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Genotype–environment interactions shape leaf functional traits of cacao in agroforests

Marie Sauvadet^{1,2,3} · Adam K. Dickinson⁴ · Eduardo Somarriba⁵ · Wilbert Phillips-Mora⁵ · Rolando H. Cerda⁵ · Adam R. Martin¹ · Marney E. Isaac^{1,4} 

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Abstract

The outbreak of frosty (*Moniliophthora roreri*) and black pod (*Phytophthora palmivora*) in Central American cacao agroforests throughout the 1980s led to the breeding of *Theobroma cacao* clones resistant to these diseases. However, while clonal disease resistance has been well-documented, clonal adaptability to contrasting pedoclimates remains less understood. Plant functional responses to environmental constraints can be assessed by evaluating intraspecific trait variation (ITV), but trait-based approaches have rarely been implemented in genotype assessments. The objective of our study was to determine cacao clone ITV in contrasting environments. Here, we used bivariate, multivariate, and functional trait space analyses to quantify ITV in nine leaf functional traits, among six disease-resistant cacao clones, growing in two clonal gardens with distinct environmental characteristics (a “mild dry season” with near-optimal cacao growing conditions and a “harsh dry season” site with sub-optimal conditions). All leaf traits varied among the six clones, though trait differences among clones differed depending on the site: physiological trait differences among clones were larger at the “mild dry season” site, chemical trait differences were wider at the “harsh dry season” site, and morphological trait differences among clones were similar at both sites. Multivariate and hypervolume trait space analyses revealed greater site influence on trait values of the least productive clones, while the most productive clones were characterized by higher specific leaf area, independent of the site. To our knowledge, our paper is among the first to show evidence that functional traits provide new insights into genotype × environment interactions and clonal selection in agroforestry systems and support in favor of applying functional trait-based research to plant breeding paradigms.

Keywords Agroforestry · Clone · Domestication · Functional traits · Intraspecific trait variation · Leaf traits · *Theobroma cacao*

1 Introduction

1.1 Functional trait plasticity among crop genotypes

The development of new crop varieties has been a pillar of agriculture since the earliest times of plant domestication (e.g.,

Meyers et al., 2012). Adapting cultivars to local environmental conditions is key to ensuring food security and financial sustainability of smallholder farms (e.g., Nhamo et al., 2014). However, predicting how cultivars are likely to adapt to local environmental conditions (i.e., genotype × environment (G × E) impacts) remains difficult, due to complex interactions between plant growth-limiting factors [e.g., water, soil N limitations (Teixeira et al., 2014)] and plant responses to environmental constraints [e.g., alterations to plant functional traits (Lemaire et al., 2008)]. For example, recent work has shown that artificial selection not only influences agronomic traits (Milla et al., 2015)—i.e., the specific targets of crop breeding programs—but also shapes a much broader suite of crop functional traits and trait syndromes that cumulatively determine plant responses to environmental conditions (Martin and Isaac, 2015, Isaac and Martin, 2019).

In diversified agroecosystems, a growing number of studies have quantified how environmental and management

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conditions constrain or influence crop functional trait expression—i.e., the morphological, chemical, physiological, and/or phenological properties of plants that mechanistically influence plant performance (Garnier and Navas, 2012; Martin and Isaac, 2015). This area of “trait-based agroecology” research has contributed to a better understanding of (1) how artificial selection has shaped functional trait expression in crops vs. their wild ancestors (e.g., Milla et al., 2015; Montazeaud et al., 2020), (2) the environmental and/or management conditions that induce crop trait variation (e.g., Buchanan et al., 2019), and (3) the crop functional traits that mechanistically govern rates of agroecosystem functioning such as yield (e.g., Gagliardi et al., 2015) and plant–soil interactions (Isaac and Borden, 2019). Moreover, across all these lines of research, trait-based approaches point to the overarching importance of crop intraspecific trait variation (ITV) in driving agroecosystem processes.

While our understanding of the causes and consequences of ITV in crops has advanced greatly in recent years, important gaps remain (Martin and Isaac, 2015). Specifically, to date, variation in crop traits has seldom been explored in the context of breeding programs, where $G \times E$ interactions are of critical importance; this is despite the potential for trait-based studies to assess the intentional and unintentional consequences of domestication (Isaac and Martin, 2019; Milla et al., 2015). Crop genotypes adapt to contrasting environmental conditions via trait plasticity—i.e., the ability of a genotype to modify trait expression in response to local conditions—but this adaptability, in terms of predicted trait values, remains hard to predict. Even closely related genotypes may express different adaptations to cope with environmental stresses, which may in turn vary widely across geographic and environmental contexts (Sultan, 2003). This unpredictability is especially high in artificially selected crop genotypes, which have consistently shown weaker patterns of relationships between or among traits, resulting in less predictable trait expression than wild plants or ancestors (Martin et al., 2017; Milla et al., 2015). Amplified climate variability, pest and pathogen outbreaks, and alterations to soil nutrient and water status underscore the importance of improving our understanding of how crop genotypes adapt to contrasting environmental conditions. However, while trait plasticity has increasingly been considered in breeding new herbaceous crop varieties (Barot et al., 2017), there remain very few studies assessing trait variation in clonal varieties of woody perennial crops (but see Doaré et al., 2020). For example, cacao—the focus of our study—shows substantial trait plasticity in response to management and environmental conditions (Borden et al., 2020; Saavedra et al., 2020), and this trait plasticity has been used to assess the viability of cultivars under drought (Araque et al., 2012; Tezara et al., 2020). However, climate change is casting doubt as to whether or not cacao genotypes are viable within current cultivation

zones (Baca et al., 2014; de Sousa et al., 2019). Assessments of functional trait plasticity across environmental gradients can therefore contribute to our understanding of the long-term suitability of cacao genotypes throughout different regions (e.g., Isaac and Martin, 2019).

