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Article

New cocoa varieties selected in Colombia

Genaro Andrés Agudelo-Castañeda[1]
Eduar Yovany Antolinez-Sandoval[2]
Eliana Yadira Báez-Daza[1]
Yeirme Yaneth Jaimes-Suárez[1]
Gladys Alejandra Romero-Guerrero[1§]

[1]Colombian Corporation for Agricultural Research-Agrosavia. La Suiza Research Center. Bucaramanga, Colombia. CP. 250047. [2]Colombian Corporation for Agricultural Research-Agrosavia. Tibaitatá Research Center, Cimpa headquarters, Barbosa, Colombia. CP. 250047.

[§]Corresponding author: gromerog@unal.edu.co.

Abstract

In recent years, the increasing cultivation of cocoa in Colombia has been consolidated into the country's agricultural economy, requiring further technological developments to improve productivity and profitability. Lack of genotypes and adapted and selected productive varieties is one of the causes for low yield. The aim of this study is the agronomic evaluation of eight cocoa genotypes TCS13, TCS19 (Theobroma Corpoica La Suiza), SCC53, SCC82, SCC83 (Selección Colombia Corpoica) and CCS 73, CCS77, CCS80 (Colección Corpoica La Suiza), compared to a commercial control ICS95 (Imperial Collage Selection), in four producing localities within the natural subregions of Montaña Santandereana and Magdalena Medio in northeastern Colombia, established in agroforestry systems with fine woods. The results identified two genotypes- TCS13 and TCS19- which were superior in yield (with production of 1.5 and 1.8 kg tree^{-1} year^{-1}, respectively), low incidence of limiting diseases such as Frosty Pod Rot, and self-compatible. These results allowed the inclusion in the national registry of commercial cultivars in the Colombian Agricultural Institute (ICA) in 2017.

Key words: sexual compatibility, tropical fine wood, yield components.

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Introduction

Cocoa (*Theobroma cacao*) is a *commodity* cultivation, originally from the Amazon region in South America. Currently, its cultivation extends in the humid tropical region worldwide, being West Africa the largest producer at 76%. In South America, Colombia is the fifth largest producer after Brazil and Ecuador (ICCO, 2021), with a production of 64 281 t of cocoa dry grain for year 2019-2020. At present, cocoa in Colombia is grown in four agroecological zones corresponding to Montaña Santandereana, tropical humid forest, dry inter-andean valleys and the low coffee marginal zone (Quintana *et al*., 2018).

In these areas there are differential conditions in altitude, climate, soil, and topography (Meena *et al*., 2019), offering states of greater and lesser aptitude for crop, demanding technological developments and a supply of varieties with general and specific adaptation. Montaña Santandereana is the agroecological zone with the largest cocoa production area in Colombia. It is located in the departments of Santander, Boyacá and Norte de Santander, in warm and temperate thermal floors, its altitude varies between 200 to 1 200 m, average annual precipitation between 1 500 and 2 500 mm, irradiance from between 1 800 and 2 100 µmol m^{-2} s^{-1} and average temperature between 25 and 30 °C (Agudelo *et al.*, 2018), it is a region of undulating topography and cocoa production is generally established in an agroforestry system.

The Magdalena Medio sub-region is another region of importance for cocoa cultivation, it is located in the inter-andean dry valleys (Quintana *et al*., 2018), characterized by annual rainfall between 1 800 and 2 300 mm, average temperature between 23 and 28 °C, altitude between 200 and 700 m above sea level, geographically, it is composed of an undulating relief with a smooth topography.

Cocoa cultivation has a long tradition. It represents great importance for the socioeconomic sustainability of the nation as well as for rural livelihoods, especially since it is a peasant economy crop, with 65 000 producer families in 189 000 ha of crop, which generates 167 000 direct and indirect jobs (García *et al.*, 2020). Currently, this is a key crop for substitution of illicit crops in a post-conflict context (Abbott *et al.*, 2018), and efforts are focused on increasing productivity and competitiveness.

