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ORIGINAL ARTICLE

Selecting cacao clones with high productivity potential and tolerance to Black Pod Rot (*Phytophthora* sp.) and Frosty Pod Rot (*Moniliophthora roreri*)

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Abstract

Cacao (*Theobroma cacao*) is significantly impacted each year by Frosty Pod Rot (FPR) caused by *Moniliophthora roreri* and Black Pod Rot (BPR) caused by *Phytophthora* species. The losses from these diseases pose a severe threat to cacao production worldwide. Consequently, cacao breeding programs focus on developing new clones that demonstrate high productivity potential and disease resistance. However, achieving this goal is challenging due to the lengthy selection process, the influence of environmental conditions on disease severity, and the need to avoid chemical control methods. Genetic resistance is, therefore, the most viable option for selecting and introducing new cacao clones to farmers. In this study, 40 cacao clones were evaluated from 2013 to 2017, with 20 clones sourced from the "Centro Agronómico Tropical de Investigación y Enseñanza" (CATIE) breeding program and 20 from the Fundación Hondureña de Investigación Agrícola (FHIA) breeding program. Three criteria were employed for clone selection: yield, percentage of diseased pods (PDP), and disease and production index (DPI). The results indicated that, depending on the objectives of the breeding program, these criteria can effectively be used to select new cacao clones that are both highly productive and disease-resistant. It was noted that cacao clones with high productivity are not always the most resistant to diseases, and vice versa. However, by combining these criteria, it is possible to identify cacao clones exhibiting high productivity potential and resistance to FPR and BPR.

Keywords: BPR-FPR, CATIE, disease and production index (DPI), FHIA, selection, yield

Introduction

Cacao production extends throughout the world and is threatened by pests and diseases; approximately one-third of global production is lost annually (Marelli *et al*. 2019). Four diseases account for the most significant losses worldwide: Black Pod Rot (BPR), caused by four *Phytophthora* spp.: witches broom (WB), caused by *Moniliophthora perniciosa*, cacao swollen shoot virus (CSV), caused by a member of the genus Badnavirus, and frosty pod rot (FPR), caused by *Moniliophthora roreri*. Some of the causal agents are globally distributed, but others have geographically restricted distribution (Gutiérrez *et al.* 2016; Marelli *et al.* 2019).

In Central America, more than half of the cacao production currently occurs in isolated rural areas on small-scale subsistence farms of fewer than 5 hectares. Consequently, the crop is seriously affected by the impact of diseases and the low-yielding potential of most plantations due to self and cross-incompatibility issues, pests, and diseases, as well as agronomical management. FPR and BPR are the two major diseases affecting cacao production, causing 30–100% yield losses (Phillips-Mora *et al.* 2006; Thevenin *et al.* 2012).

FPR disease was first officially reported in 1917 in Ecuador (Rorer 1918). The fungus was formally named when this specimen was sent to R. Ciferri who 'confirmed' it as a new species of Monilia, *Monilia roreri* Cif. (Ciferri and Parodi 1933). The disease is present in 13 countries in Latin America, including all countries of Central America (Sánchez-Mora *et al.* 2015). *M. roreri* only affects pods, with young pods 2 to 3 months old being the most susceptible and dependent on climatic conditions (Sánchez and González 1989). Farmers recognize *M. roreri* primarily by external symptoms on the fruits of cacao, especially by the appearance of signs of the pathogen, such as white mycelium, an ashen appearance, or the complete sporulation of the pathogen on the affected fruit tissue (Fig. 1A) (Phillips-Mora and Wilkinson 2007). Although the origin of the pathogen remains unknown, recent findings with the help of molecular tools confirm that it was initially introduced to the coastal zone of Ecuador and the Magdalena Valley region in Colombia, which were areas of intensive production of the crop (Díaz-Valderrama *et al.* 2022).

In the Central American region, *M. roreri* was first reported in Panamá in 1956. After that, it was successively detected in Costa Rica in 1978, Nicaragua in 1980, Honduras in 1997, Guatemala in 2002, Belize in 2004, and Mexico in 2005 (Phillips-Mora *et al.* 2006; 2012). As the disease was reported in each country, cacao production decreased considerably. For example, the total cacao production in Honduras in 1997 was approximately 5,500 tons; 5 years later, *M. roreri* arrived and the total cacao production decreased to 2,200 tons until it was reduced to 1000 tons in 2011 (FHIA 2012). The impact on cacao production in other Central American countries was like Honduras, especially because cacao producers were unaware of disease management and the genetic material used was not resistant to the disease.

Four species of *Phytophthora* cause BPR disease (*P. palmivora, P. megakarya, P. capcisi, and P. citrophtora*). *P. palmivora* is the most common (Drenth and Guest 2004) and it is present in all cacao-producing areas (Ndubuaku and Asogwa 2006). In Central America, BPR is caused mainly by *Phytophthora palmivora* (Ploetz 2007) and spreads rapidly, covering the entire pod surface 2 weeks after infection. The disease primarily affects pods (Fig. 1B) but it can also be observed in any part of the cacao plants. BPR is visually described as small, hard, dark lesions (Phillips-Mora and Cerda 2009). According to Marelli *et al.* (2019), BPR is responsible for losses of 873,000 tons of cacao per year worldwide, being the most harmful disease compared to other diseases affecting cacao production. Wet conditions like rainfall seasons, high relative humidity, and low temperatures are ideal allies of the disease (Dakwa 1973). BPR is present in all Central American countries, and the incidence is higher when the fruit development stage coincides with ideal humidity and temperature conditions for the disease's development.