1.2 *Theobroma cacao* varietal development in Central America

Theobroma cacao (cacao) is an understory tree native to the western Amazon, whose domestication is reported as early as 400 BC by the Maya (Monteiro et al., 2009). Despite its long domestication history in this area, Central American cacao cultivars contributed less than 1% of the total world production in 2017. The current cacao sector is typically characterized by old plantations with low yields (Cerdeira et al., 2014; de Sousa et al., 2019). In addition, cacao production in Central America has been highly impacted during the 1980s by the outbreak of several diseases, namely, Frosty pod “moniliasis” (*Moniliophthora roreri*) and black pod (*Phytophthora palmivora*). These diseases are currently the main limiting factor in Central American cacao development, resulting in 30–100% yield losses in some areas, and are very costly to control (Phillips-Mora et al., 2009).

In response to this outbreak, regional agricultural institutions such as the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) in Costa Rica have bred new *T. cacao* clonal varieties that are both regionally adapted to environmental conditions and resistant to frosty and black pod (Phillips-Mora et al., 2013; Somarriba et al., 2013). Specifically, six *T. cacao* clones were developed beginning in 1996 and released for cultivation in 2007. These clones presented promising, albeit varying productivity and quality potential. They are now currently cultivated in seven Latin American countries and considered as “good candidates” to allow smallholders to maintain low-input cacao production in frosty- and black pod-infested zones. However, because of the novelty of these clones, little is known about their performance across the region (Phillips-Mora et al., 2013) or the interactive effects of $G \times E$ on cacao functional trait expression. In particular, drought resistance is an increasingly targeted property in perennial crops (Schroth et al., 2016) due to the increasing extreme climate change (Baca et al., 2014; Lyra et al., 2017; de Sousa et al., 2019). Key leaf functional traits, including morphological traits such as specific leaf area (SLA) and physiological traits such as water use efficiency (WUE), have been linked to water stress resistance (Acheampong et al., 2015; Tezara et al., 2020). However, little is known about the genotype expression of leaf functional traits, especially those related to abiotic constraints.

In this study, our objective was to quantify ITV among cacao clones and evaluate whether functional trait expression is systematically linked to environment conditions. To do so,

we studied six clones of cacao in two contrasting clonal garden sites: (1) near-optimal climatic conditions for cacao production and (2) sub-optimal climatic conditions for cacao production. We measured nine leaf functional traits and used bivariate and multivariate analyses and functional trait space analyses, to describe the relationships and variability among cacao clone traits. Our findings offer a unique approach to better understand $G \times E$ responses of crops to environmental conditions and thereby inform cultivar selection under environmental constraints in agroecosystems.

2 Material and methods

2.1 Clone descriptions

The CATIE cacao breeding program resulted in the selection of six cacao genotypes according to their yield potential (789–2363 $\text{kg ha}^{-1} \text{ year}^{-1}$) and resistance to frosty pod rot and black pod disease, which were subsequently planted in clonal gardens throughout Central America. These clones are named ICS-95 type 1 (abbreviated as “ICS-95” here), PMCT-58, CC-137, CATIE-R1, CATIE-R4, and CATIE-R6 (Fig. 1 and Table S1). The oldest clone, ICS-95, is a Trinitario-Criollo hybrid developed in Trinidad and Tobago in the 1940s; while this is one of the least productive clones evaluated in our study (i.e., potential yield < 1000 $\text{kg ha}^{-1} \text{ year}^{-1}$), it presents a broad geographic

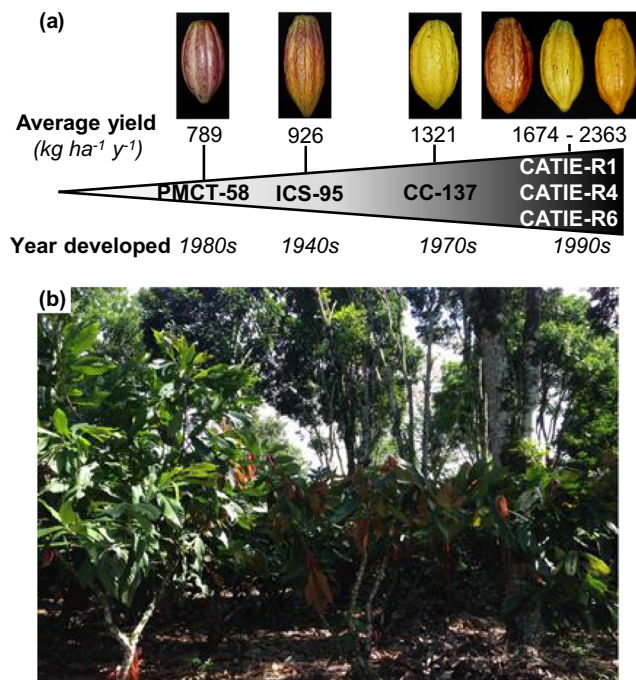


Fig. 1 Description of six *Theobroma cacao* clones (a) and clonal garden site in Turrialba, Costa Rica (“mild dry season” site) (b). Clones’ origin, average annual yield data, and pod pictures are issued from Phillips-Mora et al. (2013), and b is issued from a personal picture taken by A. K. Dickinson

applicability due to its disease tolerance (Phillips-Mora et al., 2013). The five other clones were developed by CATIE in Costa Rica. Specifically, CC-137 was developed in the 1970s under CATIE’s Cacao Center and PMCT-58 in the 1980s under the Tropical Crop Improvement program. Finally, CATIE-R1, CATIE-R4, and CATIE-R6 were developed by the CATIE in the 1990s by crossing existing disease-resistant clones from CATIE’s International Cacao Genebank; these clones present the highest yield potential (i.e., > 1600 $\text{kg ha}^{-1} \text{ year}^{-1}$, Table S1).

2.2 Study sites

We worked in clonal gardens that were established in 2007 to test the six clones’ suitability for cultivation in Central American agroecosystems (Somarriba et al., 2013). These clonal gardens have similar crop management systems but contrasting environmental conditions: one site in Turrialba, Costa Rica, managed by CATIE, and the other in Waslala, Nicaragua, managed by the cacao cooperative Cacaonica (Table 1). Cacao trees grow in both sites under mature shade trees, are fertilized and pruned twice a year, and are harvested approximately once a week during the productive period (April to December) and once a month the rest of the year.