Despite being an expanding crop, cocoa has low yields according to the productive potential due to various factors, including agroclimatic conditions, aging plantation, lack of high-yield varieties, increased pressure from diseases and pests (García *et al.*, 2014) and poor agronomic management (Vaast and Somarriba, 2014). Colombia is within the geographical origin of cocoa, so it holds a wide genetic diversity (Thomas *et al.*, 2012; Osorio *et al.*, 2017).

The use of highly productive genotypes is essential to increase cocoa yield, decrease the incidence of diseases and contribute to added value by increasing the quality of flavor and aroma. The objective of this study was to evaluate the agronomic performance of eight cocoa genotypes set, established in an agroforestry system with tropical fine woods.

Material and methods

Eight cocoa genotypes were evaluated, identified: (Theobroma Corpoica La Suiza) TCS) SCC (selección Colombia Corpoica) and CCS (colección Corpoica La Suiza) accompanied by a denomination number, as listed: TCS13, TCS19, SCC53, SCC82, SCC83, CCS73 CCS77, CCS80. They were evaluated in comparison to introduced commercial control ICS95 (imperial collage selection), selected for its acceptance by producers, its wide distribution and good agronomic performance throughout the country, as well as low incidence of frosty pod rot. The evaluation of the clones was carried out in the Andean natural region, in four localities, belonging to the subregions of Montaña Santandereana and Magdalena Medio (Table 1), located between 300 and 900 m altitude, during four years of production.

Table 1. Climate characteristics of the regions used for the agronomic and phytosanitary evaluation of cocoa clones.

Localities	Village	Latitude	Longitude	HR (%)	T° (°C)	Precipitation (mm)	Altitude (m)	Natural subregion
El Carmen	San Luis	06°47’8.2”	73°36’7.5”	75	28-32	1 500-1 800	300	Magdalena Medio
Muzo	Ejidos	05°32’26.7”	74°05’48.1”	80	22-25	1 800-2 200	900	Montaña Santandereana
Rionegro	Alto Bello	07°22’12.5”	73°10’49.6”	85	27-30	1 800-2 000	530	Montaña Santandereana
Santa Rosa	El Diamante	07°58’12.8”	74°03’49.2”	75	22-25	1 500-2 000	629	Magdalena Medio

In the four localities, the agronomic evaluation tests were established in agroforestry systems with tropical fine woods, under an experimental design of complete random blocks, with three permanent shade arrangements, Abarco (*Cariniana piryformis*), teak (*Tectona grandis*), rubber tree (*Hevea brasiliensis*) and plantain (*Musa* sp.) used as transitory shade. In the experimental design, nine treatments were evaluated corresponding to the nine cocoa genotypes, two repetitions per treatment and four effective plants.

The cocoa genotypes were established at density with trees spaced at 3 m x 3 m in triangle and distributed randomly in each plot. The permanent shade was established at density with trees spaced at 4 m x 4 m, in double-line barriers, incorporating five lines of cocoa between barriers. Under greenhouse conditions, the cocoa was grafted in its entirety onto the IMC67 to avoid variation caused by rootstock. The agronomic management in each plot of the localities was carried out according to the results of the previous analysis of soil fertility and crop requirements. For each year, two maintenance pruning were carried out in the crop, according to the growth and development of the plants, a main pruning at the end of the dry season between the months of March and April and a second pruning to a lesser extent between the months of August and September as rainfall decreases.

Weed control was performed four times a year, mainly in the months of March, June, September, and December. A cultural control of diseases was carried out with weekly removal of diseased fruits, which coincided with the health records made. The yield variable expressed in kilograms per tree for one year (kg tree^{-1} year^{-1}) was quantified after the fifth year of establishment of the genotypes (when the plants reach their productive potential), an evaluation recorded during four consecutive years. Additionally, the yield components were quantified, related to the number of cocoa pods per tree, pod index (number of cocoa pods necessary to obtain 1kg of fermented and dry cocoa), number of grains per cocoa pod and grain index (average weight of a grain of cocoa, obtained from a sample of 100 fermented and dried beans).

