moreover, Acheampong et al. [63], reported that no clear pattern was observed for leaf chlorophyll in cacao genotypes grown under three shade levels provided by plants and under fertilizer application. Our results show either a reduction of leaf chlorophyll content while PPFD increases or little variation between shade levels (Table 3).

Acheampong et al. [9] conducted a study with four cacao genotypes (T 79/501, PA-150, SCA-6 and P-30) and subjected them to three shade levels (32.5, 55 and 76%) at two seasons of growth (dry and rainy). They reported that stomatal conductance was higher in seedlings under heavy shade during the dry season and lower in the rainy season for seedlings under lighter shade. As a consequence, photosynthesis rates were higher in the wet season for cacao under medium and light shade. Results from one crop year for the same genotypes grown under three shade levels provided by plants and under fertilizer application, showed that a higher stomatal conductance was related to an increase in shade but only for two genotypes [63]. However, the opposite trend is observed in our study where stomatal conductance decreases with higher shade considering the overall results, as well as specific values for SCA-6 cacao genotype (Table 3). Overall stomatal conductance was higher in plants grown at low shade (50% shade), however there were some exceptions in genotypes of the PFC and the NIC (Table 3).

Table 3. Physiological parameters (mean values)- and Shade Tolerance Index (STI) for 58 cacao genotypes subjected to two levels of shade (50 and 80%)

Genotype	CHO (SPAD)		STC (mmol $m^{-2}s^{-1}$)		WUE (g l^{-1})		STI
	50%	80%	50%	80%	50%	80%	
PWC							
AYP-15	33.10b*	34.50a	245.10a	51.54b	1.21b	0.92c	47.43c
AYP-20	33.30b	33.93a	179.10b	54.47b	1.38b	1.14b	51.78c
AYP-22	33.03b	33.97a	226.13a	81.30b	1.16b	0.40d	25.72d
PAS-100	33.80a	33.43b	237.53a	84.63b	1.30b	0.98c	47.07c
PAS-105	33.33b	33.70b	125.13b	78.10b	1.30b	0.70d	33.54d
PAS-91	34.63a	33.20b	107.23b	58.20b	1.76a	0.91c	31.53d
PAS-93	34.60a	33.20b	151.80b	102.20b	1.09c	0.89c	44.22c
UGU-112	33.57b	33.40b	104.57b	49.73b	1.23b	0.72d	34.12d
UGU-126	33.57b	33.20b	142.63b	47.33b	1.46a	1.09c	43.79c
UGU-130	34.70a	33.47b	127.03b	62.30b	0.99c	0.99c	61.85a
UNG-53	32.90b	34.07a	259.70a	84.37b	1.09c	0.75d	43.35c
UNG-73	34.43a	33.23b	140.33b	73.70b	1.66a	1.26b	45.88c
UNG-76	34.30a	33.47b	261.33a	97.00b	1.68a	1.57a	55.37b
UNG-77	33.07b	33.00b	329.97a	62.97b	1.24b	1.37b	68.34a
PFC							
ICT-1026	27.80g	31.07d	218.80a	166.33b	1.44a	0.79d	32.43d
ICT-1087	29.10e	30.40d	159.23b	122.47b	1.77a	1.81a	63.85a
ICT-1092	28.33f	30.40d	189.17a	158.63b	1.74a	1.23b	42.67c
ICT-1112	27.40g	30.50d	229.80a	150.93b	1.52a	1.33b	52.41c
ICT-1189	28.57f	30.17d	249.50a	181.60a	1.55a	1.34b	49.82c
ICT-1251	29.23e	28.97f	146.67b	173.77b	1.33b	1.10c	48.59c
ICT-1281	30.47d	29.93e	210.97a	142.67b	1.36b	1.19b	48.43c
ICT-1292	28.63f	31.37d	131.80b	166.83b	1.42a	1.00c	41.62c
ICT-1506	24.60h	31.60c	145.83b	210.20a	1.25b	1.50a	67.30a
ICT-2142	33.03b	31.67c	131.73b	190.40a	1.04c	1.37b	67.75a
ICT-2161	30.13d	29.50e	280.97a	168.87b	1.24b	1.09c	50.50c
ICT-2171	28.57f	30.30d	146.77b	150.17b	1.30b	1.42a	61.81a
ICT-2172	29.27e	29.90e	148.67b	108.50b	1.23b	1.55a	67.60a
ICT-2173	32.60c	31.53c	109.47b	217.90a	1.18b	1.44a	73.90a
ICT-2653	27.80g	31.07d	260.83a	231.83a	1.44a	0.83d	35.89d
BCC							
BN-34	29.80e	29.27e	208.97a	85.20b	1.30b	1.07c	50.21c
BS-01	30.90d	33.53b	274.73a	88.37b	1.34b	1.05c	49.98c
CA-14	33.07b	34.50a	264.50a	147.93b	1.12c	1.28b	65.56a
CEPEC-2002	30.43d	32.50c	157.37b	123.17b	1.17b	0.66d	38.12d
CP-49-C10	30.47d	31.70c	241.07a	164.60b	1.50a	0.85d	37.46d
CP-53-C10	32.97b	33.67b	212.03a	71.43b	1.40a	0.77d	36.00d
IPIRANGA-1	32.33c	33.77a	119.83b	73.30b	1.28b	0.68d	35.77d
PH-09	32.73c	33.57b	198.03a	183.27a	1.43a	0.69d	27.79d
PH-144	30.67d	31.37d	139.57b	94.80b	1.52a	1.59a	61.76a
PH-15	30.63d	33.57b	225.30a	145.40b	1.13c	0.93c	52.68c
PH-16	33.20b	34.47a	240.67a	175.93b	1.11c	0.93c	47.89c
PH-17	30.47d	33.83a	256.60a	149.37b	1.00c	0.79d	50.41c
PH-21	31.00d	34.05a	363.03a	47.83b	1.26b	1.26b	66.32a
PH-990	32.93b	33.97a	274.30a	60.00b	1.22b	1.22b	63.68a
NIC							
CCN-10	32.13c	34.00a	144.27b	82.25b	1.30b	1.15b	50.05c
CCN-51	30.53d	30.87d	158.07b	76.37b	1.60a	1.42a	55.63b
EET-400	28.30f	31.00d	258.13a	149.07b	1.27b	1.21b	53.97b
H-10	28.60f	30.47d	175.93b	260.67a	1.06c	0.79d	49.04c
ICS-1	30.40d	30.73d	152.13b	127.57b	1.15b	1.54a	76.15a
ICS-39	25.53h	31.70c	169.50b	128.53b	1.42a	1.50a	65.38a
ICS-6	27.10g	31.90c	239.43a	237.60a	1.24b	1.02c	53.26b
ICS-95	27.87g	31.43d	141.77b	150.70b	1.29b	1.10c	49.07c
IMC-67	28.30f	32.23c	279.97a	157.17b	1.24b	0.96c	42.77c
POUND-12	30.47d	30.47d	154.17b	126.43b	1.19b	1.15b	60.72a
SCA-6	30.70d	31.07d	192.17a	91.90b	1.16b	0.93c	49.92c
TSH-1188	30.60d	30.80d	169.40b	118.90b	1.23b	1.21b	54.32b
TSH-565	28.00g	31.07d	115.60b	94.77b	1.46a	0.78d	33.68d
UF-613	27.70g	31.77c	219.07a	148.43b	1.19b	1.26b	64.23a
UF-667	30.50d	31.10d	187.13a	146.67b	1.42a	1.36b	55.05b
Media PWC	33.74a	33.55a	188.40b	70.56d	1.33a	0.98c	45.28
Media PFC	29.17e	30.44d	184.01b	169.41b	1.39a	1.27a	53.64
Media BCC	31.71b	33.46a	226.86a	115.04c	1.27a	0.98c	48.83
Media NIC	28.66e	31.19c	183.78b	139.80c	1.28a	1.16b	54.22
Media Total	**30.75B**	**32.12A**	**195.35A****	**124.77B**	**1.31A**	**1.09B**	**50.49**