Climate data were based on the preceding 30 year averages of the CATIE meteorological station and Matagalpa climate-data.org (the nearest climate station to Waslala) for Turrialba and Waslala sites, respectively. Both sites have a dry season between January and April but with varying intensities (Fig. S1). The Waslala site (13.33 °N, 85.36 °W, 500 m a.s.l) is characterized by harsh dry–wet season cycle. The Waslala site experiences monthly precipitation during the dry and wet seasons that average 27 and 165 mm, respectively, and warm average monthly temperatures (22.6 and 22.9 °C during the dry and wet season, respectively) and is henceforth referred as the “harsh dry season” site (Table 1 and Fig. S1). On the other hand, the Turrialba site (9.88 °N, 83.65 °W, 590 m a.s.l) is characterized by relatively cool average monthly temperatures for cacao growing (21.4 and 22.2 °C during the dry and wet season, respectively), and less marked differences between dry and wet seasons (average monthly precipitation of 153 and 272 mm, respectively, Table 1 and Fig. S1), and is henceforth referred as the “mild dry season” site. Finally, the Turrialba “mild dry season” site is on a loamy soil (43% sand, 35% silt, 22% clay), while the Waslala “harsh dry season” site is on a clay-rich soil (8% sand, 32% silt, 60% clay). The Turrialba site also has both higher soil C and C:N ratios ($3.1 \pm 0.4\%$ Dry Matter (DM) and 12.5 ± 0.6 , respectively) than Waslala’s “harsh dry season” site ($2.4 \pm 0.4\%$ DM and 8.3 ± 0.7 , respectively).

2.3 Sampling design and leaf traits analyses

Both sites are organized in three blocks, with the six clones planted in alternating and randomly ordered rows. In each block, five individuals of each clone that were within a 5-m

Table 1 Description of the “mild dry season” and “harsh dry season” sites. Dry and wet seasons span from January to April and May to December, respectively

Variables	“Mild dry season” site	“Harsh dry season” site
City (country)	Turrialba (Costa Rica)	Waslala (Nicaragua)
Location	9.88° N, 83.65° W	13.33° N, 85.36° W
Elevation (m a.s.l)	590	500
Average temperature (°C): dry/wet season	21.4/22.2	22.6/23.0
Average precipitation (mm): dry/wet season	153/272	27/165
Annual precipitation (mm)	2790	1429
Soil texture	Loam	Clay
Soil C content (% DM)	3.11 ± 0.35	2.42 ± 0.43
Soil C:N ratio	12.5 ± 0.6	8.3 ± 0.7

radius of shade trees were selected. Sampling was carried out at both sites during the late dry season and early wet season in 2016. This sampling design resulted in a total of 15 individuals per clone and 90 individual cacao trees in total at the “mild dry season” site. Cacao trees at the “harsh dry season” site were distributed less regularly between shade trees and could not always meet the sampling requirements. This resulted in a slightly different sample size for certain clones at this site (i.e., 14, 16, and 7 individuals for PMCT-58, CC-137, and CATIE-R1 clones, respectively).

All selected cacao individuals were then measured for nine leaf traits. First, one representative leaf for each individual was chosen according to its position on the branch (third leaf back from the terminal bud) and based on the position of the branch on the cacao tree (at 60% of the canopy height, with a partial exposure to direct sunlight). On each leaf, physiological traits were measured between 7:00 and 11:00 with a LI-6400 gas exchange system equipped with a red/blue light source (6000-02B Red-Blue SI-0951, Li-Cor, Lincoln, Nebraska). Area-based light-saturated photosynthetic capacity (A_{sat} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and transpiration rates (E , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were taken as the mean of three replicate measurement taken 30 s apart, after leaves equilibrated to the following chamber conditions: a photosynthetic photon flux density of 1000 $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$, CO_2 concentrations of 400 $\mu\text{mol mol}^{-1}$, vapor pressure deficit (VPD) < 3 kPa, relative humidity of 30–70%, and leaf temperatures of 25 °C. Leaf stomatal conductance (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and instantaneous water use efficiency (WUE; $\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) were then calculated from these measurements as the ratio between E :VPD, and between A_{sat} : E , respectively.

The leaves measured for physiological traits were then immediately collected for morphological and chemical trait analyses in the laboratory. Pictures of the leaves were taken immediately after returning from the field and then analyzed with ImageJ software to obtain the leaf area (cm^2). Leaf lamina thickness (mm) was measured with a low-force micrometer (No. 227-101, Mitutoyo Co., Mississauga, ON, Canada).

Leaves were then dried at 60 °C to constant mass and weighted to attain leaf dry mass (mg). Specific leaf area SLA ($\text{cm}^2 \text{ g}^{-1}$) was calculated from these variables as the ratio between leaf area/dry mass. Dried leaves were then transported to the University of Toronto Scarborough, Canada, for the measurement of chemical traits. They were ground with a ball mill (Retsch Ltd., Haan, Germany), and approximately 2 mg of ground leaves were analyzed for leaf total carbon (C) and nitrogen (N) concentrations ($\text{mg g}^{-1} \text{ DM}$) using a Flash 2000 Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). This data was also used to derive leaf C:N ratios.

2.4 Statistical analyses

All statistical analyses were performed using the R software (R v.3.5.1, R Foundation for Statistical Computing, Vienna, Austria). We first tested how site, clone, and site-by-clone interactions influenced leaf trait expression, using a two-way ANOVA with interactions using the *car* R package. Then, we coupled these ANOVA models with the Tukey post hoc tests, implemented in the *emmeans* R package, to assess trait differences between clones within a single site and to test for significant differences in trait values across each site × clone combination. We then performed a principal component analysis (PCA) to evaluate multivariate ITV that occurs across both clones and sites; this analysis was completed with the assessment of trait correlations with the two first PCA eigenvectors using the *dimdesc* function of the *FactoMineR* R package. These analyses were coupled with the analysis of similarity (ANOSIM) tests implemented in the *vegan* R package, based on the Bray–Curtis distances with 999 permutations used, in order to compare inter-site differences in ITV for each clone.