The evaluation of the phytosanitary response in the genotypes was carried out under conditions of natural inoculum pressure in each one of the zones. The main diseases evaluated in their incidence were: frosty pod rot (*Moniliopthora roreri*), witch's broom (*Moniliopthora perniciosa*) and brown rot (*Pythopthora* sp.). For this, the incidence calculation was applied using the following formula: Incidence (%)= $\frac{NMA}{NMA + NMS}$ x 100. NMA= number of cocoa pods affected by frosty pod rot, witch's broom or *Pythopthora* sp., NMS= number of healthy cocoa pods.

To identify the reproductive behavior of the eight genotypes, in terms of dependence or not on pollen, the characterization of sexual compatibility was carried out for each of the genetic materials under study. The evaluations were performed in two localities, Rionegro and Muzo, selected for their differences in altitude and annual precipitation. Manual pollination was carried out in 50 flowers for each genotype in the same year, both by self-pollination and by cross-pollination, methods performed according to routine processes in the Agrosavia improvement program.

Fifteen days after pollination, the effectiveness was evaluated, quantifying the formation of fruits in each pollination. Self-compatibility and intercompatibility were determined when the percentage of successful crosses was equal to or greater than 30%, following previously described methodologies and parameters (Eskes *et al.*, 2000; Royaert *et al.*, 2011). The data of the variables were subjected to normality analysis, using the Kolmogorov-Smirnov test with 95% confidence, and homogeneity analysis using the Bartlett test at 95%. Subsequently, an analysis of variance (Anova) with a confidence interval of 98% was carried out.

To evaluate the effect of permanent shade, the analysis consisted of a full factorial design of fixed effects with completely unbalanced random field arrangement. Two factors were evaluated, the first factor corresponded to the genotype and presented nine levels, while factor two corresponded to the type of shade that consisted of three levels. A 95% confidence Kolmogorov-Smirnov test was performed to assess normality and a 95% Bartlett test was performed to assess homoscedasticity, finding differences, mean comparative analysis was performed using the least significant difference methodology (LSD) with a 95% confidence level.

For the disease indices, considering the evaluations under conditions of natural inoculum pressure, a cluster analysis was performed seeking to group similar individuals or those with the same characteristics. This statistic is not inferential, so no confidence intervals or any tests were used to find similarities. The red areas represented the places with the highest incidence of the disease, while the blue areas expressed the opposite. All analyses were done with the R statistical software package V. 3.5.3 (R Core Team, 2019).

Results

The yield results (kg tree^{-1} year^{-1}) through variance analysis indicated significant differences for the treatments (genotypes) evaluated in each year and in each locality (Figure 1). Yield increased for most genotypes during the second and third year of evaluation, for the fourth year it presented a fall. On the other hand, the mean yield, considering the localities, indicated a greater potential in environmental conditions in the municipality of Muzo with an estimated 1 241 kg ha^{-1} year^{-1}, the localities of Carmen and Rionegro presented a similar mean yield (829 and 869 kg ha^{-1} year^{-1}, respectively), while Santa Rosa presented the lowest mean yield (749.6 kg ha^{-1} year^{-1}).