Source of variation	*df*	*Pv*			
Shade (S)	1	<0.0001	0.0001	0.0003	
Genotype (G)	57	<0.0001	0.0002	<0.0001	0.004
G*S	57	<0.0001	0.0007	<0.0001	

STC: stomatal conductance, CHO: leaf chlorophyll content, WUE: water use efficiency, STI: shade tolerance index. (*) Different lower case letters on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, ($P \leq 0.05$). (**) Different capital letters on the right of each value in line of each variable indicate a significant difference between shade level (Scott & Knott test, $P \leq 0.05$). PWC: wild cacao from river basins of Peruvian Amazon collection, PFC: Peruvian farmers' collection, BCC: Brazilian cacao collection, NIC: National and International cacao collection. Different lower case letters in parenthesis on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, ($P \leq 0.05$).

The evaluation of stomatal conductance in nine cacao genotypes (TCS-13, TCS-19, SCC-53, SCC-82, SCC-83, CCS-73, CCS-77, CCS-80 and ICS-95) grown in two agroforestry systems in Colombia with a maximum irradiance of 2100 and 1800 μmol m^{-2} s^{-1} showed lower stomatal conductance for the former, due to a lower transpiration rate associated with low water bioavailability in the soil [64], which is in alignment with our overall results. Nevertheless, if we only consider ICS-95 genotype, a slight increase of stomatal conductance was observed when exposed to a higher PPFD (Table 3). Da Silva Branco et al. [10] found that a decrease in stomatal conductance in TSA-792 and TSH-774 cacao genotypes was observed when plants were subjected to flooding but not when the light intensity was attenuated. These results were explained as an accumulation of abscisic acid (ABA) with the increase in shading density, regulating the stomatal opening. The opposite was observed in our study, where in TSH cacao genotypes a decrease in stomatal conductance was recorded when seedlings where exposed to a higher shade level (Table 3).

Under field conditions, Jaimez et al. [65] report that an increase in PPFD (from 400 to 1000 μmol m^{-2} s^{-1}) in 7-year old cacao plot in Ecuador involved an increase in net photosynthetic rate while high stomatal conductance was maintained, even if in some cases greater stomatal conductance at a low PPFD (High shade) was recorded for some genotypes. Baligar et al. [42] reported that stomatal conductance (around 0.02 mol m^{-2} s^{-1}) in three cacao genotypes (CCN-51, LCT EEN 37/A and VB 1117) was not significantly affected by PPFD in the range of 50 to 400 μmol m^{-2} s^{-1}. Nonetheless, maintaining a high conductance at very low irradiance may be an advantage to understory plants, by allowing photosynthesis to respond rapidly to sunflecks.

When cacao leaves were continually exposed to light intensities higher than half of that in which instantaneous maximum photosynthesis occurred (about 350-400 μmol m^{-2} s^{-1}, which is nearly 20% of the intensity of full sunlight) the rate of photosynthesis began to decline after four hours. At light intensities higher than 100% of saturating photosynthetic intensity, the decline began almost immediately causing a certain degree of photoinhibition [38]. Taking into account these findings, our results would indicate that shade levels of 50% (PPFD of 1000±50 μmol m^{-2} s^{-1}) might be too high and cause some sort of stress in terms of chlorophyll content, while the stomatal conductance increase was probably due to an increase in leaf temperature under 50% shade.

In this study, WUE was significantly affected by shade, genotypes and their interaction. WUE tended to decrease significantly with the increase of shade (Table 3). The average observed WUE at 50% shade was 1.32 g l^{-1} which was significantly higher than1.12 g l^{-1} obtained at 80% shade. Similar tendencies were reported by Lopez-Marin et al. [66], with a negative correlation between shade levels and WUE in a greenhouse experiment with sweet pepper plants, registering the highest values under non-shaded conditions. Also, Yang [67] found similar tendencies in forages. Jaimez et al. [65] found in cacao growing under high and low PPFD that WUE showed a negative linear relationship with light level. and finally based on high WUE observed in the evaluated clones at 50% shade (Table 3), indicate the posibility of growing these cacao genotypes with less shade. On the other hand, in coffee in the dry season, Baliza et al. [68] found an increase of WUE until 50% shade and then decreases with higher levels of shade. In the current study, the maximum level of WUE 1.77 g l^{-1} and 1.81 g l^{-1} were recorded for ICT-1087 genotype (PFC) at 50% and 80% shade, respectively. Such high WUE in this genotype could be related to its overall highest shoot and root dry biomass at both levels of shade.

3.3 Nutritional status influenced by shade

Many effects of shade in cacao, are not well understood, including the differences in response to specific nutrients under shade [26]. Some cacao varieties are more nutrient demanding than others [44]. In soils with low fertility, shade acts as a buffer reducing metabolic activity which also reduces nutrient uptake. Cacao trees without shade demand higher amounts of nutrients than shaded ones: the former may contain higher levels of N and K than the latter, which had higher levels of P, Ca and Mg [69].

Murray [70] reported that under shade cacao leaves had higher levels of nutrients than unshaded. In a long term study, Ahenkorah et al. [71] reported the beneficial effects of fertilization on cacao yield without shade. Macronutrients and micronutrients concentrations as influenced by genotypes and shade levels are given in Tables 4 and 5. Overall macronutrients and micronutrients concentrations were at adequate levels, however concentrations of K, Ca and Mg were slightly higher and P concentrations were slightly lower than reported adequate levels in cacao [70-74].

In the current study, the interaction between cacao genotypes and shade levels was significant for macronutrients and micronutrients concentrations, except for N. All macronutrients, except P and K, and micronutrients concentrations were very high under 80% shade than 40% shade and significant differences were observed between two shade levels and also across genotypes. An increase of nearly 25% of the N concentration was recorded for cacao genotypes grown under the 80%shade level, contrary to the findings reported by Cabala Rosand et al. [69].

Overall, cacao genotypes from the four collections showed decreases in Ca concentration when grown under 50% shade. The highest concentrations of P were 1.77 and 0.99 g kg^{-1} for the AYP-15 (50% shade) and ICT-1281 (80% shade) genotypes, respectively (Table 4).

Slight differences were noted between cacao genotypes on leaf nitrogen concentration with respect to shade treatments which increased with increasing shade, contrary to P which had a higher concentration under low shade. For K concentration, decreases in shade were associated with higher leaf K, however, no significant differences were observed between the shade levels.