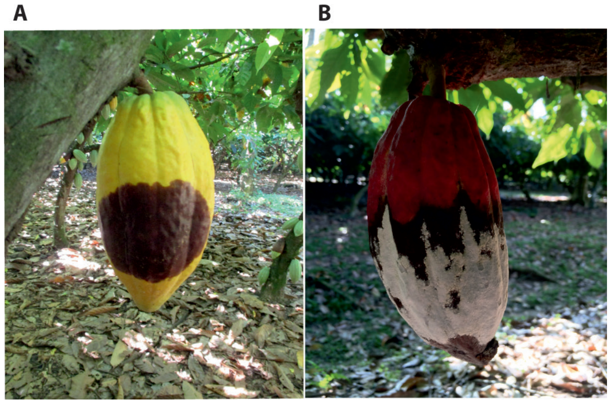

Fig. 1. *Phytophthora* – A and *Monilia* –B disease in cacao pods

One of the most critical challenges for a cacao breeding program is the evaluation time to select the desired traits in a new cacao clone. Generally, this evaluation is focused on yield and disease resistance and can take more than 15 years (Phillips-Mora *et al.* 2012). The selection of cacao clones is made by measuring the percentage of diseased fruits due to the natural incidence of the disease. However, this method does not differentiate between clones with low or high production. This is in contrast to the selection of cacao clones based on the production potential. Furthermore, the potential production method does not consider the disease management cost, which is critical when analyzing economic profitability. A third selection method could be using a subjective index that combines yield and disease resistance, identifying cacao clones with high productivity and low disease incidence(Jaimez *et al.* 2020).

The cacao breeding programs of Centro Agronómico Tropical de Investigación y Enzeñanza (CATIE) in Costa Rica and Fundación Hondureña de Investigación Agrícola (FHIA) in Honduras have developed new cacao clones with high resistance to FPR and BPR (Phillips-Mora 2015; López *et al.* 2017). These programs have validated the combination of high yield and disease resistance using artificial methods. Posteriorly, clones were evaluated for disease incidence and severity under field conditions with the natural pressure of the inoculum.

This study aimed to compare three methods using yield, percentage diseased pods, and disease production index as criteria to select cacao clones with high potential for production and tolerance to FPR and BPR. Forty cacao clones were evaluated, 20 from CATIE and 20 from FHIA. The cacao clones evaluated were part of the project "Programa Cacao Centro América" (PCC) developed between 2009 and 2015 by CATIE, and selected from the CATIE and FHIA breeding program for cacao growers in Central America (Table 1).

Material and Methods

Site, experimental design, and germplasm

Of the 40 clones used, 20 were part of the FHIA's Cacao and Agroforestry Program, and 20 were from the Cacao Breeding Program of CATIE (Table 1). The clones were planted in sandy, loamy soil (pH = 5.3) with low fertility (2.67% of organic matter) and high iron content at the Experimental and Demonstration Center-Jesus Alfonso Sanchez (CEDEC-JAS) in La Masica, department of Atlántida (15°38'42.84"N, 87°6'0.46"W, 25 m.a.s.l.) in northern Honduras from 2013 to 2017. Weather conditions from 1986 to 2019 were recorded. The annual mean temperature was 25.6°C with 2,938.1 mm annual rainfall (Díaz *et al.* 2020). In Figure 2, the temperature and rainfall conditions between 2013 and 2017 are shown.

One-year-old grafted cacao clones were planted in a square system of 3.5 m × 3.5 m, arranged in a complete randomized block design with four replicates and six plants per replicate. They were planted in agroforestry systems in association with different tropical wood species, mostly *Swietenia macrophylla, Cordia megaliths, Terminalia superba, Tabebuia rosea, Guarea grandifolia,* and *Ilex tectonic,* as permanent shade trees with no irrigation.

Mineral fertilizer was applied yearly: 136 kg of N-P-K (15-15-15), 45.4 kg of ammonium nitrate, and 45.4 kg of potassium chloride and lime amendments at a 0.5 tons/ha dose. Weather data on temperature, humidity, and precipitation were collected daily and reported as a monthly average.

In the FHIA cacao and agroforestry program, each cacao clone was assessed for yield and disease incidence of FPR and BPR.

Variables evaluated

The diseases and yield variables were evaluated 2 years after planting and continued for 5 years, relying solely on the natural pressure of both diseases without any experimental pathogen inoculation. Pod evaluations were conducted manually in each weekly harvest. Gender identification of both pathogens was done in the Plant Protection Department of FHIA.

Yield variables

Yield was recorded as kg/ha of dry beans and measured by the pod index (number of pods for 1 kg of dry cacao bean) (IPGRI 2000) and the bean index (average weight of 100 dry cacao beans) (IPGRI 2000). The mature pods were harvested and selected weekly following the commercial production process for the sale of dried cocoa, that is: harvesting, fermentation, and drying.

Diseases variables

The percentage of disease pod (PDP) was calculated as PDP = [NDP/(NHP+NDP)] × 100, where NDP = annual number of diseased pods (calculated for BPR, FPR, and the two diseases together), NHP = annual number of healthy pods (Jaimez *et al.* 2020).

Index variables

The disease and production index (DPI) was calculated as follows: DPI = [(NHP+NDP)/DPC] × 0.1, calculated separately for BPR, FPR, and two diseases together. DPC = diseased pods coefficient. The DPC was calculated using the formula DPC = (NDP+1)/(NHP+1) (Jaimez *et al.* 2020). The DPI considers the effect of FPR, BPR, and FPR + BPR separately. A high DPI value is associated with the best cacao clones.