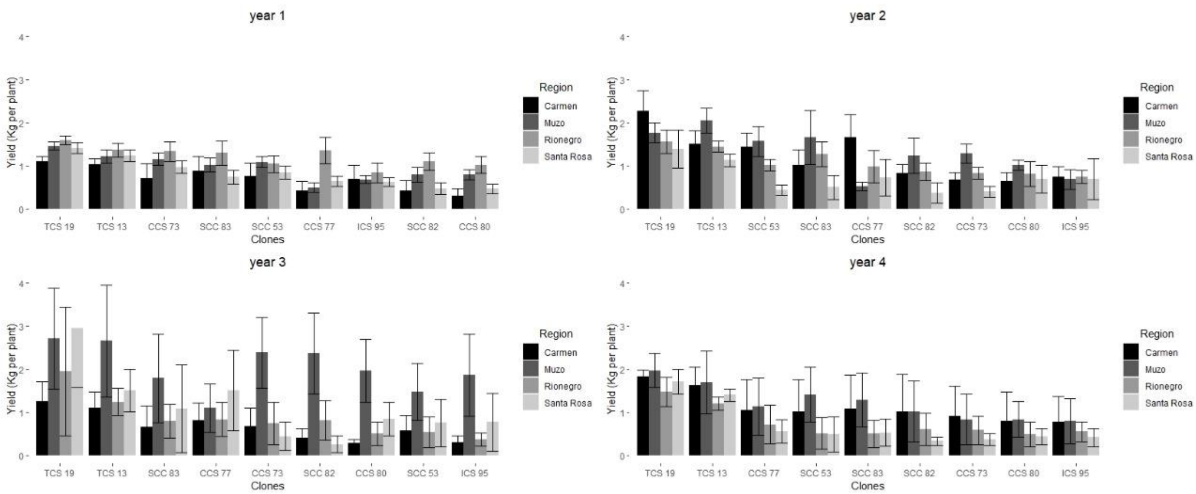

Figure 1. Yield dry bean of nine cocoa genotypes evaluated in four cocoa bean producing localities in Colombia. El Carmen (Santander), Rionegro (Santander), Muzo (Boyacá) y Santa Rosa del Sur (Bolívar) consolidated by year. Statistical differences (*p*< 0.05). Error bars correspond to standard deviation.

Regarding the treatments, the genotypes with the best response were TCS13 and TCS19 (Table 2) with a general mean of 1.5 and 1.8 kg tree^{-1} year^{-1} (projecting a yield of 1 350 and 1 620 kg ha^{-1} year^{-1}, respectively) across the evaluated years and the localities. In the different experimental plots, the productions of the evaluated genotypes were higher than the average yield in Colombia by Federacion Nacional de cacaoteros (Fedecacao).

Table 2. Averages of yield components evaluated in nine cocoa clones in four localities in the Montaña Santander and Magdalena Medio regions, producers in Colombia.

Clone	Average yield (kg plant^{-1})	Number of pods	Number of beans per pod	Index pod	Index bean
TCS19	1.8 A	27.5 A	39 B	16.5 BC	1.6 CD
TCS13	1.5 B	19.8 B	41.9 AB	14 DE	1.8 B
SCC83	1 C	12.3 CD	43.4 A	17.2 B	1.5 DE
SCC53	0.9 C	11.05 DE	43.6 A	17.3 B	1.43 E
CCS73	0.9 CD	9.59 F	37.2 C	13.75 E	2.05 A
CCS77	0.9 CD	12.31 C	38.2 C	16 CD	1.7 BC

Clone	Average yield (kg plant^{-1})	Number of pods	Number of beans per pod	Index pod	Index bean
SCC82	0.8 CD	11.63 CD	39.2 B	17 B	1.5 DE
CCS80	0.7 D	10.13 EF	37.2 C	15.2 DE	1.8 B
ICS95	0.7 D	10.04 EF	36 C	19 A	1.53 DE
Average	1.01	13.83	39.14	16.19	1.65

Mean the same letter do not differ statistically. Least significant difference (LSD) test at 95% confidence level.

The yield component variables with the greatest contribution to the total phenotypic variation were the yield and the number of pods per tree (Table 2), for this last variable the best clone was TCS19, followed by TCS13 presenting significant differences with the control ICS95. The yield obtained with the evaluated genotypes was positively correlated with a greater number of pods per tree, number of grains per pod and negatively with the incidence of frosty pod rot. Clone CCS73 presented the highest grain index and best pod index, followed by clone TCS13 presenting significant differences with the control.