Micronutrient concentrations overall increased significantly with increasing shade from 50% to 80%. Regarding micronutrients, Fe concentration at 50 % shade ranged from 72 to 346 mg kg^{-1} for ICT-1189 and CP-53-C10, respectively, and from 120 to 389 mg kg^{-1} for CEPEC-2002 and UF-667 at 80% shade, respectively. The lowest concentrations for Cu were nearly 5 mg kg^{-1} for the CP-49-C10 and UGU-112 genotypes at 50% and 80% shade, respectively. The Zn concentration at 50% shade ranged from 21.06 to 47.89 mg kg^{-1} for IPIRANGA-1 and UNG-77, respectively, wille at 80% shade the concentration was ranged from 36.85 to 76.39 mg kg^{-1} for PH-21 and AYP-22 respectively. Finaly the Mn concentration at 50% shade was ranged from 18.42 to 100.1 mg kg^{-1} for CA-14 and UF-667, respectively, wille at 80% shade was ranged from 50.23 to 155.58 mg kg^{-1} for UNG-53 and PH-17, respectively. (Table 5).

Significant differences were observed in nutrient uptake between the shade levels within each cacao collection, except for Mn content (PWC, PFC and NIC) and Cu (BCC) (Figure2). K, Mg and P NUE (NIC) and Fe NUE (BCC and NIC) (Figure 3).

At 50% shade, the highest uptake of K, Ca, Mg, P and Zn were found in PWC, with values of 0.61, 0.33, 0.14, 0.03 g plant^{-1} and 0.96 mg plant^{-1}, respectively; while at 80% shade, the highest uptake of N, K, Ca, Mg, Cu and Zn were found in PFC, values of 0.32; 0.37, 0.26, 0.11 g plant^{-1}; 0.18, 0.69 mg plant^{-1}, respectively. The NIC at 50 % and 80% shade showed the highest nutrient uptake values for Fe (4.73 and 2.98 mg plant^{-1} respectively), Mn (1.35 and 1.23 mg plant^{-1} respectively) and Cu (0.25 and 0.18 mg plant^{-1} respectively) (Figure 2).

In a field study conducted in a 8-year-old cacao plantation with different shade trees and compared to a monoculture system in Ghana, Isaac et al. [75] noted that nutrient uptake by cacao increased under shade (43–80% and 22–45% for N and P, respectively), with K (96–140%) as the most responsive nutrient.

Baligar et al. [43] conducted a greenhouse experiment where cacao was grown at three shade levels, high, medium and low shade (PPFD of 65±25 190+46 and 1050±260 μmol m^{-2} s^{-1}, respectively) combined with two levels of CO_2 (380 and 700 μmol mol^{-1}). Nutrient uptake (or content) was in the range of 158 to 168, 82 to 146, 40 to 86, 38 to 55, and 7.5 to 10 mg plant^{-1} for N, K, Ca, Mg and P respectively, and in the range of 44 to 69, 410 to 538, 1127 to 1764, and 444 to 731 μg plant^{-1} for Cu, Fe, Mn and Zn respectively. At ambient CO_2 (380 μmol mol^{-1}), an increase of PPFD to 1050 μmol m^{-2} s^{-1}, with exception

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of K and Mn content, had a negative effect on all the essential macro and micronutrient contents. Such reduction in nutrient uptake could be attributed to a reduction in dry matter accumulation at a very high level of PPFD.

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Table 4. Mean concentrations of macronutrients (g kg^{-1}) in shoots of 58 cacao genotypes subjected to two levels of shade(50 and 80%).