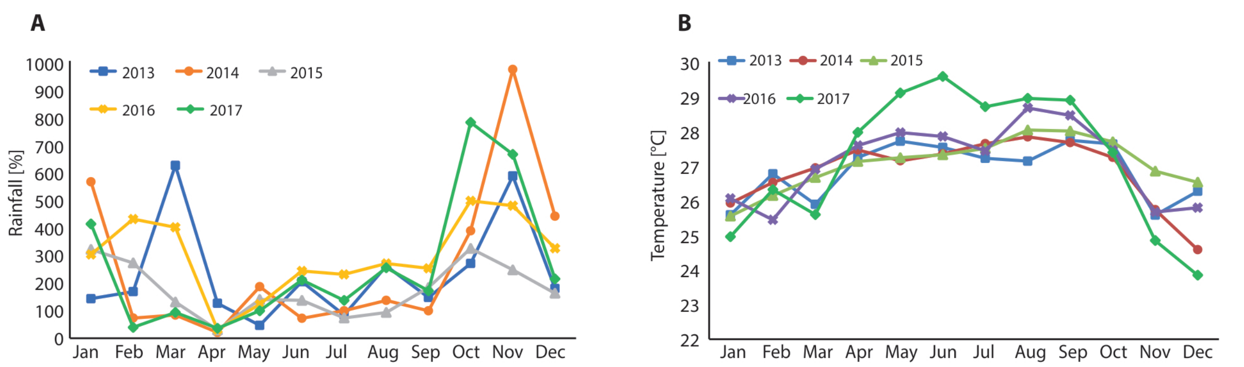

Fig. 2. Rainfall – A, and temperature – B, from 2013 to 2017

Table 1. Pedigree and origin of the selected cacao clones

Group	Source		Clone	Pedigree	
	organization	country		female	male
FHIA	FHIA´s Cacao and Agroforestry Program	Honduras	FHIA-70	ARF-22	ICS-43
			FHIA-168	PA-169	P-23
			FHIA-245	PA-169	CC-252
			FHIA-269	UF-273	PA-169
			FHIA-330	UF-273	P-23
			FHIA-485	ARF-22	UF-273
			FHIA-577	PA-169	CC-137
			FHIA-580	UF-272	P-23
			FHIA-662	ARF-22	UF-273
			FHIA-707	UF-273	PA-169
			FHIA-708	PA-169	CC-137
			FHIA-719	UF-712	CC-137
			FHIA-738	UF-712	PA-169
			Caucasia-37	unknown	unknown
			Caucasia-39	unknown	unknown
			Caucasia-43	unknown	unknown
			Caucasia-47	unknown	unknown
			FSC-A2	unknown	unknown
			CCN-51	(ICS-95 x IMC-67)	Oriente 1
			IA-RO	unknown	unknown
CATIE	CATIE´s Cacao Breeding Program	Costa Rica	CR-07	UF-712	CATIE-1000
			CR-09	UF-273	CC-137
			CR-10	UF-273	CC-137
			CR-12	UF-273	CC-137
			CR-20	UF-273	Tree-81
			CR-22	UF-273	Tree-81
			CR-26	UF-712	CC-137
			CR-27	UF-712	CC-137
			CR-29	UF-712	CC-137
			CR-31	UF-712	CC-137
			CR-32	UF-712	CC-137
			CR-38	UF-712	Tree-81
			CR-47	ICS-95	UF-273
			CR-48	ICS-95	UF-712
			CR-49	ICS-95	Pound-7
			CR-66	SCA-6	UF-712
			CR-72	PA-169	ARF-6
			CR-81	UF-712	ARF-37
			CR-82	UF-712	ARF-37
			CR-85	UF-712	ARF-37

FHIA in the table 1 is equal to F in the figures
Caucasia in the table 1 is equal to CAU in the figures

Statistical analysis

Data analysis was performed using the InfoStat software (Di Rienzo *et al.* 2020). The statistical difference was determined using the one-way ANOVA method followed by the Scott-Knott test method for grouping means of cacao clones. As many treatments were analyzed, it was important to clearly separate the differences between them, avoiding similar interpretation for statistical differences between treatments (Jaimez *et al.* 2020). The results were expressed as the mean ± standard error (SE). In addition, Spearman correlation and principal components analysis (PCA) were carried out for yield, PDP, and DPI variables using R statistical software (R core team 2019) through the Corrplot package (Wei *et al.* 2021). For correlation analysis, Factoxtra (Kassambra and Mundt 2020) and ggplot2 (Vu 2020) for biplot of principal component analysis were used.

Results

Yield

All cacao clones evaluated individually showed yields above 500 kg/ha. The Scott-Knott analysis showed that the yield variable formed three different groups (Fig. 3). Seven cacao clones were the most productive (CR-12, CR-32, CR-07, CR-26, CR-38, CR-27, and CR-72), 13 cacao clones were clustered in the second group, and 20 cacao clones in the last group. Boxplot showed that the yield average of all cacao clones was very similar throughout the 2013–2017 period (Fig. 4A), with a decreased yield in 2014. Furthermore, the results showed that bean and pod indexes were negatively correlated. When the bean index increased, the pod index decreased (Fig. 4B). The correlation between bean and pod indexes is essential for the final selection of cacao clones since it is directly associated with the dry cacao bean yield per hectare.

Percentage Diseased Pods

The incidence of BPR and FPR observed was dissimilar in the evaluation period. In general, there was a higher incidence of BPR than FPR. The num-