We then assessed how sites influenced estimates of n -dimensional trait hypervolumes in *T. cacao* clones, which describe a species niche space (Blonder, 2018). To do so, we first used Pearson correlation tests with a Benjamini–

Hochberg correction for multiple correlation errors, to identify the key traits for the hypervolume analyses. Specifically, following recommendations of Blonder (2018), trait hypervolumes were calculated based on the fewest number of traits which were least strongly correlated with one another; on our analysis, the identification of these traits was based on both PCA and Pearson correlation analyses and ultimately included A_{sat} , WUE, SLA, and leaf N. Based on these four traits, hypervolume estimates were calculated for the whole dataset ($n = 172$ leaves), “mild dry season” site ($n = 90$ leaves), and the “harsh dry season” site ($n = 82$ leaves) using the *hypervolume_gaussian* function in the *hypervolume* R package. Hypervolumes were estimated using a 0.05 quantile threshold such that each hypervolume contained 95% of the total probability distribution of random samples per points, while kernel bandwidths for each trait were estimated independently using the *estimate_bandwidth* function.

3 Results and discussion

3.1 Genetic and environmental effects on cacao leaf trait variation

Cacao trait expression differed significantly as a function of site, clone identity, and a site-by-clone interaction term, albeit differently depending on the trait considered (Table S2). Among the six cacao cultivars, environmental conditions across sites had the strongest effects on leaf physiological and chemical trait values but little influence on morphological traits (Figs. 2 and 3). Cacao individuals expressed significant differences in A_{sat} , WUE, leaf N, and g_s between sites, with average trait values being 1.2, 1.7, 1.1, and 0.7 times higher in the “mild” vs. the “harsh” site, respectively (Fig. 2). Water limitation is known as a strong driver of cacao leaf physiological traits, being negatively associated with rates of both g_s

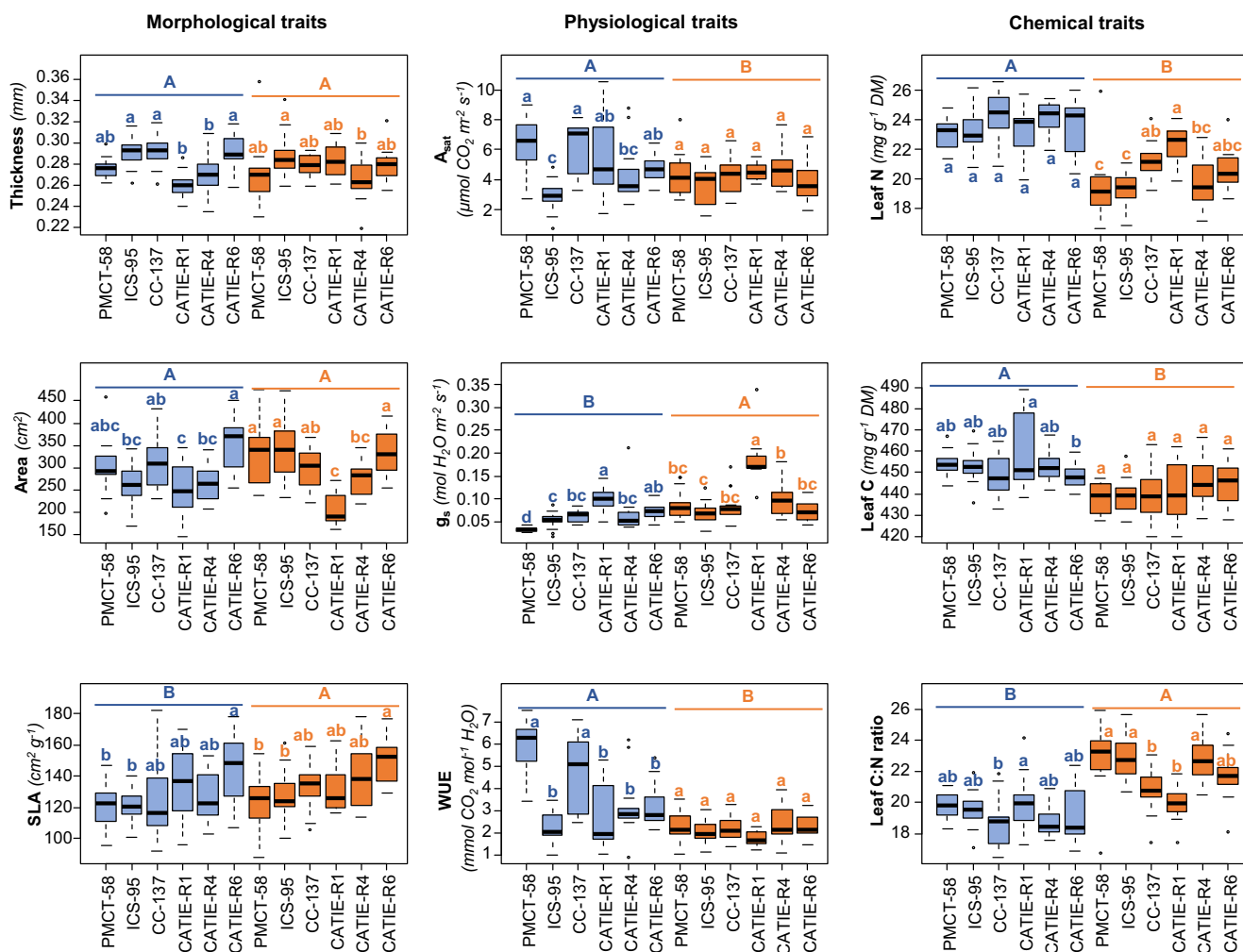
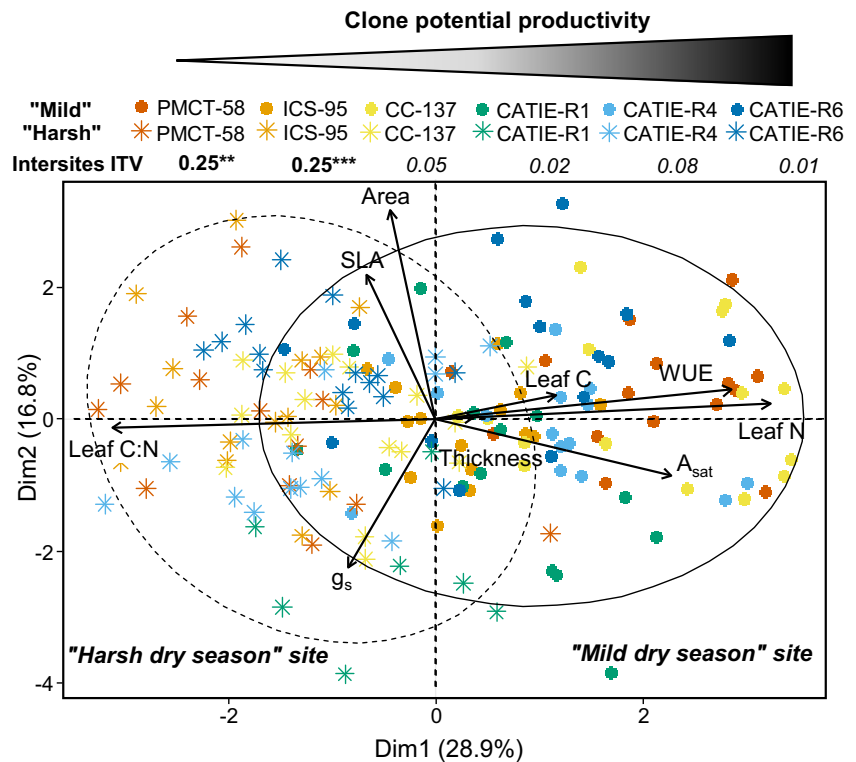