Genotype	N		K		Ca		Mg		P	
	g kg^{-1}									
	50%	80%	50%	80%	50%	80%	50%	80%	50%	80%
PWC										
AYP-15	17.44b*	20.77a	29.40a	23.16b	16.03c	18.51b	5.93b	6.35b	1.77a	0.81d
AYP-20	18.45b	22.83a	30.40a	21.62b	10.67f	16.96c	6.03b	6.47b	0.90d	0.69e
AYP-22	11.10d	13.49c	24.47b	22.56b	11.87e	22.72a	5.03c	7.63a	1.10c	0.60e
PAS-100	19.97a	15.77b	27.23a	24.28b	17.37c	18.85b	6.43b	6.57b	1.47b	0.83d
PAS-105	14.80c	16.40b	27.93a	20.96c	16.77c	17.87b	6.77b	5.92b	1.23b	0.81d
PAS-91	18.45b	22.83a	26.47a	20.91c	12.07e	16.20c	6.03b	6.20b	0.87d	0.67e
PAS-93	17.45b	20.95a	19.67c	18.39c	15.70c	17.23c	7.17a	6.35b	1.00c	0.69e
UGU-112	10.83d	23.32a	20.90c	21.71b	9.53f	17.39c	4.43c	6.20b	1.10c	0.81d
UGU-126	11.81d	11.09d	28.63a	22.70b	13.27e	16.80c	6.20b	5.74b	1.10c	0.78d
UGU-130	11.88d	18.10b	26.00a	21.03c	16.37c	15.10d	5.61b	6.60b	1.10c	0.67e
UNG-53	17.48b	21.64a	29.90a	23.11b	16.10c	20.77a	7.30a	7.50a	1.10c	0.63e
UNG-73	16.65b	20.60a	18.57c	19.72c	8.93g	14.99d	4.30c	5.76b	0.72d	0.80d
UNG-76	10.56d	16.42b	19.80c	23.19b	16.33c	15.74c	6.03b	5.72b	1.33b	0.78d
UNG-77	17.86b	22.10a	29.60a	22.16b	12.37e	15.47d	6.00b	6.59b	1.40b	0.83d
PFC										
ICT-1026	20.60a	23.06a	20.90c	23.54b	5.17h	16.95c	2.23d	6.71b	1.33b	0.75d
ICT-1087	17.39b	21.52a	19.43c	26.79a	4.43h	16.44c	1.87d	6.81b	1.30b	0.83d
ICT-1092	14.24c	17.63b	21.70b	22.14b	4.20h	17.47c	2.10d	7.84a	1.17c	0.77d
ICT-1112	18.85b	23.79a	20.17c	22.87b	4.47h	17.61c	1.97d	8.65a	1.43b	0.78d
ICT-1189	18.03b	22.31a	18.83c	24.27b	14.87d	16.59c	5.50b	6.21b	1.27b	0.84d
ICT-1251	17.46b	21.61a	21.50b	26.06a	5.00h	18.81b	2.23d	6.25b	1.10c	0.75d
ICT-1281	18.86b	27.12a	19.83c	24.87b	13.17e	16.67c	6.07b	6.69b	1.00c	0.99c
ICT-1292	18.62b	23.05a	21.50b	26.89a	4.87h	18.89b	2.43d	7.70a	1.30b	0.84d
ICT-1506	14.55c	18.01b	21.87b	26.66a	4.47h	18.45b	2.07d	7.43a	1.17c	0.88d
ICT-2142	18.18b	22.35a	21.90b	22.37b	12.87e	17.13c	6.77b	6.76b	1.10c	0.81d
ICT-2161	16.53b	20.46a	21.47b	20.87c	12.10e	16.01c	6.23b	7.10a	0.77d	0.57e
ICT-2171	18.76b	23.57a	21.10c	23.97b	4.77h	19.61b	2.17d	8.23a	1.18c	0.81d
ICT-2172	17.41b	22.30a	23.57b	27.92a	5.13h	18.77b	2.33d	7.58a	1.37b	0.77d
ICT-2173	19.40b	24.00a	22.03b	26.74a	14.03d	16.42c	6.47b	7.10a	1.30b	0.96c
ICT-2653	18.77b	23.22a	21.73b	23.96b	7.60g	19.28b	2.00d	6.90a	0.83d	0.61e
BCC										
BN-34	22.59a	23.83a	22.10b	24.55b	12.70e	19.1b	5.70b	7.41a	1.03c	0.68e
BS-01	18.86b	22.92a	22.70b	20.12c	15.23d	19.65b	6.23b	9.72a	0.97c	0.30e
CA-14	17.86b	22.11a	23.17b	24.34b	13.10e	15.88c	5.27b	6.87a	1.00c	0.69e
CEPEC-2002	18.38b	22.12a	26.07a	23.59b	11.77e	22.82a	5.43b	8.12a	1.07c	0.53e
CP-49-C10	18.86b	23.29a	21.53b	20.78c	12.90e	18.92b	5.77b	8.03a	0.77d	0.49e
CP-53-C10	19.72a	24.40a	21.63b	25.03b	12.53e	18.85b	5.60b	7.74a	0.97c	0.60e
IPIRANGA-1	17.18b	21.26a	22.37b	22.59b	10.70f	16.49c	5.40b	6.94a	0.94d	0.42e
PH-09	17.94b	21.95a	22.37b	19.06c	11.33f	20.99a	5.67b	7.47a	1.08c	0.53e
PH-144	20.06a	24.83a	21.97b	20.57c	13.27e	18.32b	5.83b	7.34a	0.97c	0.60e
PH-15	17.97b	22.72a	26.05a	21.33b	12.75e	17.57c	5.75b	7.12a	0.95c	0.62e
PH-16	18.45b	22.83a	20.53c	19.10c	14.40d	21.81a	6.30b	7.70a	1.30b	0.50e
PH-17	17.22b	21.28a	22.67b	19.54c	13.87d	18.77b	5.77b	7.42a	0.70d	0.48e
PH-21	18.28b	23.93a	23.97b	17.54c	11.70e	19.16b	5.57b	7.74a	0.93d	0.55e
PH-990	18.35b	28.83a	24.87b	23.12b	10.93f	18.73b	4.46c	7.72a	0.83d	0.63e
NIC										
CCN-10	17.63b	21.81a	12.37d	17.19c	7.23g	14.28d	4.03c	4.69c	0.63e	0.84d
CCN-51	18.73b	23.18a	13.87d	17.81c	8.33g	12.53e	3.80c	4.92c	1.10c	0.80d
EET-400	17.64b	21.83a	24.93b	19.81c	15.13d	18.46b	7.93a	6.60b	0.93d	0.78d
H-10	14.97c	18.52b	12.20d	18.98c	6.70g	17.51c	3.70c	6.42b	0.25e	0.62e
ICS-1	18.04b	26.54a	13.43d	16.44d	7.57g	12.36e	3.50c	5.72b	1.10c	0.89d
ICS-39	17.24b	21.33a	17.70c	19.68c	10.47f	15.39d	4.06c	6.18b	0.63e	0.91d
ICS-6	15.22c	21.20a	26.03a	21.18c	13.23e	15.75c	7.43a	6.35b	0.97c	0.83d
ICS-95	17.54b	21.71a	24.13b	18.97c	13.43e	13.72d	7.13a	4.66c	0.93d	0.90d
IMC-67	18.26b	22.60a	16.46d	22.13b	9.33f	17.68b	5.42b	6.95a	1.45b	0.72d
POUND-12	17.60b	21.82a	22.35b	19.63c	14.35d	14.88d	6.70b	5.97b	0.80d	0.84d
SCA-6	16.17b	20.01a	13.17d	18.25c	8.43g	16.87c	4.87c	6.37b	0.60e	0.72d
TSH-1188	17.27b	21.37a	24.70b	14.72d	12.9e	11.86e	7.33a	4.64c	0.87d	0.89d
TSH-565	16.83b	20.82a	23.40b	16.27d	14.70d	14.66d	7.73a	5.11b	0.97c	0.77d
UF-613	18.26b	22.59a	13.60d	17.22c	9.20f	16.55c	4.20c	6.39b	1.20c	0.98c
UF-667	16.64b	20.61a	23.53b	19.73c	14.10d	14.67d	7.90a	5.61b	0.83d	0.96c
Media PWC	15.09d	19.02b	25.64a	21.82c	13.81d	17.47b	6.02c	6.40b	1.18a	0.74c
Media PFC	17.45c	21.78a	21.17c	24.66a	7.81f	17.67b	3.60d	7.20a	1.14a	0.80c
Media BCC	18.26b	22.53a	23.00b	21.52c	12.66d	19.08a	5.69c	7.52a	0.93b	0.54d
Media NIC	17.20c	21.45a	18.79d	18.53d	11.01e	15.14c	5.78c	5.69c	0.87b	0.83c
Media Total	**17.01B****	**21.21A**	**22.08B**	**21.63A**	**11.26B**	**17.31A**	**5.25B**	**6.69A**	**1.03A**	**0.73B**

Source of variation	*df*	*pv*				
Shade (S)	1	<0.0001	0.1034	<0.0001	<0.0001	0.0002
Genotype (G)	57	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G*S	57	0.0022	<0.0001	<0.0001	<0.0001	<0.0001

(*) Different lower case letters on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, ($P < 0.05$). (**) Different capital letters on the right of each value in line of each variable indicate a significant difference between shade level (Scott & Knott test, ($P < 0.05$). PWC: wild cacao from river basins of Peruvian Amazon collection, PFC: Peruvian farmers' collection, BCC: Brazilian cacao collection, NIC: National and International cacao collection. Different lower case letters in parenthesis on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, ($P < 0.05$).

Table 5. Mean concentration of micronutrients (mg kg^{-1}) in shoots of 58 cacao genotypes subjected to two levels of shade(50 and 80%).