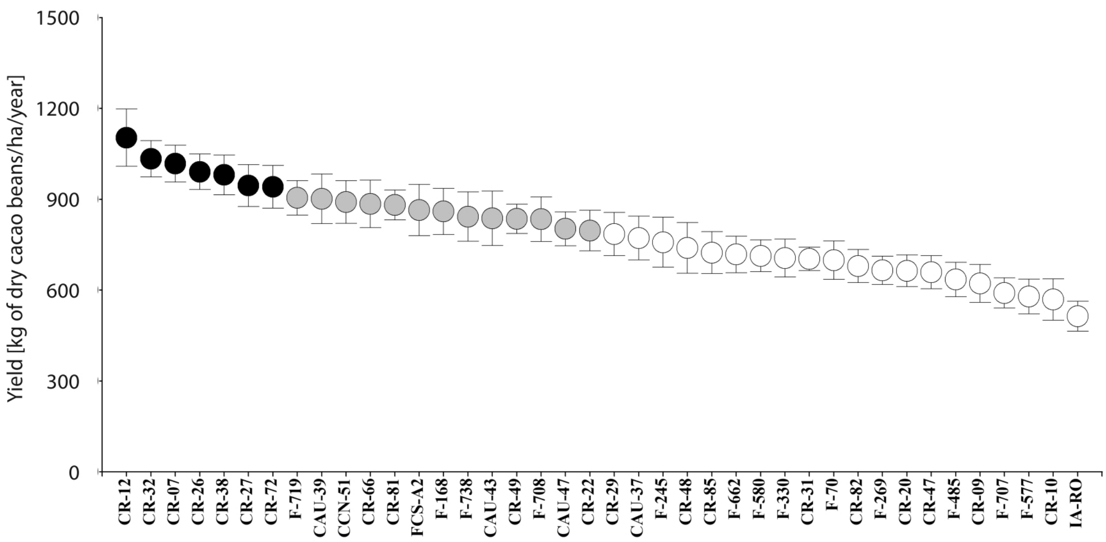

Fig. 3. The average yield of dry cacao beans in kilograms per hectare (2013–2017) of 40 cacao clones according to the Scott-Knott cluster test ($p < 0.05$), different colors (black, gray, and white) represent statistical differences between groups. Data are means ± 95% Standard Error of the mean

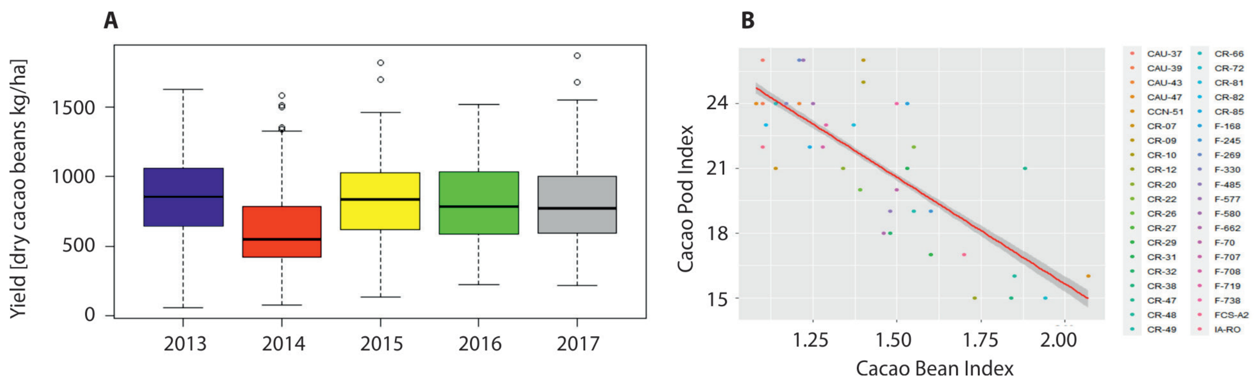

Fig. 4. Yield components. Boxplot representing total cacao yield per year – A, the relation between Pod Index and Bean Index – B

ber of diseased pods with BPR increased from 2013 to 2017 (Fig. 5A). The over-the-year averages of BPR ranked from 20 to 55% of disease incidence. Therefore, the clones could be statistically separated into two groups (Fig. 6). The first group had a range of PDP between 33.34 and 52.94% (black points), and the second group had a range of PDP from 16.64 to 31.22% (white points). The incidence of FPR remained steady throughout the years (Fig. 5B), maintaining the incidence of FPR disease lower than 5% during all years without differences between cacao clones (Fig. 7). Thus, there was a marked difference in the PDP. Furthermore, more incidences of BPR were observed when environmental conditions such as rain and temperature were high in the last quarter of the year, which is typical in tropical regions.

Disease and Production Index

The Scott-Knott test showed that the DPI for FPR was divided into two groups ($p < 0.05$). In the first group, 20 cacao clones were observed, which were less affected by FPR, whereas in the second group, the cacao clones had more incidences of the disease (Fig. 8A).

The DPI for BPR was divided into two groups ($p < 0.05$). The first group of cacao clones, F-738, CR-66, CR-07, CAU-43, CAU-47, and F-330, distinguished among the cacao group clones, had the lowest incidence of BPR (Fig. 8B). When the DPI was calculated considering the incidence of the two diseases (FPR and BPR), the cacao clones aggregated statistically into two groups($p < 0.05$). The highest DPI values were observed in the clones F-738, CAU-43, CAU-47, F-330 CR-66, and CR-07 (Fig. 8C), compared to the rest of the cultivars.

Correlation and PCA analysis

Correlation and PCA analysis were performed between production variables (pod index, bean index, and yield), disease variables (PDP, TDP, THP), and

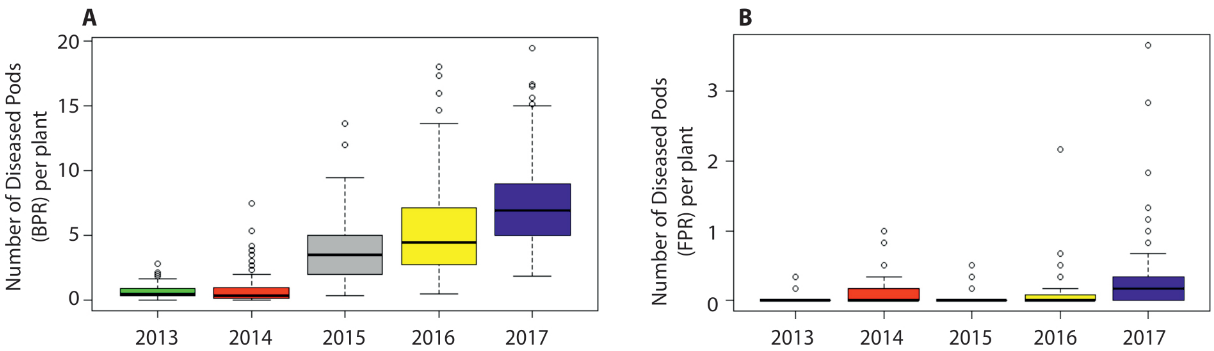

Fig. 5. Diseased pods per plant between 2013 and 2017. Black Pod Rot (BPR) – A, and Frosty Pod Rot (FPR) – B