Fig. 2 Boxplots of nine *T. cacao* leaf traits measured across six clones and two clonal garden sites. Site differences correspond to the Turrialba “mild dry season” site (in blue) and Waslala “harsh dry season” site (in orange). Significant differences ($p < 0.05$) between sites bear different capital letters, while clones with significant differences ($p < 0.05$) within

each site bear different lowercase letters. In each plot, clones are ordered from the least productive (on the left) to the most productive (one the right). SLA, specific leaf area; A_{sat} , area-based light-saturated photosynthetic capacity; g_s , leaf stomatal conductance; WUE, water use efficiency

Fig. 3 Principal component analyses of nine *T. cacao* leaf traits measured across six clones and two sites. Clone trait variation between sites was assessed using ANOSIM tests (based on Bray–Curtis distances and 999 permutations), with r -values ranging between 0 and 1, and increasing with the level of dissimilarity between sites (denoted above the PCA panel, where for each of these site comparisons, * stands for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$). ITV, intraspecific trait variation; SLA, specific leaf area; A_{sat} , area-based light-saturated photosynthetic capacity; g_s , leaf stomatal conductance; WUE, water use efficiency



(Baligar et al., 2008) and A_{sat} (Balasimha et al., 1991) that may lead to higher WUE (Acheampong et al., 2015; Araque et al., 2012). Leaf N, which is tightly coupled with A_{sat} vis-à-vis Rubisco has also been found to increase under well-watered conditions (Leibel, 2008). Cacao leaf physiological traits were therefore expected to express lower g_s and higher WUE at the “harsh dry season” site due to lower precipitation during the dry season (Table 1). However, a recent study comparing cacao clones’ physiological adaptation to drought highlighted that water limitation may lead to the opposite trend (Tezara et al., 2020), highlighting the importance of considering $G \times E$ interactive response of cacao ITV even for a single site.