Genotype	Fe		Zn		Mn		Cu	
	mg kg^{-1}							
	50%	80%	50%	80%	50%	80%	50%	80%
PWC								
AYP-15	247.80b*	259.18b	46.57b	42.23c	84.29b	63.46c	4.42c	8.89c
AYP-20	169.84c	278.63a	31.88d	45.01c	54.25c	84.93b	6.74c	9.35c
AYP-22	187.13c	222.38b	33.66d	76.39a	28.37d	84.18b	6.88c	16.50b
PAS-100	275.73a	225.13b	43.36c	50.00b	70.38b	100.18b	9.54c	7.50c
PAS-105	151.18c	239.34b	43.24c	55.80b	49.54c	131.91a	8.09c	12.92b
PAS-91	149.98c	225.64b	41.47c	47.31b	53.07c	115.15a	5.09c	6.54c
PAS-93	122.26d	225.84b	38.96c	47.19b	65.03c	75.48b	6.65c	14.33b
UGU-112	131.94d	226.99b	32.82d	50.12b	22.63d	64.79c	6.01c	4.92c
UGU-126	168.27c	245.70b	39.73c	42.50c	46.85c	80.83b	5.17c	6.78c
UGU-130	166.49c	225.14b	47.72b	47.03b	55.25c	78.62b	7.77c	6.47c
UNG-53	177.41c	182.02c	44.73c	42.59c	58.87c	50.23c	9.44c	9.13c
UNG-73	107.39d	260.63b	31.31d	41.17c	18.52d	79.68b	6.84c	7.77c
UNG-76	120.68d	303.51a	42.18c	41.53c	40.11d	77.83b	8.06c	5.95c
UNG-77	247.09b	241.41b	47.89b	41.85c	61.22c	90.83b	7.89c	14.99b
PFC								
ICT-1026	98.48d	176.44c	35.25d	48.75b	58.83c	50.67c	9.66c	24.26a
ICT-1087	109.82d	173.16c	26.16d	41.61c	47.81c	82.21b	9.80c	16.01b
ICT-1092	80.33d	185.94c	30.58d	50.88b	50.24c	105.24a	5.87c	10.70c
ICT-1112	84.37d	141.48d	40.87c	44.44c	28.94d	54.33c	6.64c	8.38c
ICT-1189	72.04d	142.24d	26.86d	42.41c	52.35c	62.81c	5.32c	8.69c
ICT-1251	177.47c	135.31d	45.55c	51.08b	63.69c	92.37b	6.56c	8.21c
ICT-1281	109.77d	134.68d	29.39d	45.75c	42.80c	67.15c	5.02c	10.97c
ICT-1292	127.43d	155.29c	30.19d	47.28b	43.96c	62.06c	6.75c	16.72b
ICT-1506	133.08d	151.98c	37.90c	44.51c	40.41d	74.93b	7.90c	9.44c
ICT-2142	127.25d	156.95c	25.04d	39.70c	39.12d	50.95c	7.61c	16.54b
ICT-2161	112.34d	138.54d	22.86d	46.56b	31.96d	81.67b	9.06c	9.38c
ICT-2171	123.40d	188.00c	42.32c	50.65b	45.54c	81.72b	8.18c	19.50b
ICT-2172	92.32d	162.03c	33.01d	45.42c	36.20d	70.56b	8.39c	9.03c
ICT-2173	110.90d	131.38d	27.19d	48.07b	47.83c	74.68b	8.72c	9.02c
ICT-2653	100.76d	168.58c	30.75d	52.14b	41.33d	81.71b	6.85c	9.49c
BCC								
BN-34	108.44d	207.65b	29.92d	38.60c	31.76d	72.46b	5.19c	9.42c
BS-01	258.90b	205.16b	24.13d	40.69c	34.11d	57.58c	4.41c	5.84c
CA-14	126.32d	155.77c	26.11d	38.29c	18.42d	52.78c	5.17c	7.01c
CEPEC-2002	159.11c	119.81d	38.74c	49.70b	21.80d	34.36d	6.04c	9.20c
CP-49-C10	97.20d	141.37d	22.02d	48.66b	33.31d	38.69d	4.13c	7.74c
CP-53-C10	345.71a	179.01c	21.64d	46.34b	38.32d	78.28b	5.72c	9.93c
IPIRANGA-1	121.31d	234.17b	21.06d	44.22c	25.86d	95.32b	5.45c	7.17c
PH-09	213.20b	171.92c	25.17d	43.18c	26.35d	122.81a	6.11c	9.18c
PH-144	243.08b	189.39c	23.46d	41.19c	32.13d	56.63c	4.48c	5.20c
PH-15	116.92d	198.69b	27.91d	40.08c	21.31d	48.97c	6.78c	7.81c
PH-16	329.32a	211.58b	21.59d	53.52b	30.70d	147.17a	6.02c	7.29c
PH-17	135.13d	156.04c	34.53d	60.42b	49.64c	155.58a	6.77c	13.69b
PH-21	194.97b	200.87b	29.07d	36.85c	22.05d	52.47c	6.30c	6.82c
PH-990	274.37a	167.23c	43.07c	41.27c	24.12d	64.56c	7.39c	10.27c
NIC								
CCN-10	262.12b	205.56b	43.74c	43.83c	84.95b	101.23b	9.07c	12.96b
CCN-51	261.34b	230.53b	26.89d	37.04c	21.36d	88.85b	9.78c	12.86b
EET-400	239.43b	256.67b	44.05c	55.18b	82.36b	73.81b	15.63b	15.86b
H-10	122.26d	152.02c	44.78c	39.49c	27.82d	65.85c	8.38c	17.54b
ICS-1	199.54b	183.69c	34.11d	37.63c	21.10d	103.17b	9.71c	11.77c
ICS-39	140.60d	167.99c	45.35c	45.45c	74.68b	109.90a	10.9c	9.02c
ICS-6	165.72c	206.67b	36.59c	40.83c	55.47c	75.83b	12.58b	15.83b
ICS-95	198.72b	205.77b	41.87c	42.48c	78.05b	126.84a	18.79b	29.92a
IMC-67	247.17b	203.83b	38.20c	44.21c	26.75d	79.31b	9.43c	12.93b
POUND-12	184.32c	183.17c	41.13c	41.83c	59.54c	64.34c	7.64c	7.23c
SCA-6	134.50d	219.24b	40.45c	44.61c	70.05b	66.24c	11.68c	20.08b
TSH-1188	189.65c	226.59b	38.94c	46.50b	54.22c	115.98a	7.16c	8.87c
TSH-565	236.09b	213.47b	36.34c	47.32b	83.48b	76.51b	8.08c	12.44b
UF-613	174.97c	229.17b	37.61c	43.42c	18.86d	93.03b	14.56b	14.16b
UF-667	286.85a	388.64a	38.00c	37.94c	100.10 b	82.76b	9.44c	10.78c
Media PWC	173.09b	240.11a	40.39c	47.91a	50.60b	84.15a	7.04d	9.43c
Media PFC	110.65d	156.13c	32.26d	46.62a	44.73b	72.87a	7.49d	12.42b
Media BCC	194.57b	181.33b	27.74e	44.50b	29.28c	76.98a	5.71d	8.33c
Media NIC	202.88b	218.20a	39.20c	43.18b	57.25b	88.24a	10.85b	14.15a
Media Total	**169.83B**	**198.54A**	**34.93B**	**45.53A**	**45.66B**	**80.56A**	**7.82B**	**11.16A**

Source of variation	*df*	*pv*			
Shade (S)	1	0.0017	<0.0001	0.0006	<0.0001
Genotype (G)	57	<0.0001	<0.0001	<0.0001	<0.0001
G*S	57	<0.0001	<0.0001	<0.0001	0.0017

(*) Different lower case letters on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, (P < *0.05*). (**) Different capital letters on the right of each value in line of each variable indicate a significant difference between shade level (Scott & Knott test, (P < *0.05*). PWC: wild cacao from river basins of Peruvian Amazon collection, PFC: Peruvian farmers' collection, BCC: Brazilian cacao collection, NIC: National and International cacao collection. Different lower case letters in parenthesis on the right of each value in both columns of each variable indicate a significant difference between genotypes and shade level, (Scott & Knott test, (P < *0.05*).