The interaction analysis between genotype and shade type was significant with 98% confidence, showing significant differences between the interactions. The best response in yield was for the genotype-*Cariniana piryformis* interactions (Figure 2), followed by genotype-*Tectona grandis* and finally the genotype-*Hevea brasilienses* interaction, presenting the lowest yields for most of the treatments. The best response genotypes were TCS19 regardless of the type of shade, followed by TCS13 with shade *T. grandis* and *C. piryformis*, surpassing the control ICS95, which presented its best performance associated with *C. piryformis* showing significant differences with *H. brasiliensis*.

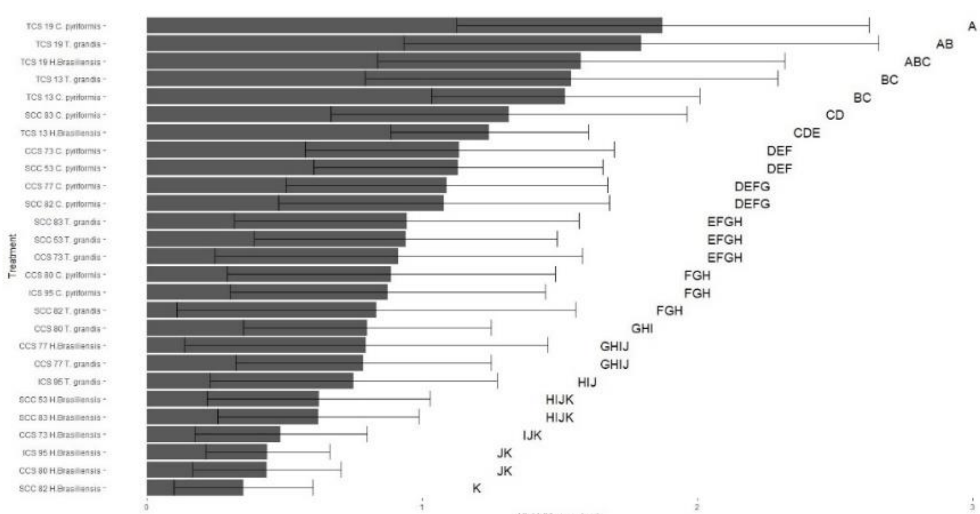

Figure 2. Yield of cocoa clones evaluated in interaction with three types of permanent shade, tropical fine woods of *Cariniana piryformis*, *Tectona grandis* and *Hevea brasilienses*. Means with the same letter do not present significant differences through LSD test. Error bars correspond to standard deviation.

The response of cocoa genotypes to the incidence of diseases under conditions of natural inoculum pressure, indicated that in the case of *M. roreri*, *M. perniciosa* and *Phythophtora* sp. different levels were presented in the studied localities, as well as among the evaluated genotypes (Figure 3). The frosty pod rot disease presented with a higher incidence in the localities of Rionegro and Santa Rosa (Figure 3 top left), forming a separate group about the localities of Carmen and Muzo where the incidence was lower.