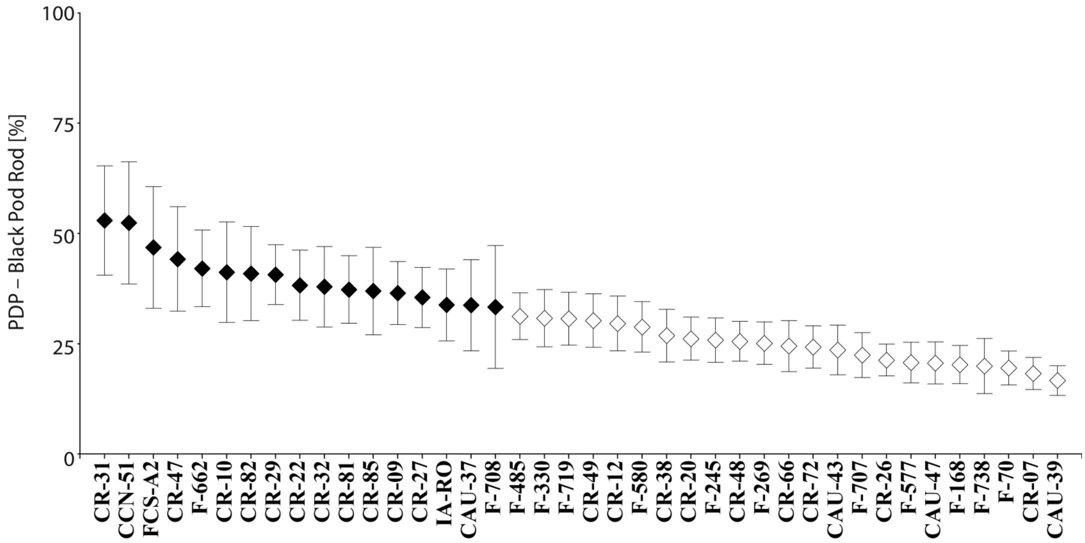

Fig. 6. The percentage of pods affected by BPR, different colors (white and black) in the boxplot shows statistical differences according to the Scott-Knott test ($p < 0.05$). Data are means ± 95% Standard Error of the mean

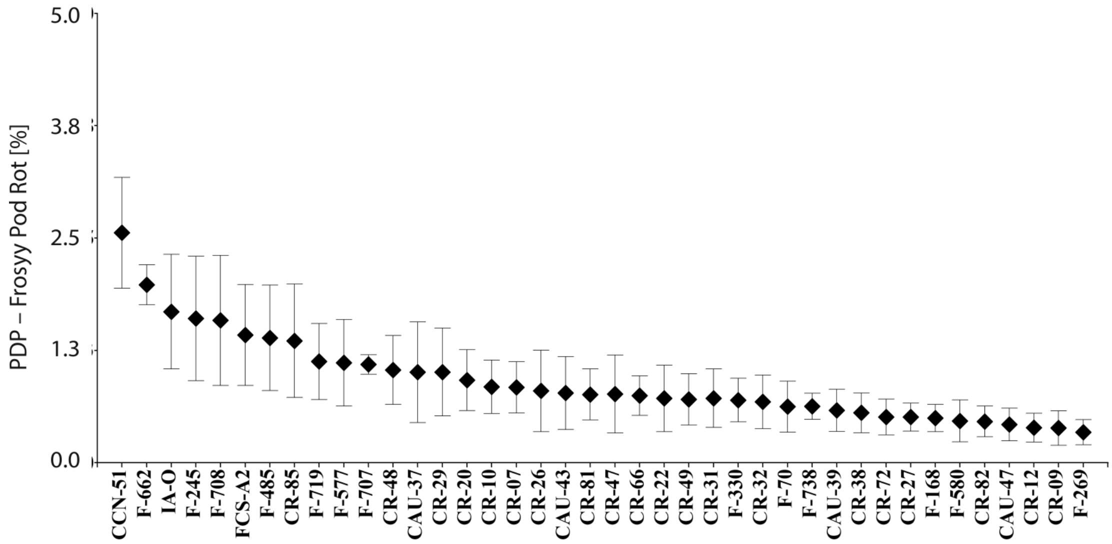

Fig. 7. According to the Scott-Knott test, the percentage of pods affected by FPR does not show statistical differences between cacao clones ($p < 0.05$). Data are means ± 95% Standard Error of the mean

disease and production index variables [(DPI-FPR, DPI-BPR, and DPI(FPR+BPR)].

Figure 9A shows different results of the correlation analysis. For the yield variables, a positive correlation between yield and THP (R = 0.92) was present, and there was a high negative correlation between the seed index and the pod index (R = –0.71). In the disease variables, various positive and negative correlations were found. As expected, THP correlated positively with yield (R = 0.92). It also correlated positively with disease and production index (DPI-(FPR+BPR) (R = 0.51), DPI-FPR (R = 0.85), and DPI-BPR (R = 0.50). The variable TDP correlated positively with the variable PDP (R = 0.94). However, a negative correlation was observed with the variables of TDP with DPI-(FPR + BPR) (R = –0.69) and with DPI-BPR (R = –0.7). The yield variable positively correlated with DPI-FPR (R = 0.78), possibly due to the low incidence of FPR disease observed during the investigation. The variable PDP showed a high negative correlation with the variables DPI (FPR+BPR) (R = –0.88) and DPI-BPR (R = –0.89). Finally, a positive correlation between DPI-(FPR+BPR) and DPI-BPR (R = 1) is shown. Using the PCA analysis, the first two dimensions explained 62.65% of the overall variation (Fig. 9B). The variation related to the first component was primarily associated with yield (THP, yield, and DPI-FPR) and DPI [(DPI-BPR and DPI-(FPR+BPR)] variables. The second component was associated with disease variables (PDP and TDP). It was observed that the DPI variables were more associated with disease than yield variables.