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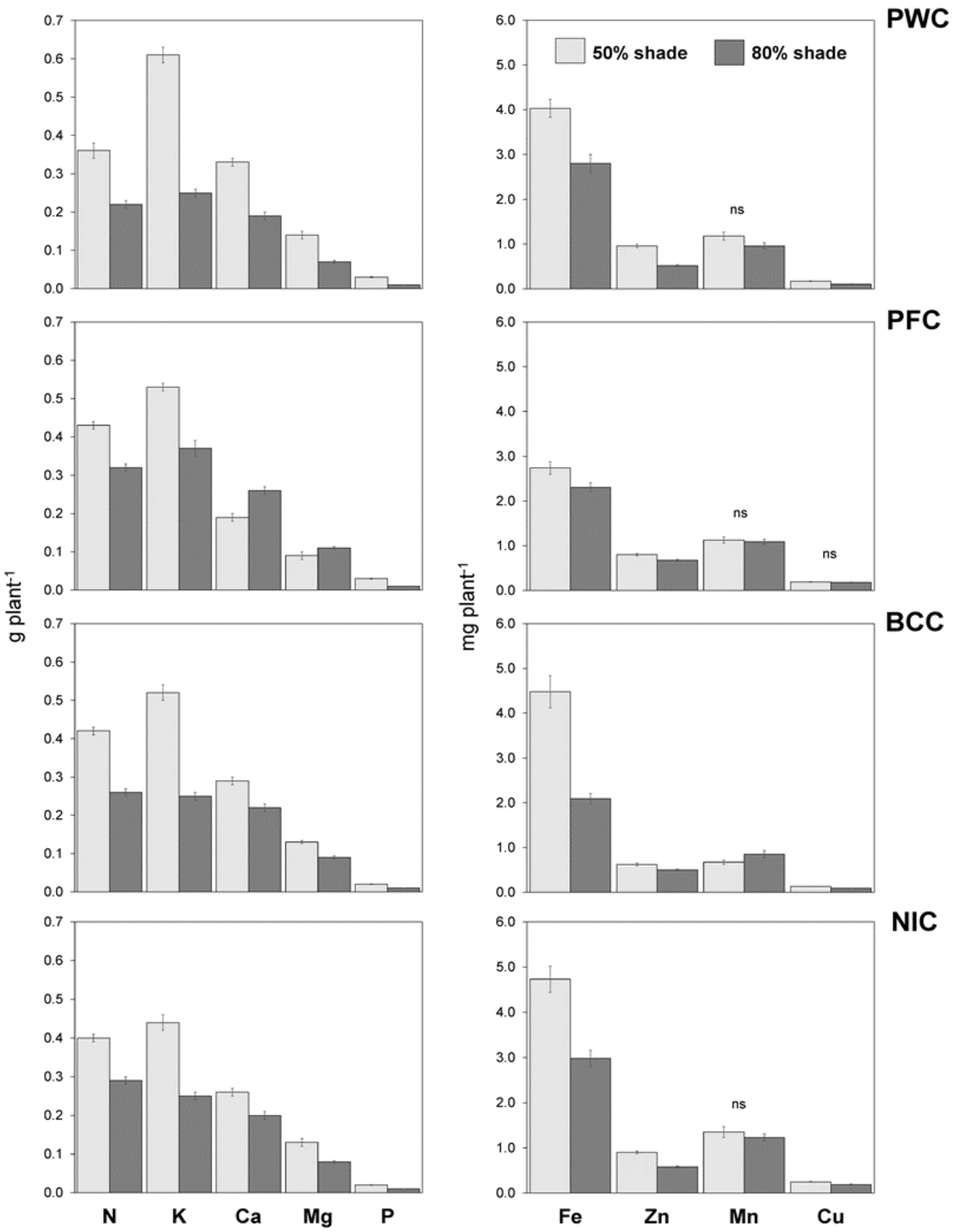

Figure 2. Nutrient uptake (U, Macro nutrients in g plant^{-1} or Micro nutrients in mg plant^{-1} (±SE) of 58 cacao genotypes subjectedto two levels of shade(50%, 80%). PWC: Wild cacao (from River basins of Peruvian Amazon), PFC: Peruvian farmers' cacao, BCC: Brazilian cacao NIC: National and International cacao collections. (ns) denote not significant differences between shade levels (Scott & Knott test, $P \leq 0.05$)

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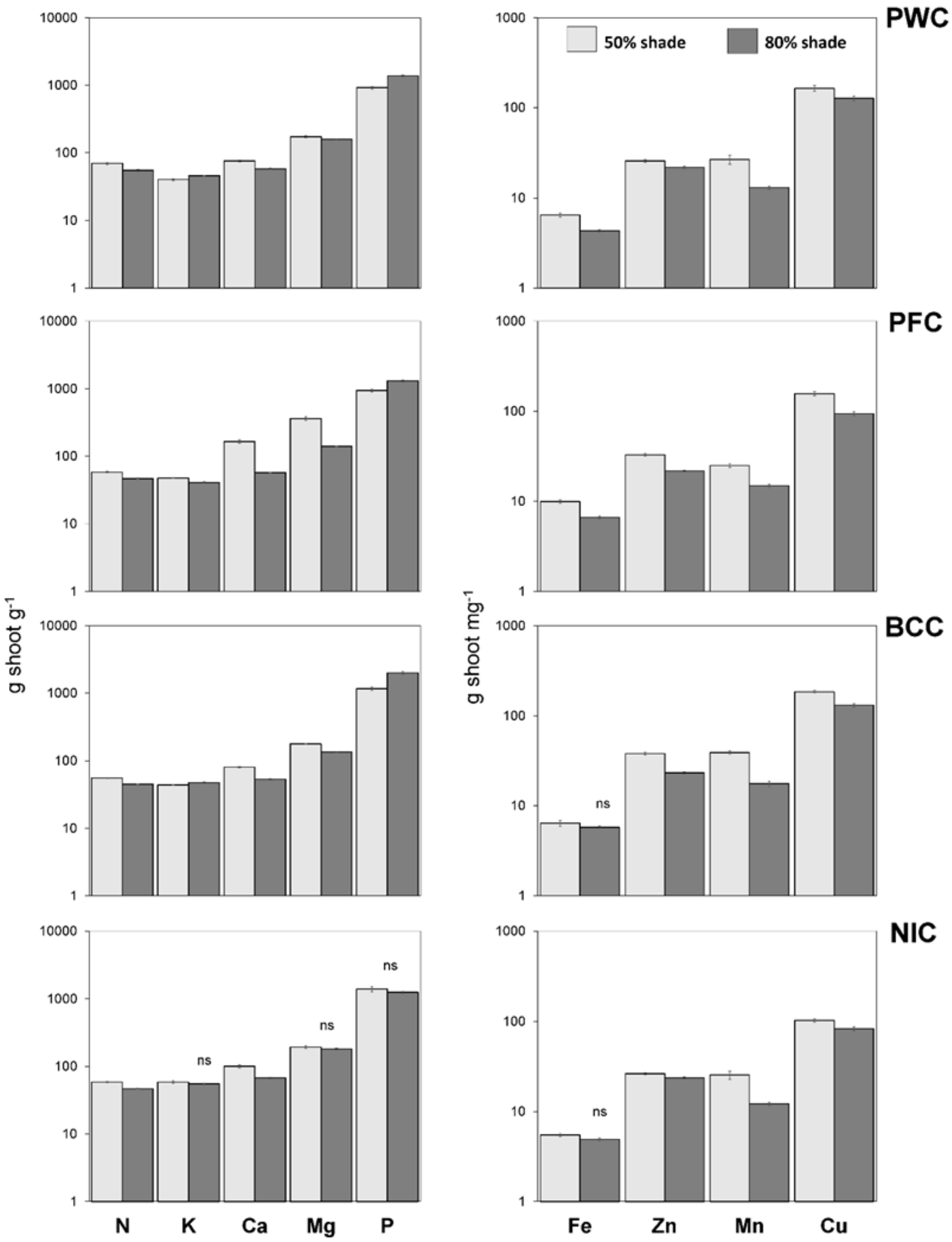

Figure 3. Nutrient uptake efficiency (NUE, Macro nutrients in g shoot g^{-1} or Micro nutrients in g shoot mg^{-1}, ±SE) 58 cacao genotypes subjected to two levels of shade (40%, 80%). PWC: Wild cacao (from River basins of Peruvian Amazon), PFC: Peruvian farmers' cacao, BCC: Brazilian cacao NIC: National and International cacao collections. (ns) denote not significant differences between shade levels (Scott & Knott test, $P \leq 0.05$)

Our values for U were higher than those reported by Baligar et al. [43], and higher at 1000±50 (50% shade) than at 400±50 μmol m^{-2} s^{-1} (80% shade). These differences could be due to the fact that in the current study plants were grown a longer period (180 days vs 57 days) and with a wide collection of cacao genotypes.