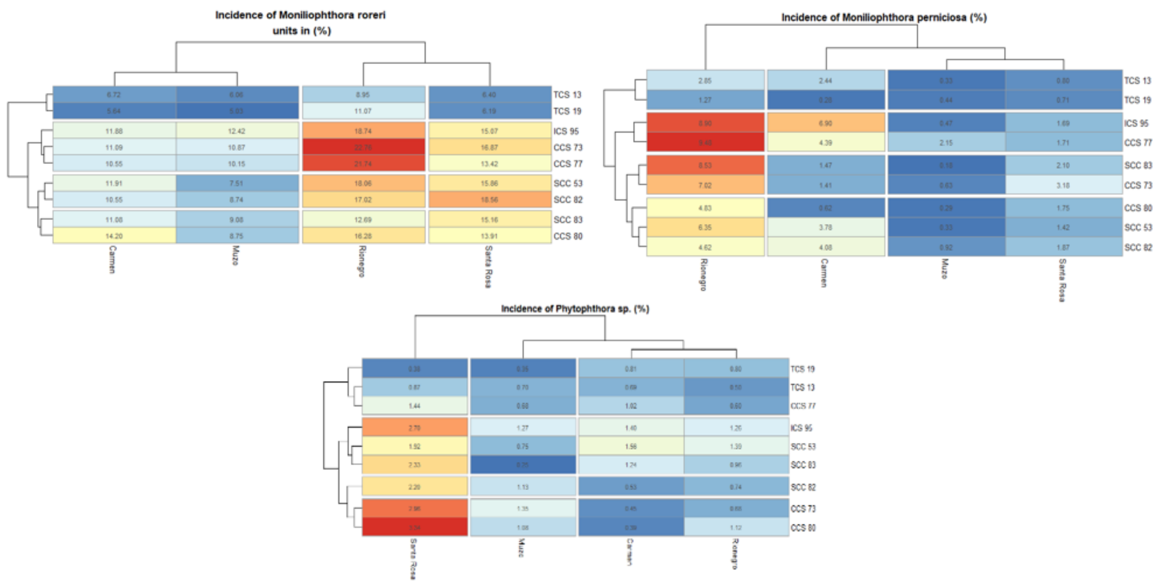

Figure 3. Incidence of diseases: top left: frosty pod rot (*Moniliophthora roreri*), top right: witch's broom (*Moniliophthora perniciosa*) and down: *Phytophthora* sp. in nine cocoa clones. Evaluations carried out under conditions of natural epidemics in four localities of Montaña Santandereana and Magdalena Medio, Andean natural region, during four years of evaluation in productive stage.

In the case of witch's broom (*M. perniciosa*), the highest incidence occurred in the municipality of Rionegro, forming an independent group compared to the other three locations, very similar to the case of *Phythophtora* sp., in the municipality of Santa Rosa (Figure 3 to right, down) although the incidence levels were very low. The genotypes with the best response to the three diseases were TCS13 and TCS19, presenting lower levels of incidence in the four evaluated localities, consolidating a separate group compared to the control ICS95.

The results in fruit formation from self-pollination indicated that the genotypes TCS13 (52%), TCS19 (40%) and SCC82 (42%) are self-compatible (SC), evaluations carried out in the localities of Rionegro and Muzo (Figure 4). In the other genotypes evaluated, their self-incompatibility (SI) was determined, given their low percentage of fruit formation, less than 30%. It should be noted that for the ICS95 and CCS 77 materials, percentages of effectiveness (SI) of fruit set of 25 and 26% respectively were obtained. However, the minimum level is decisive to classify the self-compatibility of the genotype.

Rionegro

♀ \ ♂	TCS13	TCS19	SCC53	ICS 95	SCC82	SCC83	CCS73	CCS77	CCS80
TCS13	SC	IC	IC	IC	IC	IC ✦	IC ✦	II	IC ✦
TCS19	II	SC	II	II	IC	IC ✦	IC	II	II
SCC53	IC ✦	IC	SI	IC ✦	IC	IC	IC ✦	IC	IC
ICS 95	IC	IC ✦	II	SI	II	II	IC	IC	II
SCC82	IC	IC	II	II	SC	II	IC	II	II
SCC83	IC ✦	IC ✦	IC ✦	II	IC	SI	II	II	II
CCS73	IC	IC ✦	IC ✦	II	II	IC	SI	IC ✦	IC ✦
CCS77	IC	IC ✦	IC ✦	IC	IC	IC ✦	IC	SI	II
CCS80	II	IC	II	II	IC	II	II	IC	SI