Discussion

Genetic improvement for disease-resistant cacao clones with high yield

Presenting a new cacao clone to growers with high productivity and resistance to pests and diseases is the dream of a cacao breeder. This task is never easy to achieve due to the lengthy selection process and time that this activity entails. Pests and diseases can destroy 20–30% or more of total cacao production, and high yield and disease resistance have received the most attention from breeders (Lopes *et al.* 2011; Gutiérrez *et al.* 2016a). The primary strategy of cacao genetic improvement programs is based on recurrent selection using parental trees with high yield and resistance to pests and diseases. Recurrent selections in cacao breeding programs are continuing with a broader level of diversity and are aimed at accumulating favorable alleles for yield and resistance to the four diseases genetic improvement (Pimenta *et al.* 2018). The choice of selecting desirable parents for cacao breeding traditionally depended on the availability and characterization of germplasm. However, polygenic traits significantly influenced by the environment are more challenging to measure without a tool to identify the significant genes influencing the phenotype (Bekele and Phillips-Mora 2019). Currently, with molecular biology tools, it is possible to identify molecular markers associated with resistance genes FPR and BPR and the characterization of germplasm banks that could be used for genetic improvement in the future (Legavre

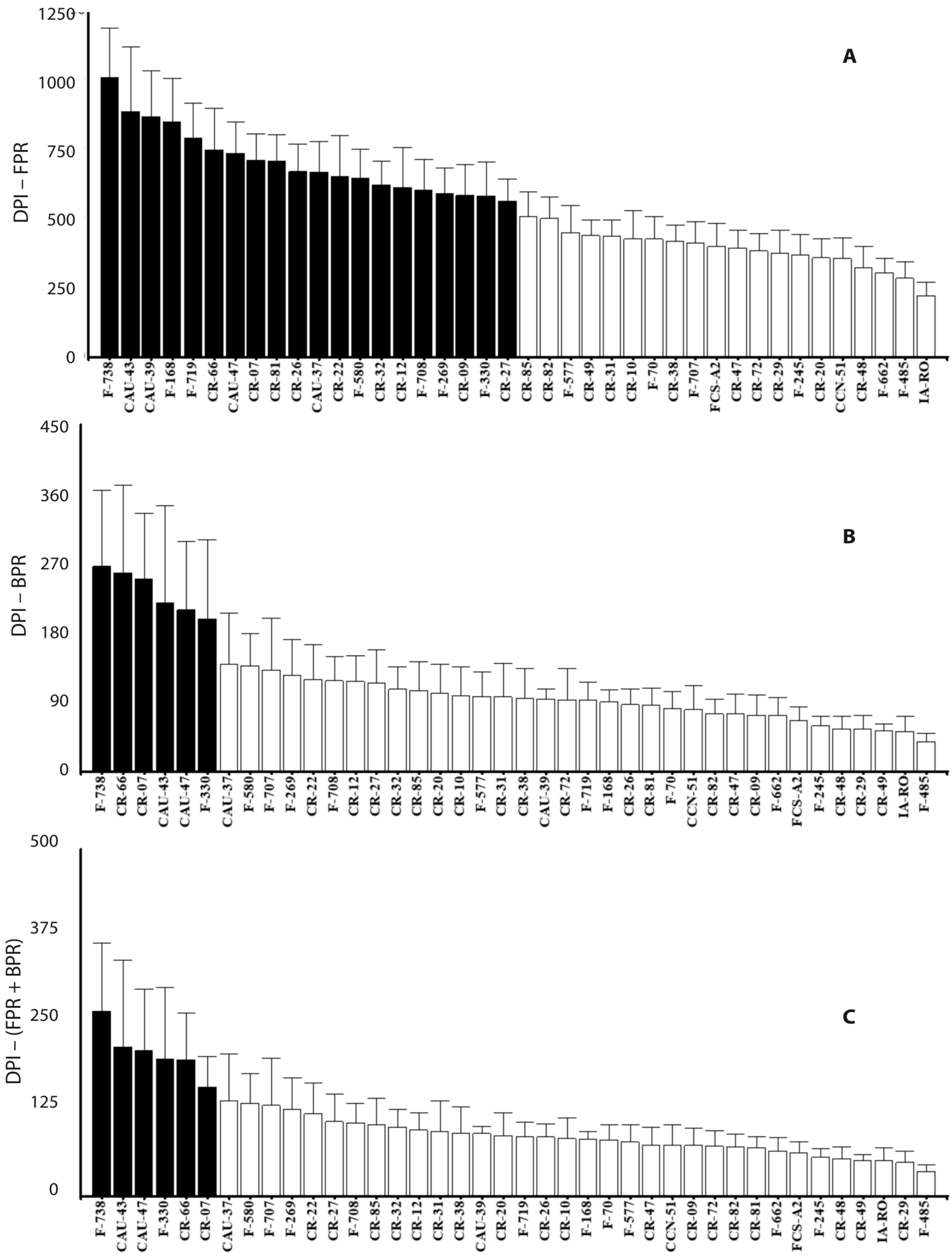

Fig. 8. Disease and Production Index Pod (DPI). FPR– A, BPR – B, and FPR + BPR – C. Different colors (black and white) represent statistical differences between groups ($p > 0.05$) according to the Scott-Knott test

et al. 2015; Osorio-Guarin *et al.* 2020; Rodríguez-Polanco *et al.* 2020).