Significant differences were observed in NUE of macronutrients and micronutrients between the shade levels and within each cacao collection (Figure 3). With the exception of NUE for P in PWC, PFC and BCC and K in PWC and BCC, overall increasing shade from 50% to 80% reduced NUE for all the nutrients. However, Baligar et al. [43], reported that at ambient CO_2 (380 μmol mol^{-1}) increasing PPFD from 65 to 1050 μmol m^{-2} s^{-1} decreased the NUE of all macronutrients and micronutrients, with the exception of NUE for N and Ca which increased. In recent study, Baligar et al. [44] showed that increasing PPFD from 100 to 400 μmol m^{-2} s^{-1} increased uptake and NUE for all macro and micronutrients in seven cacao genotypes.

3.4 Cacao genotypes tolerant to shade

Shade tolerance is a complex property of plants that is achieved by different sets of responses, such as alterations in leaf physiology and biochemistry, leaf anatomy and morphology and/or plant architecture [4]. Many methods have been proposed to measure the degree of shade tolerance of several plant species such as sapling ratios (number of saplings growing in a low-light environment over the total abundance of the species), abundance-based index (number of stems, leaf density), mortality rate and relative growth (which is assumed to be larger in shade tolerant species) [1,2]. The differences observed in shade tolerance is mostly related to variations among species in adaptation of the photosynthetic apparatus to low light intensity. The effective growth of plants at low irradiance requires capacity to efficiently catch the available light and convert it into chemical energy, maintain a low rate of respiration, and use a large fraction of the carbohydrate pool for leaf growth [38, 57]. On the other hand, shade-intolerant species tend to respond to high light regimes with much increased photosynthetic capacity, reduced leaf expansion, decreased branching and early flowering. These responses are known as shade avoidance syndrome (SAS), which is one best-studied forms of plant phenotypic plasticity [4, 76]. (Bongers et al., 2014; Martínez-García et al., 2010)

In our study significant differences were observed across cacao genotypes regarding STI as well as a moderate variability within replicates. This index varied from 25.72% to 76.15% for AYP-22 (PWC) and ICS-1 (NIC) genotype, respectively (Table 3, Figure 4).

The genotypes sensitive to shade were: AYP-22, PAS-91, PAS-105 and UGU-112 from PWC; ICT-1026 and ICT-2653 from PFC; PH-09, IPIRANGA-1, CP-53-C10, CP-49-C10 and CEPEC-2002 from BCC; TSH-565 from NIC (Figure 4). By contrast, cacao genotypes tolerant to shade were: UGU-130 and UNG-77 from PWC; ICT-2171, ICT-1087, ICT-1506 ICT-2172, ICT-2142 and ICT-2173 from PFC; PH-144, PH-990, CA-14 and PH-21 from BCC; POUND-12, UF-613, ICS-39 and ICS-1, from NIC. The rest of the genotypes were classified as medium-tolerant to shade (Figure 4).

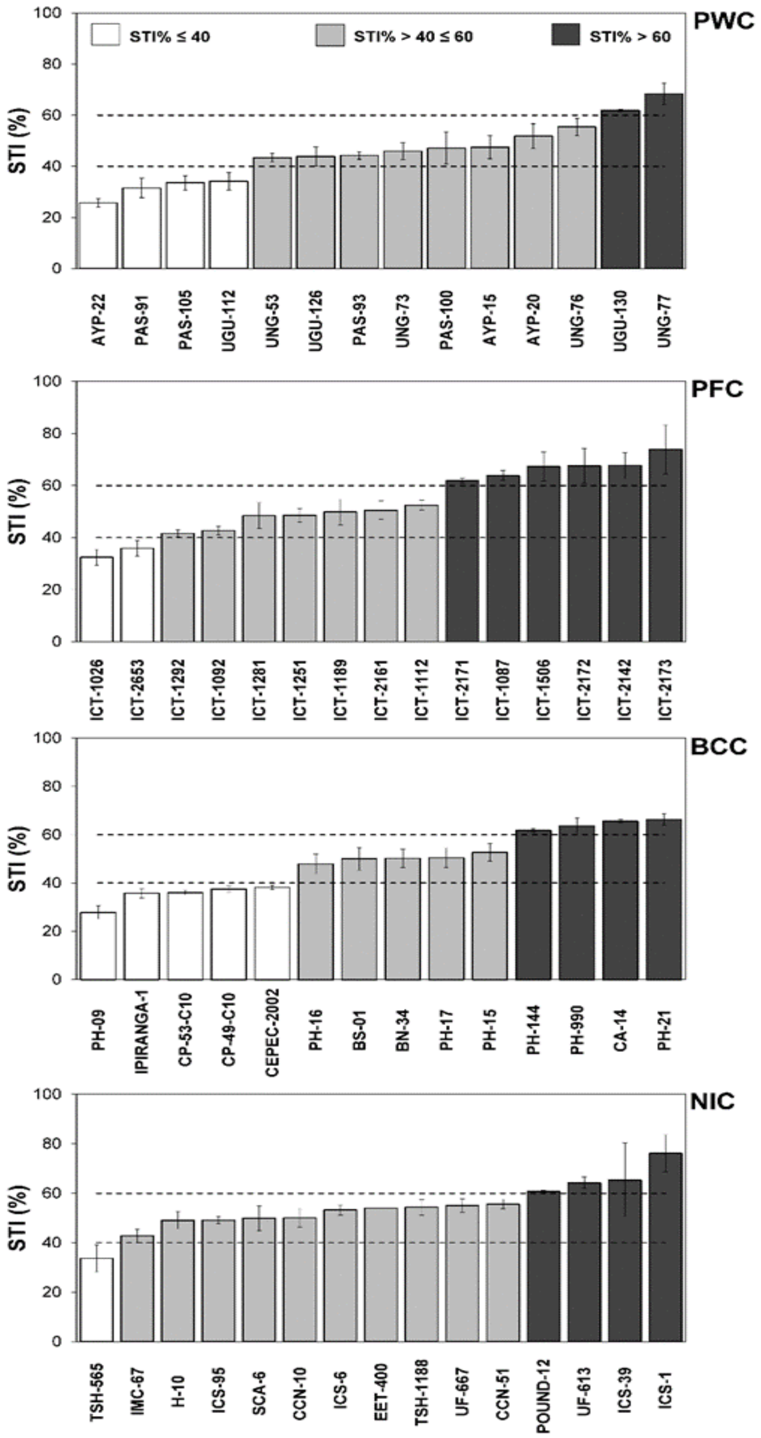

Figure 4. Shade tolerance index (STI, ±SD) of 58 cacao genotypes subjected to two levels of shade (40%, 80%). PWC: Wild cacao (from River basins of Peruvian Amazon), PFC: Peruvian farmers' cacao, BCC: Brazilian cacao NIC: National and International cacao collections. Genotypes were classified in 3 groups: sensitive to shade (STI % ≤ 40), medium shade tolerant (STI % > 40 but ≤60) and tolerant to shade (STI % > 60).