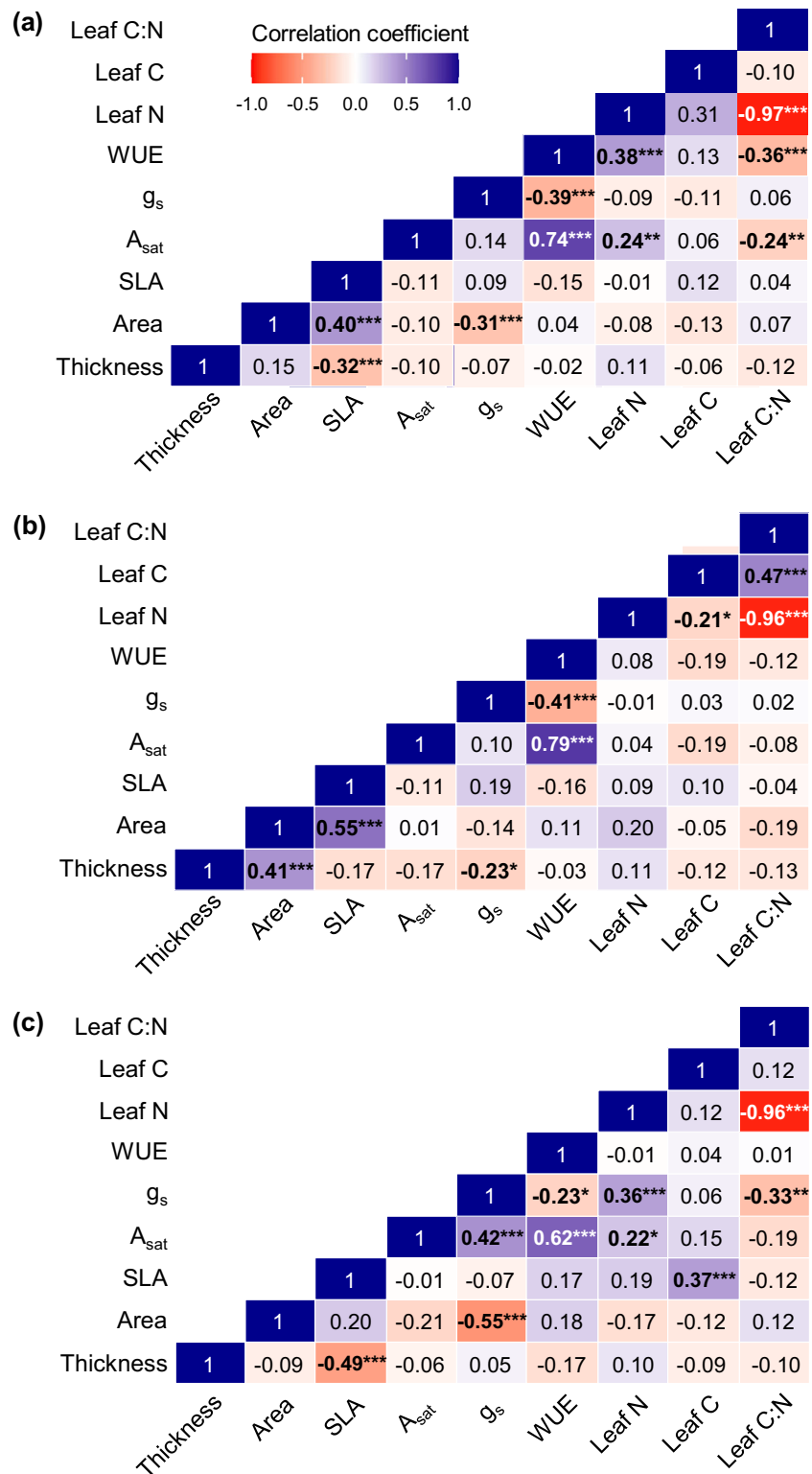
In the case of our study, other environmental factors including soil properties may have had stronger impacts on cacao physiological traits and caused the differences observed. Recent research comparing the traits of multiple species to environmental conditions suggests that leaf physiological and chemical traits are more strongly coupled with soil than with climate. For example, soil pH is more strongly correlated with A_{sat} and leaf N rather than climate variables (Maire et al., 2015), while soil hydraulic conductivity is tightly linked with g_s (Carminati and Jarvaux, 2020). Presumably, certain key soil–climate interactions such as soil available water capacity may have played an important role in driving leaf physiological trait differences between our study sites, as suggested by the coarser soil texture of the “mild dry season” site (Table 1).

Unlike leaf physiological and chemical traits, morphological traits values had a similar range in the “mild dry season”

and “harsh dry season” sites (Fig. 2). These results are consistent with studies that have shown strong site effects on leaf chemical and physiological traits vs. leaf morphological traits, in a wide range of natural and domesticated species (Siefert et al., 2015) including *Coffea arabica* (Martin et al., 2017). Leaf morphological traits including leaf area and SLA tend to be determined by incident light levels (Miyaji et al., 1997). In our study, the discrepancy of light availability within vs. between sites was reduced by selecting cacao individuals that were located within a 5-m radius from at least one shade tree. Given the light variability within a cacao agroforest (Isaac et al., 2007), it is possible that stronger variability in cacao morphological traits may be detected at greater distances from shade trees.

Among the six cacao cultivars, leaf traits varied widely with contrasting patterns between the two sites (Fig. 2 and Table S2). At the “mild dry season” site, cultivars differed primarily in their leaf physiological traits: on average g_s was lower in the less productive clones (i.e., PMCT-58 and ICS-95), while both A_{sat} and WUE were the highest for PMCT-58 ($6.3 \pm 1.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $5.9 \pm 1.2 \text{ mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, respectively) and the lowest for ICS-95 ($2.9 \pm 1.0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $2.3 \pm 0.7 \text{ mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, respectively, Fig. 2), suggesting, respectively, a high and low drought resistance (Araque et al., 2012; Tezara et al., 2020). In contrast, at the “harsh dry season” site, cacao clones differed in their chemical traits, with lower leaf N concentrations (and hence higher leaf C:N ratios) for the less productive

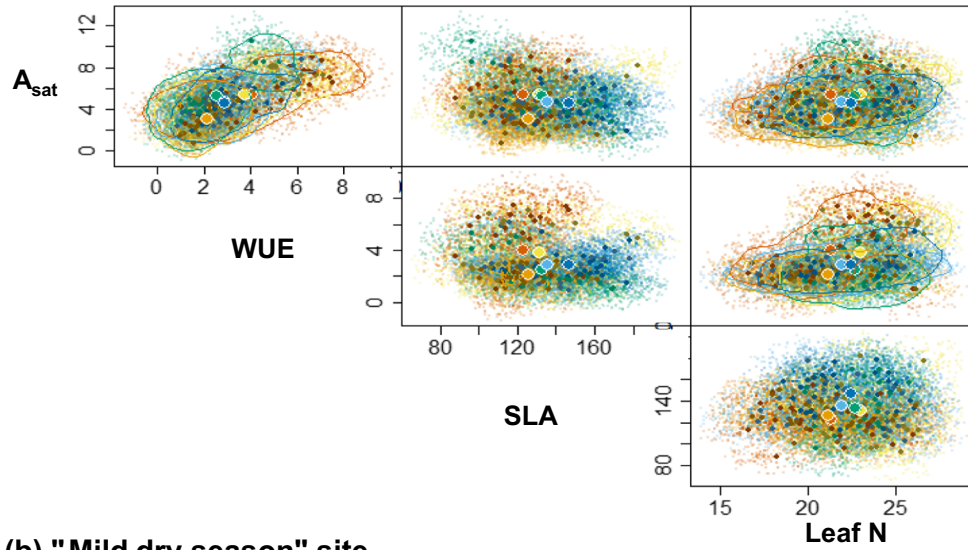
Fig. 4 Pearson correlation coefficient matrices for *T. cacao* leaf traits measured across two sites ($n = 172$) (a); the “mild dry season” site ($n = 90$) (b); and the “harsh dry season” site ($n = 82$) (c). Correlations were performed on centered reduced data and were corrected for multiple comparisons with the Benjamini–Hochberg method. Significance correlations are denoted by * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SLA, specific leaf area; A_{sat} , area-based light-saturated photosynthetic capacity; g_s , leaf stomatal conductance; WUE, water use efficiency