The two oldest and most extensive collections of cacao germplasms are in Trinidad and Tobago (CRU/UWI) and Costa Rica at CATIE. Both are used as a source of germplasm for genetic improvement (Monteiro *et al.* 2009; Laliberté *et al.* 2012). At the same time, other more recent germplasm collections, such as FHIA in Honduras, have a core of genetic material as a source of specific characteristics of resistance to diseases and yield (Somarriba and Villalobos-Rodriguez 2013; Somarriba *et al.* 2013). Therefore, 40 cacao clones from the two genetic improvement programs (FHIA and CATIE) were evaluated in the present

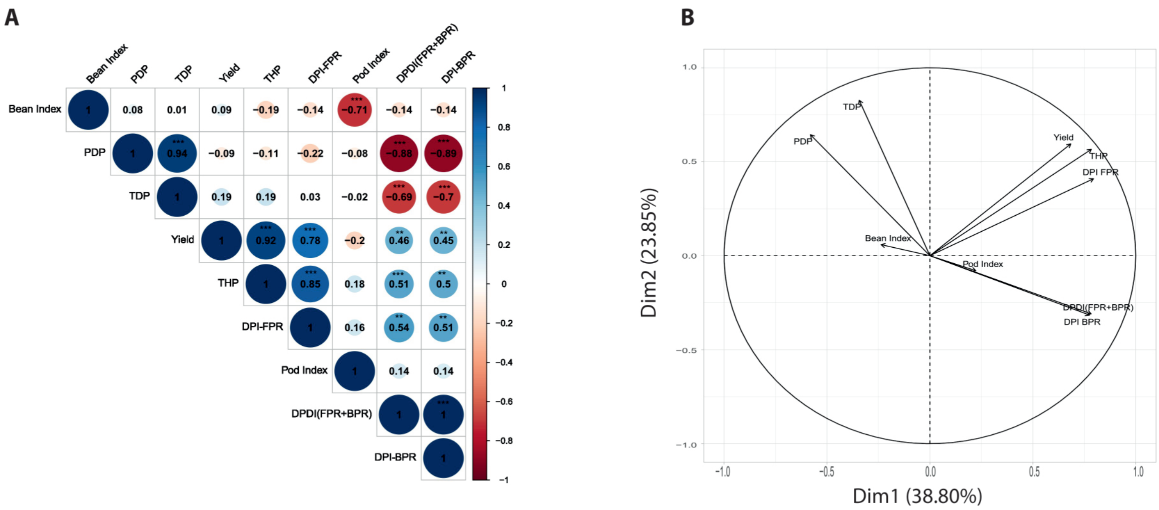

Fig. 9. Relation between Disease (PDP, TDP, and THP), Yield (Yield, Bean Index, and Pod Index), and DPI [(DPI-FPR, DPI-BPR, and DPI-(FPR+BPR)] variables. Correlation Analysis – A, Principal Components Analysis – B

study. This study aimed to select the best clones with resistance to BPR, FPR, and high productivity as an initiative to transfer the best clones to Central American growers.

The evaluated clones showed more resistance to FPR than BPR based on natural incidence. The reason may be that when FPR arrived in Central America about 20 years ago, its appearance was so devastating that genetic improvement programs focused their efforts on selecting cacao clones with resistance to FPR, and genetic improvement for resistance to BPR was neglected (FHIA 2012; Phillips-Mora *et al.* 2005, 2006, 2012).

In contrast, while resistance to BPR has been identified in several germplasm accessions, resistance to FPR is relatively uncommon. In an evaluation of 70 new cacao clones, only two clones (3%) were characterized as moderately resistant (MR) (Phillips-Mora and Castillo 1999). Furthermore, based on their screening results, only 10 (2.3%) out of 441 clones (56%) of the CATIE collection were identified as resistant or moderately resistant to FPR. Phillips-Mora *et al.* (2017) stated that out of 1260 clones from the CATIE collection, 76 (6%) showed tolerance to FPR. On the other hand, using molecular tools, Gutiérrez *et al.* (2021) found and reconfirmed QTLs associated with FPR and BPR resistance and the expression of genes related to plant defense and disease resistance.

There are several challenges for cacao breeders, among them low productivity, higher pressure of pests and disease due to climate change, small production units, high production costs, and above all, maintaining the quality of the final product so that consumers can be satisfied (Bekele and Phillips-Mora 2019). Cacao breeding programs should design strategies that include all the possible variables, considering the long process of improving a new cacao cultivar. The main objective of a developmental program is to create new cacao cultivars with high yield and disease resistance. However, there are subdivisions within each of these desirable features, as they all come together to achieve the objective. For example, among other yield components, a new cultivar should have cacao fermented seeds > 1 g, a low pod index, a high number of seeds per pod, and high butterfat content (Soria and Enriquez 1977). Other selection criteria include vigor, self-compatibility, uniform plant type, compactness in tree size, precocity (early flowering and maturing) tolerance to drought stress, and quality expressed in terms of bean flavor, purity, and food safety (Ahnert 2009; Bekele and Phillips-Mora 2019). The second important objective is genetic disease resistance because this represents the most serious biological constraint in cacao production (Gutiérrez *et al.* 2016b).

Relation between yield, Percentage Diseased Pods, and Disease Pod Index

Cacao production and its components should be appropriately appraised since they are considered polygenic characteristics and, therefore, highly influenced by environmental factors (Monteiro *et al.* 2009). Consequently, yield continues to be the main objective in a cacao production unit. Growers, therefore, use all the technologies available to achieve higher yields, including germplasm, fertile soils, planting distance, fertilization, irrigation, and self-compatibility to make the crop profitable. However, yield can be reduced without effective pest and disease control measures. Statistical analysis showed that seven cacao clones are the most productive (CR-12, CR-32, CR-07, CR-26, CR-38, CR-27, and CR-72). The results indicate that the environmental factor significantly drives the yield. Cacao clones evaluated in Costa Rica by CATIE had higher yields than those evaluated in Honduras by FHIA (Arciniegas-Leal 2005). High yield is a characteristic that could be inherited from the parent of the clone (CC-137 is characterized as high yielding, with a low incidence of diseases, low index pod, and long grain) since five of these clones have the clone CC-137 as a mother parent (Arciniegas-Leal 2005). The yield from CR-12, CR-32, CR-07, CR-26, CR-38, CR-27, and CR-72 (950–1150 kg · ha^{-1}) is acceptable considering that the global average is between 300-400 kg/ha. Similar results were found in other studies evaluating elite cacao clones under field disease pressure for at least 4 years (Sánchez-Mora *et al.* 2015; Solis *et al.* 2015; Ofori *et al.* 2019).