Muzo

♀ \ ♂	TCS13	TCS19	SCC53	ICS 95	SCC82	SCC83	CCS73	CCS77	CCS80
TCS13	SC	IC	IC	IC	II	IC	IC	IC	IC
TCS19	II	SC	II	IC	IC	IC ✦	IC	II	II
SCC53	IC ✦	IC ✦	SI	II	IC	IC	II	IC	II
ICS 95	II	IC	II	SI	II	II	IC	IC	IC
SCC82	IC ✦	IC ✦	IC ✦	II	SC	IC	II	II	IC
SCC83	IC ✦	IC ✦	IC	IC	II	SI	IC	II	II
CCS73	IC	IC ✦	II	IC	II	IC	SI	IC ✦	II
CCS77	IC	IC	IC	II	IC	IC	II	SI	II
CCS80	II	II	IC	IC	II	II	II	IC	SI

SC = Self-Compatible; SI = Self-Incompatible; II = Inter-Incompatible; IC = Intercompatible; IC ✦ = Intercompatible greater than 70%

Figure 4. Degree of compatibility between the combinations of the nine cocoa genotypes under study. Assessments carried out in the Montaña Santandereana and Magdalena Medio natural subregion, localities corresponding to the municipalities of Rionegro and Muzo.

The TCS 19 genotype was highlighted as a pollen donor, presenting intercompatibility with the other genotypes, except with CCS80 in Muzo. In turn, the TCS 13 genotype is generally a good pollen receptor, considering that it presented intercompatibility with most treatments, except with the CCS77 genotype in Rionegro and SCC82 in Muzo, in general, its response varies regarding the conditions, it presented better compatibility percentages in Rionegro. CCS80 genotype had the highest inter-compatibility, regardless of the two evaluation conditions, in turn, along with SCC82 genotype, presented the greatest instability in their response for the two localities (ie. Rionegro and Muzo).

Discussions

The mean yield obtained for the genotypes evaluated in the present study (1.02 kg tree^{-1} year^{-1}) regarding the established planting density (900 plants ha^{-1}), indicated a yield potential of 922.4 kg ha^{-1} year^{-1}, yield higher than the national average (0.53 t ha^{-1}) in 2018. With these results, it can be inferred that the problem of low cocoa yields in the country can be increased with high-yield genetic materials together with proper agronomic management as well as the interaction with the use of shade (Lahive *et al.*, 2019). TCS19 and TCS13 genotypes presented productive potential higher than 1 t ha^{-1}. TCS19 was the one with the highest yield and the most stable across the evaluated locations, indicating a lower genotype-by-environment interaction, variety with projection of general adaptation to the different agroecological conditions of production.

The Muzo locality presented a potential average yield of 1.2 t ha^{-1} year^{-1}, considering all the evaluated genotypes, higher than the other localities, indicating possible optimal agroecological and climatic conditions (Schroth *et al.*, 2016) for the cocoa cultivation, especially annual rainfall between 2 000 and 2 600 mm and temperature between 22 and 25 °C. The decrease in yield in the fourth year of evaluation is correlated with the increase in the incidence of frosty pod rot, the main limiting disease of cocoa in Colombia, which has been registered with high levels of incidence in different cocoa-producing areas in the country (Cubillos, 2017).

In the fourth year of evaluation, there was a variation in climatic conditions, mainly with the annual decrease and monthly fluctuation in rainfall and an increase in monthly maximum temperatures. However, the distribution of rainfall and the duration of the dry period are considered more important factors in the climatic suitability for the cultivation of cocoa (Schroth *et al.*, 2016), than the accumulated precipitation, our results indicated that the distribution of rainfall in the year 4 presented a greater number of dry months, with precipitation less than 100 mm, in most localities.

The higher incidence of frosty pod rot presented in year 4, when compared to previous years, may be due to the stress that dry periods can generate on cocoa plants, since it is a condition that may not favor the development of the epidemics caused by *M. roreri*. The development of epidemic is favored by conditions with a higher frequency of precipitation (Leandro-Muñoz *et al.*, 2017) and vary according to the locality, genotypes, observation time and the number of pods produced (Jaimes *et al*., 2019).