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3.5 Interaction between growth, physiological parameters and nutrient uptake

The results of the general PCA for the growth, physiological and nutrient uptake variables under two levels of shade with sensitive and tolerant cacao genotypes are shown in Figure 5. The first two PCA axis explained 51.9 % of the overall variation from genotypes under 50% shade (Figure 5A), while under 80% shade the variability explained was 69.2% (Figure 5B)

At 50% shade, the first PCA axis accounted for 30.3% of overall variation and was related in negative values with high values of S/R, CHO, Ca Mg and Fe that were positively correlated among themselves and negatively correlated with SL, SD, RDW and Cu; the sensitive genotypes PAS-105, CP-53-C10, PH-09, CP-49-C10 were related with Ca, Mg and Fe uptake. At the positive values were genotypes with high values of BDW, SD, RDW, WUE, N, K, Mn,P and Cu, that were also positively correlated among themselves. 33% and 50% of sensitive and tolerant genotypes respectively were related with K, Mn, N, Zn, P and Cu. The second principal component, which explained 21.6% of the overall variance, was represented mainly by variations in negative values of growth parameters (RDW, SD, SL) and Cu uptake and in positive values with the rest of the nutrient and physiological parameters. The axis divides 50% of sensitive and tolerant genotypes respectively.

At 80% shade, the first PCA axis accounted for 58.4% of the overall variation and was related in negative values with CHO and S/R and divides 100% and 25% of sensitive and tolerant genotypes respectively. In positive values we found all shade tolerant genotypes related with physiological parameters and nutrient uptake.

Based on these results, we can infer that the sensitive and tolerant genotypes have a better development at 50% shade, while at 80% shade the tolerant genotypes are related directely with nutrient uptake, physiology and growth parameters. This characteristic of shade tolerant genotypes permits better development of plants and selection of genotypes that withstand the high level of shade, while the sensitive genotypes do not have a positive relationship with the nutrient uptake, physiology and growth parameters.

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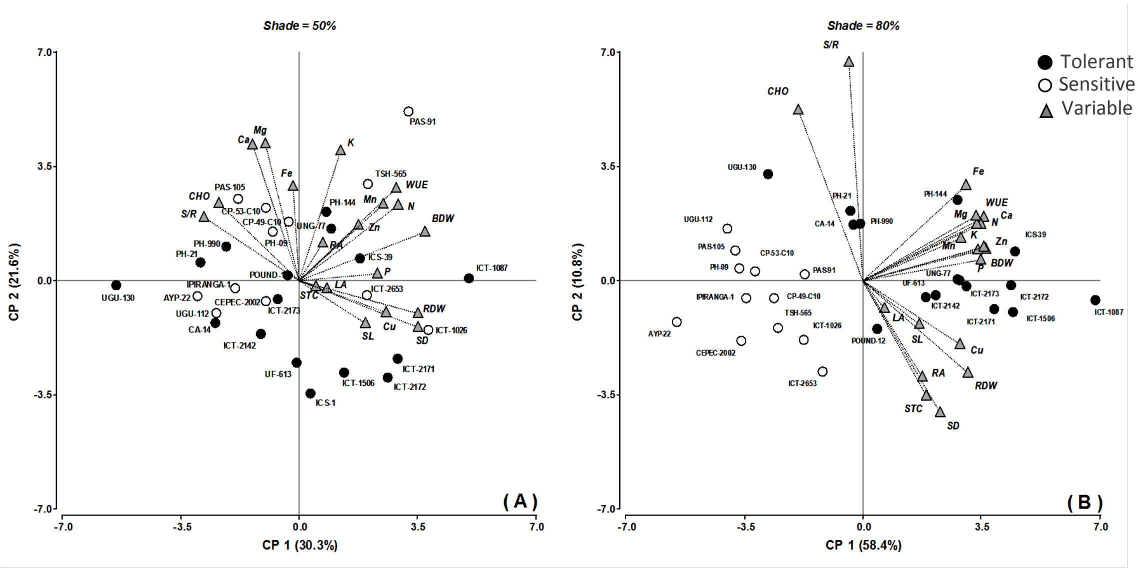

Figure 5. PCA analysis of growth, physiological parameters and nutrient uptake of shade sensitive and tolerant genotypes subjected to 50% (A) and 80% (B) shade

Usually shade intolerant species exhibit a greater physiological plasticity which allows them to achieve rapid growth rates, probably associated with more effective net assimilation rates than with structural traits [38,77] The increase of light intensity when photosynthesis and growth rate are faster causes a decrease in total N in the leaf (source for proteins, chlorophyll, etc.) and also a decrease of P, that would imply that P uptake cannot keep up with increased growth at higher light levels [57].

It would be expected that in cacao genotypes sensitive to shade, growth parameters have strong correlation with almost all nutrients, which is the case for RDW in tolerant genotypes. On the other hand, only shade sensitive cacao genotypes showed a positive correlation between RDW and N content in the aboveground tissues, probably because a long and branched root system is necessary to increase water and nutrient capture [78], that could be reduced under heavy shade conditions.

Under controlled conditions, some plants grown hydroponically have shown a decline in chlorophyll content as the Mn concentration increased [79,80], whereas in other cases it decreases as the Mn content decreased [81. In the case of cacao, all genotypes showed a negative but not significant correlation between these two parameters.

Finally, shade sensitive and tolerant cacao genotypes exhibit negative correlations between Cu and chlorophyll content. Several authors observed that increasing levels of Cu in the nutrient solution or in soil were associated to a decrease of the stomatal conductance, which causes a decline in photosynthetic gas exchange [82,83,84] or that an increase of Cu lowered the leaf chlorophyll concentration, enhancing sensitivity to photoinhibition [85].

4. Conclusions

Fifty eight cacao genotypes grown under greenhouse conditions and subjected to 50% shade (PPFD of 1000±50 μmol m^{-2} s^{-1}) and 80% shade (PPFD 400±50 μmol m^{-2} s^{-1}) were evaluated to determine their growth, physiological, nutrient use efficiency responses and tolerance to shade. For almost all growth, physiological and nutritional parameters interactions between shade levels and genotypes were statistically significant, therefore, it was not possible to establish significant differences for each factor (individually). Only maximum root length, leaf area, N concentration, Cu content and Cu efficiency seem to vary significantly between shade levels and across genotypes.

Overall results suggest that heavy shade affects cacao negatively, except for shoot length, leaf and root area, shoot/root ratio and chlorophyll content, which are usually higher at 80% than at 50% shade.

Merely 28% of the cacao genotypes evaluated were identified as tolerant to shade, from the wild cacao, Peruvian famers' (or ICT), Brazilian, National and International cacao collections, whereas 21% were sensitive to shade.

Total dry weight and WUE showed strong relation with almost all macronutrients and micronutrients content in cacao tolerant to shade. Besides, they also have a higher total dry biomass than sensitive genotypes.

Finally, the possibility of cacao genotypes adapted to unfavorable conditions such as high shade could be used in breeding programs as a strategy to breed shade tolerant cacao cultivars to maintain sustainable cacao production under agroforestry systems.

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Author contributions

E.A-G implemented the original idea, planned the experimental details and carried out the research

A.F collected and compiled reported data.

F.B took the lead in writing the manuscript, organized and interpreted the data and conducted the statistical analysis.

C.O.A-H. did plant analysis and worked the correlation matrix of all the observed parameters.

J.A. assisted with planning and implementation of the experiment.

V.C.B. conceived the study and was in charge of overall direction and planning.

All authors provided critical feedback in writing process.

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