Another way to quantify cacao production is to consider the pod number affected by diseases and their effect on the total production. In this study, all the cacao clones used showed a high level of genetic resistance to FPR, and there were no differences between the clones. However, a high percentage of pods was affected by BPR, and within the group of clones evaluated, two groups with different infection levels were observed. Since BPR is a disease in all cacao-producing areas of the world (Ploetz 2007), it is unsurprising that BPR causes more damage during some humid seasons than FPR (Phillips-Mora and Cerda 2009). However, in this case, the clones had higher genetic resistance to FPR than BPR.

Since chemical control is not commonly used in managing FPR and BPR, selecting genetically resistant cacao clones is the most effective strategy. It also helps to avoid environmental contamination by reducing the use of pesticides. Although more resistant clones to FPR have been found (Torres *et al.* 2011; Phillips--Mora *et al.* 2012), it is still a global challenge to develop cacao clones with resistance to different BPR strains (McMahon *et al.* 2018; Fister *et al.* 2020; Decloquement *et al.* 2021)

Yield and percentage of diseased pods can be used as criteria for cacao clone selection between genotypes with high and low production potential (Jaimez *et al.* 2020). In this sense, it can be the most valuable as a method that includes both criteria for yield and percentage of diseased pods as an index for cacao clone selection. Some studies have been carried out to select new cacao clones using both criteria: yield and percentage of diseased pods (Nyassé *et al.* 2003; Cervantes--Martinez *et al.* 2006; Efombagn *et al.* 2007, 2011; Ofori and Padi 2020). On the other hand, this criteria combination has been used in other species such as *Zea mays* (Horne *et al.* 2016), *Saccharum officinarum* (Magarey *et al.* 2003), *Arachis hypogaea* L. (Iroume and Knauft 1987), *Cicer arietinum* (Toker and Çanci 2003), and *Capsicum annuum* L. (Sreenivas *et al.* 2020).

Jaimez *et al* (2020) developed a disease and production index to select cacao clones that are highly productive and tolerant to pod rot diseases. When this index was evaluated for cacao clone classification, two groups were formed, especially when yield, BPR, and FPR were included. This index is valid for breeders and growers because it balances cacao crops' production potential and disease resistance. The cacao clones that combine the best yield and disease resistance have the highest index value.

Correlation analysis, PCA, and final selection

Finally, a correlation analysis between production potential and disease variables was carried out. The analysis results are consistent with those shown in selecting cacao clones by yield, percentage of diseased pods, and disease pod index.

As shown in the PCA analysis, the pod index variable negatively correlated with the seed index, an important characteristic to use as a criterion for selecting new cacao clones. The variables yield, THP, and TDP were grouped as the main factors contributing to cacao yield performance. In this group, the disease and production index for FPR (DPI-FPR) correlated due to the low incidence of FPR in this study. On the other hand, DPI-BPR and DPI-(FPR+BPR) were on the opposite side of PDP and TDP, suggesting that both indexes depend on PDP and TDP. PCA demonstrated how variables are grouped to explain yield and disease incidence for cacao selection.

In the correlation analysis, variables for cacao yield performance correlated positively, finding statistical correlations from 0.76 to 0.86, and index variables correlated negatively with disease variables showing statistical correlations from –0.69 to –0.89. Both analyses were complemented in their results. The final decision in the cacao clone's selection process must always be balanced, including yield and disease resistance components. The cacao clones that show the highest yield are not necessarily the most resistant to diseases because the genetic yield potential (Ofori and Padi 2020) is different from the accumulation of genes with the total or partial expression of resistance to pests and diseases and have environmental influence (Nyadanu *et al.* 2017).

Finally, after a lengthy selection period, the breeder should select the best cacao clones according to the objectives of his genetic improvement program. In conclusion, the top 10 cacao clones selected for this study, based on three criteria are presented: yield, diseases, and DPI, illustrating the process of final selection (Table 2). In the criteria for disease and DPI, there are subdivisions based on the specific disease on which

Table 2. The top 10 cacaos were selected using three criteria [Yield, Disease Percentage, and Disease and Production Index (DPI)]

No	Yield	Disease percentage			Disease and production index [dpi]		
		FPR	BPR	FPFR + BPR	DPI-FPR	DPI-BPR	DPI-[FPR + BPR]
1	CR-12	F-269	CAU-39	CAU-39	F-738	F-738	F-738
2	CR-32	CR-09	CR-07	F-738	CAU-43	CR-66	CAU-43
3	CR-07	CR-12	F-70	CR-07	CAU-39	CR-07	CAU-47
4	CR-26	CAU-47	F-738	F-70	F-168	CAU-43	F-330
5	CR-38	CR-82	F-168	CAU-47	F-719	CAU-47	CR-66
6	CR-27	F-580	CAU-47	F-168	CR-66	F-330	CR-07
7	CR-72	F-168	F-577	F-577	CAU-47	CAU-37	CAU-37
8	F-719	CR-27	CR-26	F-707	CR-07	F-580	F-580
9	CAU-39	CR-72	F-707	CAU-43	CR-81	F-707	F-707
10	CCN-51	CR-38	CAU-43	CR-26	CR-26	F-269	F-269

selection is to be focused. The cacao clones selected by yield criteria differ from those selected using the other two criteria because those cacao clones with high yield are not always associated with disease resistance. The final decision as to which method to use depends on the breeding program objectives and the environmental conditions in which the cacao clones selected will be planted. Environmental conditions highly influence disease incidence. However, in tropical areas, production coinciding with high humidity and low temperatures suggests using a disease percentage or DPI criteria.

Conclusions

Selecting new cacao clones with high yield and disease resistance presents challenges due to the influence of environmental conditions and the time required for the process. In this study, it was demonstrated that it is feasible to make the final selection of cacao clones using three specific criteria tailored to the goals of the breeding program. While the most common methods focus on yield or disease incidence, it was also found that utilizing a DPI, which combines both criteria, can effectively select new cacao clones for growers.

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