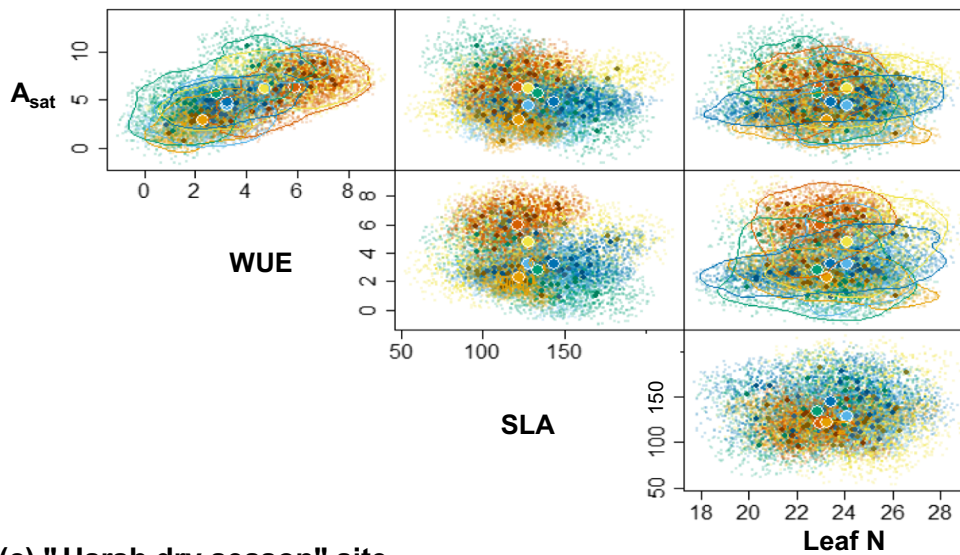
clones PMCT-58 and ICS-95 ($> 20 \text{ mg N g}^{-1} \text{ DM}$, Fig. 2). Leaf area and SLA differed among clones, though these differences followed the same pattern at both sites: SLA increased with the potential productivity and was highest for CATIE-R1, CATIE-R4, and CATIE-R6 ($>130 \text{ cm}^2 \text{ g}^{-1}$, Fig.

2). SLA is a desirable trait for plant selection for its importance in maximizing plant C acquisition (Milla et al., 2015), and the low degree of site influence on this trait makes it a promising indicator of clone potential across multiple pedoclimates.

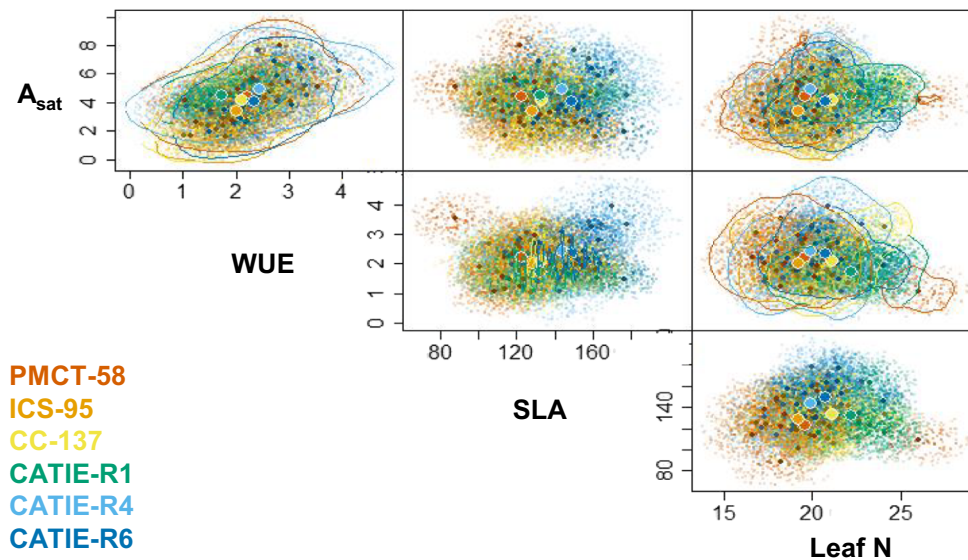
(a) All sites



(b) "Mild dry season" site



(c) "Harsh dry season" site



PMCT-58
ICS-95
CC-137
CATIE-R1
CATIE-R4
CATIE-R6

◀ **Fig. 5** Trait hypervolume estimates for multiple bivariate functional trait axes across six *T. cacao* clones, measured in two sites (a), a “mild dry season” site (b) and a “harsh dry season” site (c). Larger points correspond to observations in bivariate trait space, while the solid lines represent 2-dimensional hypervolume trait space, estimated as capturing 95% of randomized points. Clone legends are ordered from the least to the most productive. A_{sat} , area-based light-saturated photosynthetic capacity; WUE, water use efficiency; SLA, specific leaf area