In plants, the stresses generated by non-optimal climate conditions for their growth cause a homeostatic imbalance, inducing the plant to compensate. This can predispose plants to severe attacks by hemibiotrophic pathogens with low inoculum concentrations (Bostock *et al.*, 2014), as could occur for *M. roreri* during this period. The non-availability of water resources according to the requirements of the plant, as well as salinity, are environmental factors that cause the greatest impact on the development and productivity of the plants, presenting a reduction in crop yields (Golldack *et al.*, 2014).

In the cultivation of cocoa in Colombia the use of shade predominates by 75% (Somarriba and Lopez, 2018), offering ecophysiological advantages where the type of shade influences the response in gas exchange variables such as photosynthesis, which contributes directly on the growth and productivity of plants (Agudelo *et al.*, 2018), added to the adaptation and mitigation to conditions of climatic variability (Lahive *et al*., 2019).

Our results show a superior productive response for the genotypes evaluated in the agroforestry system with *Cariniana piryformis*, a native species commonly known as ‘abarco’, representing a potential forester to incorporate as permanent shade, although the three foresters project a level of shade between 60 and 70%, *C. Piryformis* does not present strong defoliation in the dry season that can affect radiation levels and consequently the photosynthetic rate in cocoa (Agudelo *et al.*, 2018), the added value for its quality is also highlighted in fine wood and contribution to forest conservation given that it is in a threatened state (Cárdenas *et al.*, 2015; Sarmiento *et al.*, 2019), additionally lower levels of moniliasis were evidenced compared to agroforestry systems with teak and rubber tree.

Although in the localities the incidence of the diseases was low, the highest percentages of incidence of *M. roreri* and *M. perniciosa* occurred in the experimental plot of Rionegro (Figure 3). In the conditions of this municipality, high incidences of 48.8% of moniliasis have been reported (Cubillos, 2017). In this regard, most genotypes under the conditions of the APS evaluated have a good response to this disease, compared to the control ICS95, which is reported as moderately resistant in the same locality (Jaimes *et al.*, 2019) and where a greater variability of the pathogen is estimated due to the location of the cocoa germplasm bank.

The genotypes with the best response to the presence of these three diseases were TCS19 and TCS13 (Figure 3), this added to the best grain yield and greater number of cocoa pods per tree, TCS13 compared to TCS19 presented a better cocoa pod index, requiring an average of 14 cocoa pods per kg of dry grain, this due to a higher grain index exceeding the control ICS95, These characteristics led to their selection as varieties for the establishment of commercial production in the regions of Montaña Santandereana and Magdalena Medio, additionally, to be incorporated as parental lines in the Agrosavia breeding program.

Cocoa is considered a preferentially cross-pollinated species, evidenced by its high rate of self-incompatibility and its floral morphology. The inter-compatibility between genetic materials is very important to define the planting arrangements that allow increasing fruit production. The two superior genotypes obtained in this research presented self-compatibility and inter-compatibility for TCS13-TCS19, but TCS19 does not receive pollen from TCS13.

This indicates that it is possible to establish homogeneous productions with each clone. In the case of planting designs, it is recommended to establish productions with combinations of specific varieties, especially for the TCS19 clone which presented a greater inter-incompatibility compared to TCS13, this with the goal of increasing production per unit area, cocoa yields can be improved with the optimal selection of clonal varieties during the establishment of a new plantation (López *et al.*, 2021) by presenting a greater opportunity for compatible combinations.

Conclusions

The genotypes TCS13 and TCS19 stood out in average yield, number of pods, low natural incidence of diseases, under agroforestry system with *Cariniana piryformis*. These genotypes were registered for commercial use, but they can also be considered as progenitors in breeding programs and should be tested in crosses with other genotypes as CCS73 genotype for its outstanding grain index (2.05), whit the aim of increased productivity in cocoa.

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