3.2 Cacao leaf trait coordination and plasticity

Leaf trait syndromes of cacao follow the general hypotheses of universal “plant economics spectra” that exists across species, which has more recently been applied to describe ITV in plants and crops (Martin et al., 2017; Siefert et al., 2015). We show a positive covariation between leaf N, WUE, and A_{sat} , all of which covaried negatively with leaf C:N ratios (Fig. 3). These four traits were highly correlated to axis 1 of the PCA (Table S3) and differed significantly across both genotypes and environment (Fig. 3). Cacao leaf area negatively covaried with g_s , which in turn both contributed to PCA axis 2 (Fig. 3; Table S3). The positive correlation between WUE and A_{sat} ($r = 0.74$, $p < 0.001$) suggests that drought resistance is an important property for cacao growth for both sites (Tezara et al., 2020). In addition, the positive correlation between A_{sat} and leaf N concentrations ($r = 0.24$, $p < 0.01$, Fig. 4a) is consistent with findings in literature for other perennial crop species (e.g., Martin et al., 2017) and is mainly attributed to the high N concentrations in foliar structures associated with photosynthesis, namely, Rubisco (Ávila-Lovera et al., 2016). Nonetheless, this relationship was not observed at the “mild dry season” site (Fig. 4b). Leaf N concentrations were similar for all clones at the “mild dry season” site and were 1.2 times higher than at the “harsh dry season” site (Fig. 2); these high leaf N concentrations suggest a lack of N limitation for cacao growth at this site. We assumed that the lack of N limitation resulted in the absence of trait coordination or very weak relationships (as previously shown in domesticated species, e.g., Martin et al., 2017), between leaf photosynthetic activity and N concentration at the “mild dry season” site. Such changes in trait coordination between the “mild dry season” and “harsh dry season” sites make the prediction of clonal trait plasticity more complex. Indeed, the least productive clones

(PMCT-58 and ICS-95) had the lowest within-site functional trait variation (Fig. 3) while also presenting the highest trait dissimilarity between sites (ANOSIM $r = 0.25$, $p < 0.01$, Fig. 3). On the opposite, the more productive clones (CC-137, CATIE-R1, CATIE-R4, and CATIE-R6) presented inverted trends, with higher within-site trait plasticity (Fig. 3) but lower trait dissimilarity between sites (ANOSIM $r < 0.08$, $p > 0.05$, Fig. 3).

3.3 Effects of artificial selection on leaf functional trait space in cacao

Understanding crop trait relationships is a major challenge for plant breeders, since the promotion of desirable traits in new varieties is often hindered by trade-offs between traits (Denison, 2012). This understanding is hampered by two main factors: (1) the low availability of varietal trait measurements in multiple different environments (Barot et al., 2017) and (2) the weaker trait patterns in artificially selected crops and varieties vs. wild plants (Martin et al., 2017; Milla et al., 2015). Similar to other crops, cacao clones showed a decoupling of morphological, chemical, and physiological traits (Fig. 4). Across all plants in our study, cacao trait space overlapped for all cultivars (Fig. 5a). Different patterns could nonetheless be observed between clones: for example, PMCT-58 showed high disconnection between leaf N and all the other traits, with two distinct hypervolumes notable at the “harsh dry season” site (Fig. 5c).

A notable exception however was ICS-95, the oldest cultivar. This low-yielding cultivar expressed the lowest trait plasticity among cultivars and between sites (1.4 to 13.4 times smaller than the trait space in other clones, Table 2), a finding that differs from previous work showing that environmental variability drives larger trait hypervolumes, as compared to variety identity (Isaac and Martin, 2019). This may suggest that the geographic applicability of the oldest cultivars may be limited by their lower adaptability/variability in resource acquisition strategies, despite their overall favorable disease resistance. Trait hypervolume estimates were also influenced by the environmental conditions. Strong coordination of WUE and A_{sat} were detected, especially at the “mild dry season” site (Fig. 5b), highlighting the importance of breeding clones with

Table 2 Hypervolume values from Fig. 5 for all sites, the “mild dry season” site, and the “harsh dry season” site

Clone identity	All sites	“Mild dry season” site	“Harsh dry season” site
PMCT-58	40,057	9922	10,521
ICS-95	6265	2763	3651
CC-137	35,902	36,950	4136
CATIE-R1	21,000	31,906	2458
CATIE-R4	21,334	11,433	9762
CATIE-R6	11,037	10,579	3796

high WUE, even in favorable climatic conditions. This trait coordination was especially high for PMCT-58 and CATIE-R1 (Fig. 5b), suggesting that trait hypervolume analyses within a specific trait category may be more revealing of clonal adaptability, as compared to univariate traits analysis. Our study therefore shows for the first time that evaluating ITV in the context of $G \times E$ can be a viable tool for assessing clonal adaptability to local conditions such as drought.

4 Conclusion

Our approach and findings underline several promising avenues for optimizing cacao cultivar selection in contrasting environments. First, we found that cacao leaf physiological and chemical functional trait variation could be indicative of local environmental constraints, as has been shown for other domesticated (Martin et al., 2017) and wild species (Siefert et al., 2015). Further, environmental conditions highly constrain phenotypic trait expression, since clones differed primarily in their physiological traits at one site (likely due to water limitations), while clones mainly differed in their chemical traits at the other site (likely due to nutrient limitations). These significant $G \times E$ interactive effects on cacao functional trait expression provide new opportunities for selecting clones while providing insight into the consequences of these decisions for agroecosystem functions. Our work here also highlights the need to test new cultivar adaptation in sites of contrasting environmental constraints. For instance, we suggest that morphological traits, namely, SLA, appear well suited to indicate clone viability across different soil conditions. Our results also confirm that physiological traits such as WUE are more appropriate indicators in water-limited sites, as shown previously in single-site studies (Araque et al., 2012; Tezara et al., 2020).

Clonal ITV can be linked with the productivity potential of individual cultivars. In this study, the least productive clones, PMCT-58 and ICS-95, presented the lowest within-site trait variation, yet the highest trait changes between sites. The underlying processes driving trait plasticity and coordination between clones require further evaluation. Low trait space of ICS-95 indicated low within-site ITV, while for PMCT-58, this was mainly attributed to a pronounced decoupling of leaf N from other traits in the face of environmental constraints. Whether functional trait hypervolume analyses may be more revealing of certain clones' adaptability than univariate traits analysis needs to be tested across a wider range of environmental conditions. We highlight the role that functional traits and quantification of ITV can play, in assessing clonal adaptability and suitability to local and regional environmental conditions in cacao agroforestry landscapes. To our knowledge, our paper is among the first to show evidence that functional traits provide new insights into genotype \times environment

interactions and clonal selection in agroforestry systems and support in favor of applying functional trait-based research to plant breeding paradigms.

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Code availability The code used to analyze the datasets is available in the Dryad repository, <https://doi.org/10.5061/dryad.zkh18939d>.

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Declarations

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